Anti-oxidative stress of red and black rice bran extracts against H_2O_2, *t*-BHP, and UVA radiation

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Abstract

Natural beauty products have become a major trend in recent years. Rice has been used as a natural beauty treatment to relief inflammation, for cleansing and softening of the skin. Several antioxidant compounds of colored rice bran, especially flavonoid could then be valuable ingredient in cosmetic recipe. We have previously demonstrated that Red rice bran of Hom Dang and Hom Red Rose offered higher antioxidant activity than the black rice bran of Klam Doi-saked and Hom Dum Sukhothai II. However, antioxidant activity may exhibit different capacity to different chemical stressors. Thus, total flavonoid contents of black and red rice bran extracts were determined and anti-oxidative stress was evaluated under different stressors of H_2O_2, *t*-BHP and UVA radiation. Human melanoma cells were used and viability of the cell was measured by MTT assay. It was observed that Hom Dang contained the highest total flavonoid contents as compared to Hom Red Rose, Klam Doi-saked and Hom Dum Sukhothai II, consecutively. All these rice extracts were able to protect the cells upon H_2O_2, *t*-BHP and UVA treatment. Hom Dang and Hom Red Rose demonstrated better protection activity from H_2O_2 stressor than the black rice as presented from higher cell viability. Interestingly, cell protection of Hom Dang and Hom Red Rose from *t*-BHP was also superior to the rests extracts. Furthermore, cell treatment with Hom Dang demonstrated the most protective capacity against UVA. As a result, Hom Dang seemed to be the most potent anti-oxidative stress and could be considered as a candidate extract for cosmetic preparation.

Keywords: black and red rice bran, flavonoid, anti-oxidative stress, UVA radiation

Introduction

Herbal extracts are principally added to the cosmetic preparations due to several associated properties such as UV protection and antioxidant properties. The fascinated antioxidant botanicals include carotenoids, flavonoids and polyphenols. Rice bran, one of the most important by-products in the rice milling, contains many beneficial anti-oxidative phytochemicals including gamma-oryzanol, anthocyanins and phenolic compounds. Thus, it was possible to derive a health-promoting value-added product from the rice bran. Flavonoids have been reported on their scavenging effects on super-oxide radicals and hydroxyl radicals (Zhishen et al. 1999; Ji-Wu et al. 2002). Anthocynins in black rice are also well-known to lessen oxidative stress and inflammation (Hou et al. 2010; Min et al., 2010). Furthermore, a water-soluble enzymatic extract of rice bran exhibited anti-oxidant effect to cell by reducing the UVB cellular damage (Santa-Maria et al. 2010). However, anti-oxidant capacity could vary depending on variety of rice bran. The total phenolic, total flavonoid, and antioxidant capacities have been shown to be correlated with the intensity of bran color (Min et al. 2011). However, Shen and colleague (2009) demonstrated that the grain color parameters had

negative correlations with the phenolics, flavonoid contents and antioxidant capacity. We have previously found that red rice bran of Hom Dang and Hom Red Rose exhibited relatively high antioxidant activity as compared to the black rice bran of Klam Doi-saked and Hom Dum Sukhothai II (Butchan et al. 2013). This was also in agreement with the report by Muntana and Prasong (2010) that red rice bran extracts had stronger antioxidation capacities than the black and white rice bran extract.

Measurement on mechanism of antioxidant activity of the rice bran extract could be varied depending on methodology, cell types and oxidative stress inducers. This work was aimed to determine anti-oxidative stress of rice bran extracts on human melanoma (A375) cells using MTT assay. Various stress inducers including hydrogen peroxide (H_2O_2), *tert*-Butyl hydroperoxide (*t*-BHP) and UVA radiation were used. H_2O_2 and *t*-BHP is commonly used for evaluation of oxidative stress in cells and tissues due to its capability of free radicals production. UVA irradiation also generates reactive oxygen species (ROS) including singlet oxygen, superoxide anions (O_2^-), hydrogen peroxide (H_2O_2) and the hydroxyl radical ($^{\bullet}OH$). These free radicals lead to cell damage by the oxidation of DNA, proteins and cell membrane, which can be finally determined based on cell viability assay. Therefore, total flavonoids content of red rice bran (Hom Dang and Hom Red Rose) and black rice bran (Klam Doi-saked and Hom Dum Sukhothai II) extracts were determined. Then, antioxidant capacity of the extracts was examined based on its potential to protect the cell from various stress inducers of H_2O_2, *t*-BHP and UVA.

Methodology

Sample

The red and black rice bran extracts were obtained from the S&J international enterprises public company limited (Bangkok, Thailand). The red rices were Hom Dang, Hom Red Rose strains and the black rices were Klam Doi-saked and Hom Dum Sukhothai II strains. These extracts were 50% hydroglycol and stored at -20°C.

Cell culture

Human A375 skin malignant melanoma cells were cultured in Dulbecco medium supplemented with 10% fetal bovine serum and 1 mM pyruvate. They were maintained at 37°C in a humidified atmosphere incubator containing 5% CO_2.

Total flavonoids

Total flavonoid content was determined by a colorimetric method. Two hundred fifty micro-milliliter of rice bran extract was added in a test tube, following with 150 µL of 5% $NaNO_2$ and 150 µL of 10% $AlCl_3.6H_2O$. After 5 min, 500 µL of 1 M NaOH was added. The mixture was incubated at room temperature for 15 min. The absorbance was measured using microplate reader at OD 415 nm. The standard curve of total flavonoids was made using catechin standard (0 to 300 µg/mL) under the same procedure as described earlier. The total flavonoid content was expressed as mg catechin equivalents/g samples.

Cellular oxidative stress

1) Chemical stressor

A375 cells (3×10^5 cells/mL) in DMEM were seeded onto 96 well-plate and grown for 24 h. Cells were washed and treated with H_2O_2 (80 µM) or *t*-BHP (300 µM) for 30 min. After treatment, cells were incubated with new medium containing rice bran extracts (10 mg/mL) for 24 h. The effect of oxidative stress on cell viability was monitored by MTT colorimetric assay. Briefly, 50 µL/well of MTT solution was added and incubated for 4 h. Then, DMSO

was added to dissolve the formazan product. The color intensity of formazan solution reflecting viable cells was measured at 570 nm using microplate reader.

2) UVA treatment

A375 cells (3×10^5 cells/mL) were seeded onto 96 well plate. After cultivated for 24 h, cells were washed with PBS before exposed to UVA radiation at 10 J/cm^2. After that, the treated cells were further incubated with new medium containing rice bran extracts (15 mg/mL) for 24 h. After which the effect of anti-oxidative stress was monitored in the same manner by MTT colorimetric assay.

Statistical Analysis

All analysis were conducted in triplicate (n = 3), and one way ANOVA test (using SPSS statistical software) was used to compare the mean values of each treatment. Significant differences between the means of parameters were determined by using Least Significant Difference (LSD) Test ($p < 0.05$).

Results

Total flavonoid content

The total flavonoid content of each extract expressed as mg catechin equivalent /g samples was shown in Figure 1. Hom Dang revealed higher flavonoid content than Hom Red Rose, Klam Doi-saked and Hom Dum Sukhothai II, respectively. Flavonoid contents in the rice extracts ranged from 6.183-9.046 mg catechin equivalent /g samples. Statistical analysis revealed similar range of flavonoid content in most of the rice extract except Hom Dum Sukhothai II having the least.

Anti-oxidative stress induced by H_2O_2 and *t*-BHP of rice bran extracts

The cytotoxic effect of H_2O_2 and *t*-BHP after post-treatment with rice bran extracts to A375 cells were determined by MTT assay. While the rice bran extracts showed no cytotoxic effect to A375 cells, H_2O_2 (80 µM) could reduce cell viability to 70% as presented in Figure 2. However, post-treatment with rice bran extracts demonstrated that Hom Dang helped alleviate the stress induced by H_2O_2 and provided higher cell viability than those of Hom Red Rose, Klam Doi-saked and Hom Dum Sukhothai II, respectively. The extracts of Hom Dang, Hom Red Rose and Klam Doi-saked prevented cell stress from H_2O_2 more significantly than Hom Dum Sukhothai II. Similarly, *t*-BHP (300 µM) reduced cell viability to 64% as shown in Figure 3 and Hom Dang, Hom Red Rose and Klam Doi-saked seemed to provide well protective effect against *t*-BHP. However, statistical analysis revealed that only Hom Dang and Hom Red Rose could protect *t*-BHP stressor. Therefore, it seemed that improvement from cellular oxidative stress of the extracts was well correlated to total flavonoid content as showed by the highest flavonoid content in red rice, the Hom Dang strain.

Anti-oxidative stress induced by UVA-irradiated of rice bran extracts

UVA irradiation at 10 J/cm^2 could decrease A375 cell viability to 65.82% as shown in Figure 4. Viability of the treated cells with 15 mg/mL Hom Dang was slightly higher than the rest. This revealed that Hom Dang was the most protective effect from UVA stress and could improve cell viability to approximately 71.56 %. Statistical analysis also confirmed that Hom Dang could revive cell from UVA stressor better than the rest.

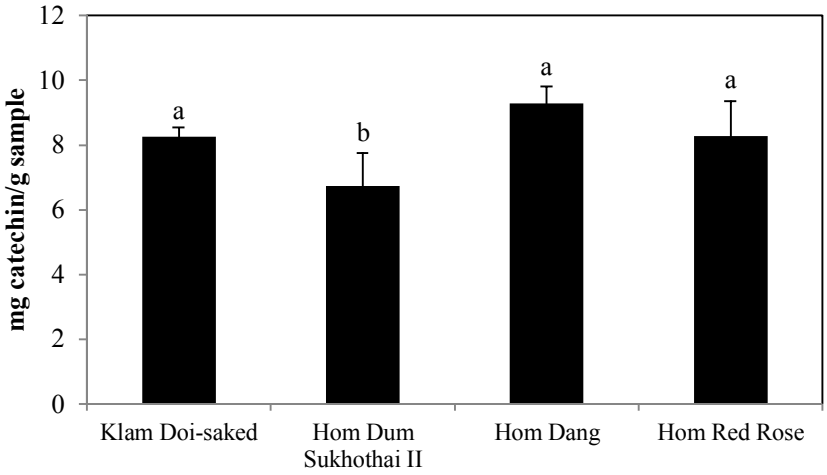

Figure 1 Total flavonoid content of color rice bran extracts. Values are means ± SD (n = 3).

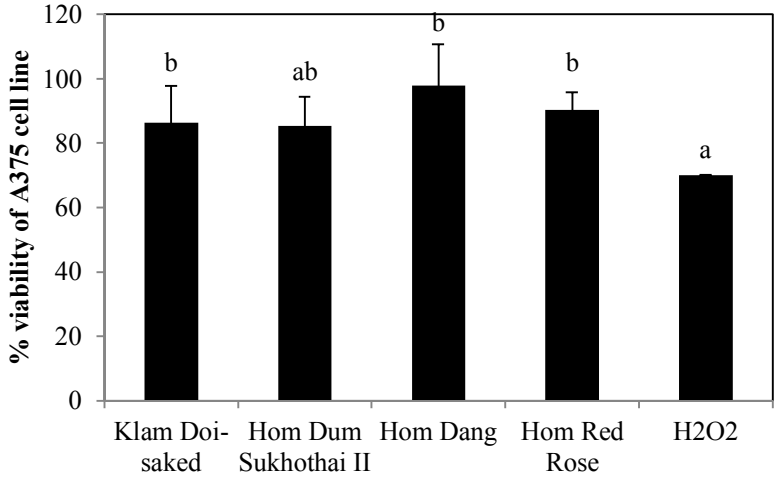

Figure 2 Cell viability of A375 cells pretreated with H_2O_2 (80 µM) following with medium incubation at the presence of 10 mg/mL of rice bran extracts for 24 h. Values are means ± SD (n = 3).

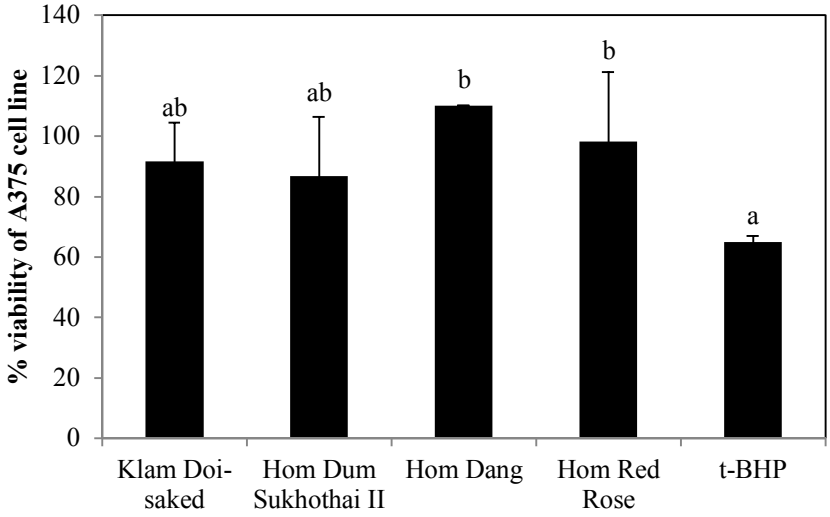

Figure 3 Cell viability of A375 cells pretreated with *t*-BHP (300 µM) following with medium incubation at the presence of 10 mg/mL of rice bran extracts for 24 h. Values are means ± SD (n = 3).

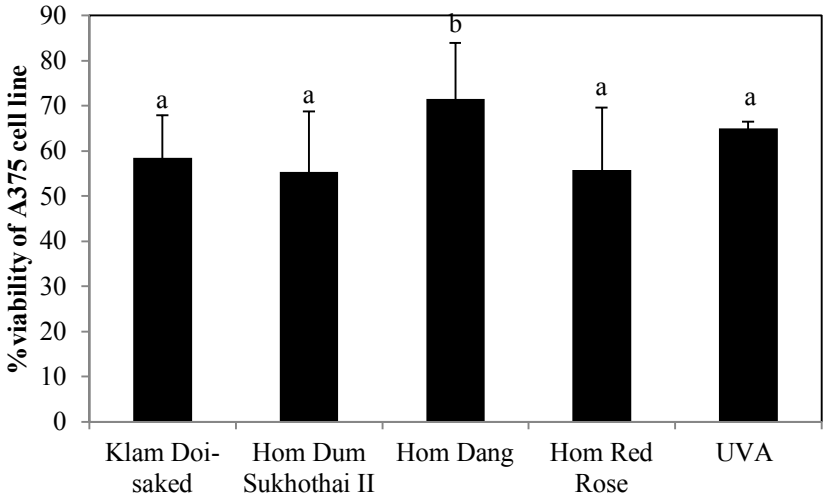

Figure 4 Cell viability of UVA irradiated cells of A375 cells at 10 J/cm^2 prior to post-treatment with 15 mg/mL rice bran extracts for 24 h Values are means ± SD (n = 3).

Discussion

Colored rice bran was regarded as a health-promoting food owing to its higher antioxidant activity than the white rice (Rattanachitthawat et al. 2010). Various antioxidant compounds have also been reported in red and black rice. For example, red pigment of proanthocyanidin in red rice has been claimed for anti-inflammatory and anti-allergic activity (Gunaratne et al. 2013). Oki et al. (2002) reported that procyanidin in red-hulled rice was the major components that scavenged free radicals of DPPH$^\bullet$ and t-BuOO$^\bullet$. Anthocyanins such as cyaniding-3-glucoside, peonidin-3-glucosied, and its metabolites cyanidin and protocatechuic acid from black rice could also alleviate oxidative stress and inflammation (Hou et al. 2010; Min. et al. 2010). However, antioxidant activity of the colored rice could be varied depending on varieties of the rice. Previously, we have reported that red rice (Hom Dang, Hom Red Rose) contained higher total phenolic content and present higher antioxidant activity than the black rice (Klam Doi-saked, Hom Dum Sukhuthai II) as determined by Folin-Ciocalteau method as well as DPPH and ABTS assay (Butchan et al. 2013). In the present work, red rice bran extracts with higher total phenolic content, also showed higher total flavonoid contents than black rice bran extracts (Figures 1). This was in agreement with the report on color natural substances that they are mostly members of flavonoid groups (Tapas et al. 2008).

Free radical is known to cause cellular oxidative stress in cell by the harmful effects to intracellular biomolecules, such as lipids, proteins and DNA. A cell model could then be used to screen for anti-oxidative stress activity of an agent in coupling with cell viability assay. However, mechanism for radical prevention of antioxidants may different depending on types of free radicals and its cellular damaging mechanism. Thus, different stress inducers were chosen and performed on A375 cells. The *tert*-butyl hydroperoxide (*t*-BHP) produced peroxyl and alkoxyl radicals leading to membrane damaging by lipid peroxidation, and glutathione (GSH) depletion. Hydrogen peroxide permeated cell membrane and led cytotoxicity also via lipid peroxidation and GSH depletion; however, the more dangerous of hydroxyl radical could be generated through Fenton reaction with available metal ions. While UV light directly donates an electron to molecular oxygen leading to superoxide anion ($O_2^{\cdot-}$) which eventually transform to hydroxyl radical.

We found that Hom Dang could better protect cell damage from H_2O_2 than Hom Red Rose, Klam Doi-saked and Hom Dum Sukhothai II, respectively (Figures 2). This also implied that

Hom Dang extract had the highest scavenging activity for hydroxyl radical. In addition, Hom Dang and Klam Doi-saked also exhibit more potent activity against peroxyl and alkoxyl radicals from *t*-BHP than Hom Red Rose and Hom Dum Sukhothai II (Figures 3). It could be observed that all rice bran extracts could revive cell viability from toxicity induced by *t*-BHP better than those by H_2O_2 as indicated by the amount of percent cell viability. This implied that rice extracts may obtain higher scavenging activity of organic hydroperoxides than the H_2O_2. On the other hand, short term exposure to UVA could provide estimation of superoxide anion accumulation. Hom Dang also showed the most potent superoxide anion prevention among the tested rice bran extracts. In this scenario, the results from the present work indicated that Hom Dang was the most potential extract for anti-oxidative stress. Considering on anti-stress activity of the rice bran extract, phytochemicals in rice bran may help revive cell by many functions. These include scavenging activity, metal chelating activity and maintenance of cellular antioxidant system, especially reduced glutathione, catalase and glutathione peroxidase. Choi et al., (2012) reported on beneficial effect of black rice under stress-induced by hydrogen peroxide (H_2O_2) of premature senescence (SIPS) of WI-38 human diploid fibroblasts cells and showed that it could possibly be used as for anti-aging. Therefore, outcome from the present work also demonstrated that rice extract with high potential for anti-oxidative stress could also be an appropriate ingredient for cosmetic preparation.

Conclusion

Among the 4 tested rice strains, red rice (Hom Dang, Hom Red Rose) exhibited higher total flavonoid content than the black rice (Klam Doi-saked, Hom Dum Sukhothai II). Hom Dang extract seemed to be the most potent anti-oxidative stress induced by H_2O_2, *t*-BHP and UVA irradiation in human melanoma (A375) cells. Therefore, Thai colored rice bran extracts could perfectly serve as natural source of anti-oxidant which was applicable as ingredient for cosmetics and pharmaceutical industries.

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