Determination of Phenolic Compounds, Flavonoids, and Antioxidant Activities in Water Extracts of Thai Red and White Rice Cultivars

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Background: *Free radical-induced oxidative stress damages cellular components leading to many human diseases. Plant-derived antioxidant compounds have become a profitable alternative to prevent oxidative stress in cells.*
Objective: *To determine and compare total phenolic and flavonoid contents as well as antioxidant activity using both chemical and cell assays in the water extracts of brown rice and rice bran from two Thai rice cultivars: Sangyod, a red pigmented rice typically grown in Southern Thailand, and Dawk Mali 105, a commercial white-colored rice.*
Material and Method: *All the rice water extracts were analyzed for their total phenolic and flavonoid contents using the colorimetric assays, as well as for their antioxidant activity through two chemical assays: DPPH radical-scavenging and inhibition of lipid peroxidation assays, as well as through cell-based assays: scavenging capacity of intracellular ROS in HL-60 cells using the fluorescent DCF and the NBT reduction.*
Results: *The two chemical assays detected free radical scavenging and free radical chain breaking activities in all the rice extracts with EC_{50} values ranging from 26 to 357 µg/ml. Moreover, the cell-based assays detected ROS scavenging activities of these extracts with EC_{50} values in the range of 0.6 - 5 mg/ml. All these assays indicated that the water extracts of Sangyod exerted significantly higher antioxidant activity than those of Dawk Mali 105, which exhibited only moderate to low activity. Furthermore, high levels of antioxidant activity of the water extracts of Sangyod were closely correlated to their flavonoid and phenolic contents, which were approximately 2.5 and 3 times higher, respectively, than those of Dawk Mali 105.*
Conclusion: *These findings suggest that water extracts from colored brown rice or colored rice bran can be promising sources of potential natural antioxidants.*

Keywords: *Antioxidant activity, Red rice, Brown rice, Rice bran, Water extract*

J Med Assoc Thai 2010; 93 (Suppl. 7) : S83-S91
Full text. e-Journal: *http://www.mat.or.th/journal*

An imbalance between free radical formation and radical scavenging capacities causes oxidative stress that extensively damages all components of the cell including proteins, lipids, and DNA, ultimately leading to many diseases such as cancer, atherosclerosis, cardiovascular diseases, ageing and inflammatory diseases[1-4]. Since some synthetic antioxidants have been documented to exhibit adverse effects such as carcinogenic effects in animals[5,6], antioxidants from natural sources such as vegetables, fruits, and cereals have become a profitable alternative to prevent oxidative stress.

Rice bran is a rich source of natural antioxidants including phenolic compounds, plant-based materials such as phenolic acids and derivatives, phenylpropanoids, tannins, lignins, flavonoids, and so forth. Flavonoids are the most common group of phenolic compounds and are water-soluble plant

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pigments with many colors. Both phenolic compounds and flavonoids are powerful antioxidants that can act as free radical scavengers[7], reducing agents, and/or metal ion chelators[8], thus providing various human health benefits[9]. Previous studies have shown that bran of red and black rice cultivars exhibits higher antioxidant activity and higher phenolic contents than bran of nonpigmented rice seen in light brown[10,11]. Further, such pigmented rice bran effectively decreases atherosclerotic lesions as well as reduces oxidative stress and inflammation[12,13]. There are many varieties of colored rice mainly produced in Southeast Asian countries. Among these, Sangyod is a Thai red rice cultivar, typically grown in Southern Thailand.

Studies have been reported concerning identification of phenolic contents and antioxidant properties in some Thai nonpigmented cultivars[8], but such scientific information is limited for Thai colored rice varieties. Therefore, the objectives of this study were to determine phenolic compound content, including polyphenols and flavonoids, and to evaluate antioxidant properties, specifically in the rice bran water extract and in the washed water extract of brown rice from Sangyod using both chemical and cell assays. All data obtained from these assays were compared with those of Thai Dawk Mali 105, a commercial nonpigmented rice cultivar.

Material and Method

Folin-Ciocateu reagent, gallic acid, ascorbic acid, 2, 2-Diphenyl-1-picrylhydrazyl (DPPH□), linoleic acid, nitrotetrazolium blue chloride (NBT) 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 and 2,6-Di-tert-butyl-4-methylphenol (BHT) were purchased from Fluka, Spain. DCF-DA was purchased from Invitrogen, USA. The analytical grade methanol and other organic solvents were purchased from Merck, Germany. Other chemicals used in this study were of analytical grade and used without further purification.

Preparation of brown rice and rice bran extracts

Brown rice and rice bran powder of Sangyod and Dawk Mali 105 were obtained by milling rice grains in a local grinding mill (Fig. 1). The water-soluble extract of rice bran of each cultivar was prepared by heating the rice bran powder (1 kg) in 60-75°C distilled water (4 L) for 15-30 minutes. Next, the extract was left for 1 h at room temperature to cool down and was then filtered through a 300-mesh screen and Whatman No.1 filter paper. The washed water extract of brown rice of each cultivar was obtained by mixing brown rice with 2 times weight of distilled water and collecting the washed water (repeat 3 times). The extracts of brown rice and rice bran were then freeze-dried (Lyophilization Systems, Inc., USA) to remove the water. For Sangyod, the percentage yield of the water-soluble extract of rice bran and the washed water extract of brown rice is 6.99% and 0.17%, respectively. For Dawk Mali 105, the percentage yield of the water-soluble extract of rice bran and the washed water extract of brown rice is 16.98% and 1.00%, respectively.

Fig. 1 Brown rice and rice bran of Dawk Mali 105 and Sangyod used in this study.

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 1,640 medium (HyClone, UK) supplemented with 15% heat-inactivated fetal bovine serum (FBS) (HyClone, UK), 100 unites/ml penicillin, and 0.1 mg/ml streptomycin (Gibco, USA) at 37°C and 5% CO_2 atmosphere. To induce myeloid differentiation, HL-60 cells (5 x 10^5 cells/ml) were cultivated for 7 days in RPMI 1,640 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

The total phenolic content was determined using the Folin-Ciocalteu reagent according to the method of Amin et al[14]. The reaction mixture contained 100 µl of the diluted rice extract, 500 µl of freshly prepared diluted Folin Ciocalteu reagent, and 400 µl of 7.5% sodium carbonate. Mixtures were kept in dark for 2 h at room temperature to complete the reaction. The absorbance at 750 nm was measured with a UV-Vis spectrophotometer (Shimadzu Corp., Bara Scientific Co., Ltd.). Gallic acid was used as a standard and the results were expressed as µg gallic acid (GAE)/g rice extract.

Determination of total flavonoid content

Total flavonoid content was determined according to the method of Jia et al[15]. Briefly, an appropriate dilution of the rice extract dissolved in distilled water (250 µl) was added to 1.25 ml distilled water followed by 75 µl of 5% $NaNO_2$. After 5 min at room temperature, 150 µl of 10% $AlCl_3$ was added. After further 5 min, the reaction mixture was treated with 0.5 ml of 1 M NaOH. The absorbance was measured at 510 nm with a UV-Vis spectrophotometer (Shimadzu Corp., Bara Scientific Co., Ltd.). Rutin hydrate was used as a standard and the results of total flavonoid content were expressed as µg rutin hydrate/g rice extract.

Determination of DPPH radical-scavenging activity

The radical-scavenging activity of rice extract was determined by the method of Brand-Williams et al[16]. Briefly, 100 mL of 1 mg % DPPH solution in ethanol was added to 100 ml of various concentrations of the rice extract in distilled water. The mixtures were shaken and allowed to stand for 30 min in the dark at room temperature. A decrease in absorbance of these samples was measured at 517 nm using a microplate reader (PowerWave XS, BioTek) and compared to that of the control (without the extract only). DPPH free radical scavenging ability of each concentration of the rice extract was calculated by the following formula: scavenging ability (%) = 100 x [$A_{517\ nm\ of\ control} - A_{517\ nm\ of\ sample}$] / $A_{517\ nm\ of\ control}$. The scavenging activity was expressed as 50% effective concentration, EC_{50} (mg/ml) described in Statistical Analyses. BHT was used for comparison.

Determination of lipid peroxidation inhibition

Inhibition of lipid peroxidation was measured according to the method of Lingnert et al[17]. Briefly, various concentrations of the rice extract in distilled water (200 ml) were mixed with 3.8 ml of linoleic acid emulsion (3.22 mM) in sodium phosphate buffer (0.2 M), and were then incubated at 60°C in the dark for 17 h to accelerate lipid peroxidation. At the end of incubation, 4 ml of 60% methanol was added. The absorbance of these samples was measured at 234 nm using a UV-vis spectrophotometer (Shimadzu Corp., Bara Scientific Co., Ltd.) and compared to that of the control (without the extract only). The inhibition of each concentration of the rice extract against lipid peroxidation was calculated by the following formula: inhibition of lipid peroxidation (%) = 100 x [$A_{234\ nm\ of\ control} - A_{234\ nm\ of\ sample}$] / $A_{234\ nm\ of\ control}$. The inhibition was expressed as EC_{50} (mg/ml) described in Statistical Analyses. BHT was used for comparison.

Determination of intracellular peroxide level in HL-60 cells by NBT reduction

Intracellular superoxide formation was quantified by nitroblue tetrazolium reduction assay (NBT) according to the method of Makishima et al 1996[18]. Briefly, HL-60 $1x10^6$ cells were incubated with various dilutions of the rice extract dissolved in HBSS (500 µl) in the dark at 37°C for 30 min. Then, they were incubated with 500 ng/ml PMA and 1.25 mg/ml NBT solution for another 30 min. At the end of the incubation time, 2 ml of 1N HCl was added. After vortexing and centrifugation at 12,000 x g for 5 min, the precipitate of insoluble formazan deposits was washed with PBS and dissolved in 250 ml DMSO. The absorbance was measured at 572 nm using a microplate reader (PowerWave XS, BioTek) and compared to that of the control (without the extract only). The inhibition of each concentration of the rice extract against superoxide formation measured by NBT reduction was calculated by the following formula:

NBT reduction (%) = 100 x [($A_{234\ nm\ of\ control} - A_{234\ nm\ of\ background}$) - ($A_{234\ nm\ of\ sample} - A_{234\ nm\ of\ background}$)] / ($A_{234\ nm\ of\ control} - A_{234\ nm\ of\ background}$)

The background absorbance was determined by incubating cells without activation with PMA. The inhibition was expressed as EC_{50} (mg/ml) described in Statistical Analyses. Vitamin C was used for comparison.

Determination of intracellular peroxide level in HL-60 cells by DCF-DA

The inhibition of hydrogen peroxide and

superoxide production of the rice extracts was carried out according to the method of Lin et al[19]. DCF-DA, a nonfluorescence probe was used to determine the intracellular peroxide level. It becomes fluorescent following oxidation by hydrogen peroxide produced during respiratory burst. HL-60 $1x10^6$ cells were suspended in various concentrations of the rice extract with a final concentration of 0.75 mM DCF-DA in the dark at 37°C for 30 min. The cells were stimulated with the addition of 100 ng/ml PMA and incubated for another 30 min. Flow cytometric analysis was then performed using the FACSCalibur flow cytometer (Becton Dickinson) and CellQuest 3.0.1 software (Becton Dickinson). The mean fluorescence intensity (MFI) of more than 1×10^4 cells was collected and analyzed for the cells producing hydrogen peroxide and superoxide. The remaining mean fluorescence intensity (MFI) of treated cells relative to that of non-treated control cells (without the extract only) indicated percentage inhibition of peroxide production and was calculated by the following formula:

Inhibition of O_2 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 activation with PMA. The inhibition was expressed as EC_{50} (mg/ml) described in Statistical Analyses. Vitamin C was used for comparison.

Statistical analyses

The effective dose concentration (EC_{50}) value of each extract was calculated by generating dose-response curves, plotting the percentage of antioxidant activity versus its corresponding concentration (five to six different concentrations) using GraphPad Prism software and cubic spine interpolation. All results were expressed as mean ± SD of three or four separate experiments. All statistical analyses were carried out using SPSS for Windows. Analysis of variance was performed by the ANOVA procedure. Significant differences between means were determined by LSD at a level of $p \leq 0.05$.

Results

Total phenolic content of rice extracts

With the Folin-Ciocalteu method, the total phenolic content of the washed water extract of brown rice and the water-soluble extract of rice bran from Dawk Mali 105 and Sangyod were in the range of 228.10-753.48 µg gallic acid eq/g rice extract (Table 1). The content of phenolic compounds was the highest in the water-soluble extract of Sangyod rice bran and the lowest in the water-soluble extract of Dawk Mali 105 rice bran with significant difference between all the extracts ($p \leq 0.05$). In addition, the phenolic content of the water extracts obtained from Sangyod red rice was approximately three times higher than that obtained from Dawk Mali 105 white rice.

Total flavonoid content of rice extracts

The total phenolic content of the washed water extract of brown rice and the water-soluble extract of rice bran from Dawk Mali 105 and Sangyod were in the range of 39.59-135.09 µg rutin hydrate eq/g rice extract (Table 1). The content of flavonoids was the highest in the water-soluble extract of Sangyod rice bran and the lowest in the washed water extract of Dawk Mali 105 brown rice with significant difference

Table 1. Total phenolic and flavonoid contents of the water 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 hydrated eq/g rice extract)
Dawk Mali 105		
Washed water extract of brown rice	258.30 ± 6.44d	39.59 ± 2.30d
Water-soluble extract of rice bran	228.10 ± 4.47c	53.97 ± 6.49c
Sangyod		
Washed water extract of brown rice	616.30 ± 7.25b	95.17 ± 5.91b
Water-soluble extract of rice bran	753.48 ± 8.98a	135.09 ± 4.15a

Results represent means ± standard deviation ($n > 5$). In each column, different letters mean significant differences ($p \leq 0.05$).

between all the extracts ($p \leq 0.05$). In addition, the flavonoid content of the water extracts obtained from Sangyod red rice was nearly two-and-one half times greater than that of Dawk Mali 105 (Table 1).

DPPH radical-scavenging activity of rice extracts

DPPH assay was used to assess antioxidant efficiency of all the rice extracts that scavenged the free radical DPPH with purple color, resulting in its stable nonradical form with yellow color. This scavenging activity was shown to increase with the increasing concentrations of all the rice extracts, suggesting that these extracts scavenged radical DPPH in a dose dependent manner. The water-soluble extract of rice bran and the washed water extract of brown rice from Sangyod showed the best free-radical scavenging activity with EC_{50} values of 32 and 35 µg/ml, respectively with no significant difference. These activities were significantly higher than those of Dawk Mali 105 ($p \leq 0.05$) (Fig. 2A). The water-soluble extract of rice bran from this white rice exhibited moderate antioxidant activity with an EC_{50} value of 186 µg/ml, whereas its washed water extract of brown rice had the lowest activity with an EC_{50} value of 357 µg/ml with significant difference ($p \leq 0.05$) (Fig. 2A). The free-radical scavenging activity of all the rice extracts was higher than that of BHT (EC_{50} 13 µg/ml).

Inhibition of lipid peroxidation by rice extracts

Linoleic acid test system was used to measure inhibitory capacity of all the rice extracts that can inhibit hydrogen peroxide production in the peroxidation of linoleic acid. The inhibitory activity of the extract against lipid peroxidation was shown by a gradual decrease of absorbance at 234 nm, due to the diminution of the lipid oxidation products of linoleic acid, specially the conjugated dienes. In this study, all the rice extracts inhibited linoleic peroxidation in a dose dependent manner. The water-soluble extract of rice bran and the washed water extract of brown rice from Sangyod exhibited potent antioxidant activity with EC_{50} values of 26 and 31 µg/ml, respectively with no significant difference. These activities were significantly higher than those of Dawk Mali 105 ($p \leq 0.05$) (Fig. 2B). The water-soluble extract of rice bran from this white rice showed moderate antioxidant activity against linoleic peroxidation with an EC_{50} value of 69 µg/ml, while its washed water extract of brown rice had the lowest activity with an EC_{50} value of 88 µg/ml ($p \leq 0.05$) (Fig. 2B). Only the water extracts of Sangyod had higher inhibitory capacity than BHT (EC_{50} 50 µg/ml).

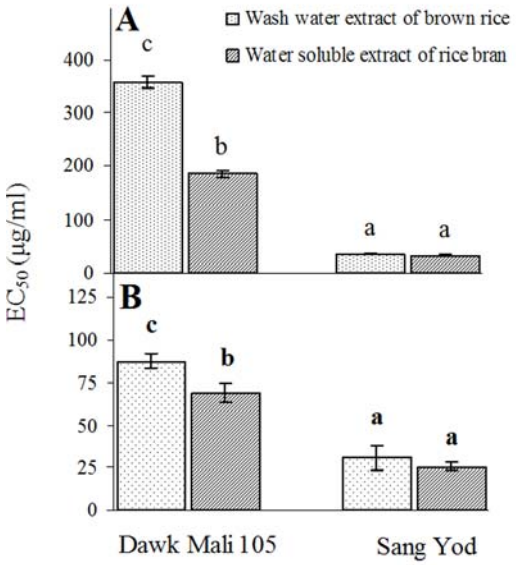

Fig. 2 EC_{50} values of the water extracts from Dawk Mali 105 and Sangyod rice cultivars in scavenging stable free DPPH radical (A), and in inhibiting linoleic acid peroxidation (B). Bars marked by different letters are significantly different ($p \leq 0.05$).

Inhibition of superoxide generation by NBT reduction assay

The scavenging activity of all the rice extracts against PMA-induced ROS production in differentiated HL-60 cells was quantified by the reduction of NBT to formazan, which was observed by a gradual decrease of absorbance at 572 nm. Similar to the results obtained by the two chemical assays above, the rice extracts showed dose-dependent increase in scavenging activity on superoxide production. The washed water extract of brown rice and the water-soluble extract of rice bran from Sangyod possessed the strongest superoxide scavenging activity with EC_{50} values of 614 and 637 µg/ml, respectively with no significant difference (Fig. 3A). These activities were significantly greater than those of Dawk Mali 105 ($p \leq 0.05$). The water-soluble extract of rice bran from this white rice showed moderate scavenging effect with an EC_{50} value of 723 µg/ml, which was significantly higher than that of its washed water extract of brown rice with very high EC_{50} value of 5 mg/ml ($p \leq 0.05$) (Fig. 3A). All the rice extracts expressed lower superoxide scavenging activity than ascorbic acid (EC_{50} 141 µg/ml).

Inhibition of superoxide production by DCF assay

The scavenging activity of all the rice extracts

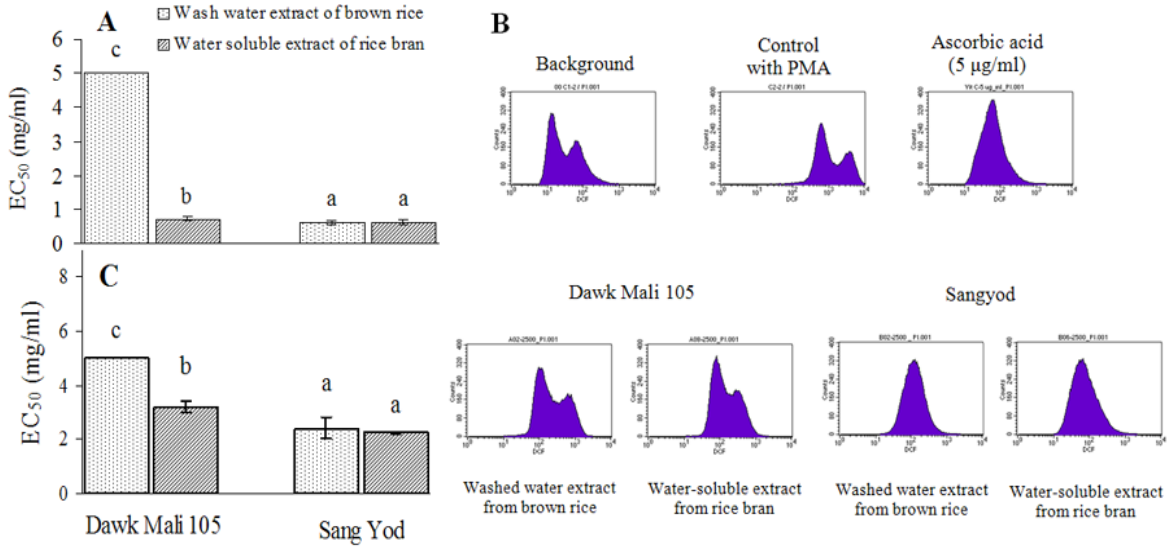

Fig. 3 EC_{50} values of the water extracts from Dawk Mali 105 and Sangyod rice cultivars in scavenging PMA-stimulated superoxide production in HL-60 cells measured by the NBT reduction (A) and DCF fluorescence intensity (C). Bars marked by different letters are significantly different ($p \leq 0.05$). The histograms of DCF fluorescence intensity in HL-60 cells after incubation with 2,500 mg/ml of each extract listed in the figure except for ascorbic acid in comparison to the background and the control (B).

against PMA-induced ROS production in differentiated HL-60 cells was also quantified by measuring DCF fluorescence intensity after these cells were treated with each extract of various concentrations ranging from 125-5,000 mg/ml. Fig. 3B depicts the histograms of the fluorescence intensity of the cells only treated with 2,500 mg/ml of each extract as representatives of the other concentrations. These histograms showed the shift in fluorescence to the left with varying degrees, resulting in different intensities of the remaining fluorescence as compared to the background and the control. Ascorbic acid only at 5 mg/ml was shown for comparison. Percentage inhibition on superoxide production at each of those concentrations was calculated by using the formula listed in Materials and Methods, as well as was expressed as EC_{50} (mg/ml) described in Statistical analyses.

Consistent with the NBT reduction assay, all the rice extracts scavenged superoxide produced in HL-60 cells in a dose dependent manner. The water-soluble extract of rice bran and the washed-water extract of brown rice from Sangyod exhibited the strongest scavenging effect with EC_{50} values of 2.25 and 2.4 mg/ml, respectively with no significant difference (Fig. 3C). These scavenging effects, however, were significantly higher than those of Dawk Mali 105 ($p \leq 0.05$), which showed moderate and low scavenging activity in its water extracts of rice bran and brown rice, with EC_{50} values of 3.21 and 5 mg/ml, respectively ($p \leq 0.05$) (Fig. 3C). Besides, all the rice extracts exhibited lower antioxidant activity than ascorbic acid (EC_{50} 2.7 µg/ml).

Discussion

Many previous studies have reported the use of different solvent systems to extract rice bran such as water at room temperature[20], ethanol-water (70:30 v/v)[21], methanol[8,22], and hexane[23]. In this study, two novel methods using water were developed as follows: (i) the method of heating rice bran in 60-75°C water, followed by freeze-drying to obtain water-soluble extract of rice bran, and (ii) the method of mixing brown rice with distilled water and collecting the washed water, followed by freeze-drying to obtain the washed-water extract of brown rice. These two water-extraction methods were shown to be efficient for extracting water-soluble substances containing phenolic compounds and flavonoids from both Thai red and white rice cultivars. Contents of those compounds in the water extracts of Sangyod red rice were higher than those found in Dawk Mali 105 white rice. These data are consistent with previous studies showing high level of phenolic compounds in pigmented rice, such as red and black rice[10,11]. Among all of the rice extracts, the washed water extracts of Sangyod brown rice contained

the second highest level of polyphenols and flavonoids, after the water-soluble extracts of Sangyod rice bran. These findings suggest that water from washing colored brown rice is highly nutritious and should therefore be saved for cooking brown rice. However, one concern is heat effects on the total phenolic content. Previous studies have shown conflicting results on this matter. Some studies have reported increased phenolic contents with increasing extraction temperature[24] whereas other studies have shown some heat-labile phenolic compounds with varying degrees[25].

Regarding the antioxidant activity, both chemical and cell assays were used to analyze this activity of all the rice extracts. A combination of different methods is necessary because natural antioxidants from plant materials and their actions are complex, as well as antioxidant analytical methods employ different reaction mechanisms. Furthermore, the cell-based methods tend to more closely reflect antioxidant effects of the rice extracts *in vivo*. In this study, the results revealed that both chemical and cell culture systems detected the antioxidant activity of all the rice extracts with different degrees depending on the extract, and nevertheless showed parallel results. These findings indicated that these rice extracts contained substances with various antioxidant activities including free radical scavenging, free radical chain breaking, and ROS scavenging activities, which were monitored by DPPH radical scavenging assay, inhibition of lipid peroxidation assay, as well as NBT reduction and DCF assays, respectively, on the basis of their specific reaction mechanisms in detecting a particular mode of antioxidant actions.

From those results, all the rice extracts showed different levels of antioxidant activity that can be classified into three groups according to their EC_{50} values; (i) the washed water extract of brown rice and the water-soluble extract of rice bran from Sangyod possessed high antioxidant activity with no significant difference, (ii) the water-soluble extract of Dawk Mali 105 rice bran exhibited moderate antioxidant activity, and (iii) the washed water extract of Dawk Mali 105 brown rice showed the lowest antioxidant activity. The same rice extract, however, displayed relatively higher EC_{50} values obtained by the cell assays than those obtained by the chemical assays, partly due to the complexity of these natural antioxidant materials and their inhibitory actions in the cells. These data are consistent with those of previous studies indicating that bran extracts of colored rice have higher antioxidant activity than those of white rice[10,11,21]. In addition, the results of this study reveal for the first time that the washed water extract of colored brown rice exhibits the same antioxidant capacity as the water-soluble extract of colored rice bran.

With respect to the correlation between total contents of phenolic compounds and flavonoids, as well as the antioxidant capacity, the results indicated that the antioxidant activity of Sangyod red rice extracts was greater than that of Dawk Mali 105 white rice extracts. These activities were closely correlated to their phenolic and flavonoid contents. In contrast, the washed water extract of Sangyod brown rice exhibited the same degree of antioxidant activity as that of the water-soluble extract of Sangyod rice bran despite having lower phenolic and flavonoid contents. This could likely be due to the fact that apart from polyphenols and flavonoids, other antioxidant compounds such as vitamin C and carotenoids present in the part of colored rice grain may partly contribute to the overall antioxidant activity.

In summary, the findings of this study suggest that appropriately prepared water crude extracts from colored brown rice or colored rice bran have potential application as preventive agents for degenerative diseases because of their potent antioxidant activity.

Acknowledgements

This work was supported by the National Research Council of Thailand.

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การวิเคราะห์ปริมาณสารฟีโนลิค และฟลาโวนอยด์ และทดสอบฤทธิ์ต้านอนุมูลอิสระของสารสกัดน้ำจากข้าวไทยพันธุ์มีสี และข้าวขาว

อัมรัตน์ ศรีสวัสดิ์, วัชรินทร์ ปะนันโต, นพมาศ แก่นดี, เสริมเกียรติ ทานุชิต, อรุณพร อิฐรัฐ, นุชสิริ เลิศวุฒิโสภณ, พินทุสร หาญสกุล

ภูมิหลัง: *สารอนุมูลอิสระเป็นอันตรายต่อเซลล์ของร่างกาย โดยจะทำลายดีเอ็นเอและองค์ประกอบอื่นๆ ของเซลล์ ก่อให้เกิดโรคต่างๆ มากมาย สารต้านอนุมูลอิสระที่ได้จากพืช เช่น ข้าว ซึ่งเป็นอาหารหลักของชาวเอเชีย เป็นอีกทางเลือกหนึ่งที่ถูกนำมาใช้ประโยชน์ในด้านการป้องกันและยับยั้งปฏิกิริยาของสารอนุมูลอิสระที่เกิดขึ้นภายในเซลล์*

วัตถุประสงค์: *เพื่อวิเคราะห์และเปรียบเทียบปริมาณสารฟีโนลิคและฟลาโวนอยด์ รวมทั้งฤทธิ์ในการต้านอนุมูลอิสระของสารสกัดจากน้ำซาวข้าวกล้องและสารสกัดน้ำจากรำข้าว ของข้าวไทยสองสายพันธุ์ ได้แก่ ข้าวสังข์หยด ซึ่งมีเมล็ดข้าวสีน้ำตาลแดง และข้าวดอกมะลิ 105 มีเมล็ดข้าวสีขาว โดยทำการทดสอบฤทธิ์ต้านอนุมูลอิสระด้วยระบบสารเคมีและระบบเซลล์เพาะเลี้ยง*

ระเบียบวิธีวิจัย: *วิเคราะห์หาปริมาณสารฟีโนลิคและฟลาโวนอยด์ในสารสกัดน้ำจากข้าวทั้งสองสายพันธุ์โดย colorimetric assays และวิเคราะห์หาฤทธิ์ต้านอนุมูลอิสระโดยใช้ระบบสารเคมี ได้แก่ DPPH radical-scavenging assay และ lipid peroxidation inhibition assay นอกจากนี้ยังได้ทดสอบฤทธิ์ต้านอนุมูลอิสระโดยใช้ระบบเซลล์ ซึ่งเป็นการทดสอบฤทธิ์ scavenging activity ของสารสกัดในการยับยั้งการสร้าง ROS ในเซลล์ HL-60 ซึ่งตรวจวัดได้โดย DCF assay และ NBT reduction assay*

ผลการวิจัย: *การทดสอบฤทธิ์ต้านอนุมูลอิสระด้วยระบบสารเคมีทั้งสองวิธีพบว่าสารสกัดน้ำจากข้าวสองสายพันธุ์มีฤทธิ์เป็น free-radical scavenger และมีฤทธิ์ยับยั้ง free-radical chain reaction โดยมี EC_{50} อยู่ในช่วง 26-357 mg/ml สำหรับการทดสอบด้วยระบบเซลล์พบว่าสารสกัดมีฤทธิ์เป็น ROS scavenger โดยมี EC_{50} อยู่ในช่วง 0.6-5 mg/ml จากการทดสอบทั้งสี่วิธีพบว่าฤทธิ์ของสารสกัดทั้งหมดเป็นไปในแนวเดียวกัน คือ สารสกัดน้ำจากรำข้าวและข้าวกล้องสังข์หยดมีฤทธิ์ต้านอนุมูลอิสระสูงที่สุด สารสกัดน้ำจากรำข้าวดอกมะลิ 105 มีฤทธิ์ต้านอนุมูลอิสระในระดับปานกลาง และสารสกัดจากน้ำซาวข้าวกล้องดอกมะลิ 105 มีฤทธิ์ต้านอนุมูลอิสระต่ำสุด ฤทธิ์ต้านอนุมูลอิสระในสารสกัดดังกล่าวมีความสอดคล้องกับปริมาณสารฟลาโวนอยด์และฟีโนลิค ซึ่งพบว่าในสารสกัดจากข้าวสังข์หยดมีปริมาณสูงกว่าสารสกัดจากดอกมะลิ 105 ประมาณ 2.5 และ 3 เท่าตามลำดับ*

สรุป: *จากผลการวิจัยชี้ให้เห็นว่าสารสกัดน้ำจากข้าวสังข์หยดซึ่งเป็นข้าวมีสี เป็นแหล่งของสารต้านอนุมูลอิสระจากธรรมชาติที่มีศักยภาพในการนำไปใช้ประโยชน์ในด้านต่างๆ*