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In vitro Antioxidant Potentials of Four Thai Colored Rice Cultivars

Wiladda Sinthorn[1], Waranya Chatuphonprasert[2], Kanokwan Jarukamjorn[3*]

Abstract

Introduction: Extensive researches showed several pharmacological activities of dietary plants, especially rice grains. Hence, development of health supplements form rice might be the strength of Thailand. **Methods:** In this study, the four extracts from Thai rice cultivars, two from red (Hom-Dang, Hom-Kularb-Dang) and two from black rice (Kum-Doi-Saket, Hom-Dum-Sukhothai2) were determined of total phenolic and flavonoids contents followed by antioxidant properties *in vitro* using 2,2-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS) radical scavenging, and lipid peroxidation assays. **Results:** The Hom-Dang cultivar extract with the highest contents of phenolics and flavonoids showed the highest antioxidant activities. **Conclusion:** These findings indicated that Thai colored rice cultivars, especially the Hom-Dang variety, possess ultimate benefits to develop as health supplements due to their antioxidative potentials.

Keywords: ABTS, antioxidant, flavonoids content, phenolic content, lipid peroxidation,

[1] M.S. student, Pharmaceutical Chemistry and Natural Product Program, Faculty of Pharmaceutical Sciences, Khon Kaen University, Khon Kaen 40002 Thailand

[2] Ph.D., Lecturer, Faculty of Medicine, Mahasarakham University, Mahasarakham 44000 Thailand

[3] Ph.D., Associate Professor, Research Group for Pharmaceutical Activities of Natural Products using Pharmaceutical Biotechnology (PANPB), Faculty of Pharmaceutical Sciences, Khon Kaen University, Khon Kaen 40002 Thailand

* **Corresponding author:** Tel: 043-202305 E-mail: kanok_ja@kku.ac.th

1. Introduction

There are some special cultivars of colored rice which are composed of color pigments, namely black, red, and brown rice. The names of the cultivars refer to the kernel color (black, red, or purple) formed by deposition of anthocyanins in different layers of the pericarp, seed coat, and aleurone layer (Chaudhary, 2003). Previous report noted the anti-oxidative effects of phenolic and flavonoid containing in fruits, vegetables, and grain products (Velioglu *et al*., 1998). Colored rice is reported as a potent source of antioxidants and therefore, considered as a valuable source of antioxidants in functional foods (Muntana and Prasong, 2010;Yawadio *etal*., 2007). In the present study, we demonstrated the contents of phenolics and flavonoids in the four extracts from Thai rice cultivars, two from red rice (Hom-Dang

and Hom-Kularb-Dang) and two from black rice (Kum-Doi-Saket and Hom-Dum-Sukhothai 2).The 2,2-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS) radical scavenging and lipid peroxidation activities of all cultivars were additionally tested for *in vitro* antioxidant potentials.

2. Materials and Methods

Chemicals and reagents

The extracts of four Thai colored rice cultivars, namely Hom-Dang, Hom-Kularb-Dang, Kum-Doi-Saket, and Hom-Dum-Sukhothai 2 were kindly supplied by S&J International Enterprises Public Co., Ltd., Bangkok, Thailand. ABTS, Folin-Ciocalteu reagent, quercetin, gallic acid, vitamin C, malondialdehyde (MDA), and thiobarbituric acid (TBA) were purchased from Sigma-Aldrich Chemicals (St. Louis, MO, USA). Other chemicals were of the highest purity available from commercial suppliers.

Determination of total phenolic and total flavonoids contents

The content of total phenolics of the colored rice extract was determined using the Folin-Ciocalteu method (Chatuphonprasert *et al*., 2012). The rice extract was thoroughly mixed with the Folin-Ciocalteu reagent before subjected to spectrophotometry at a wavelength of 750 nm. Total phenolics content was calculated as the weight unit equivalent to the dry weight of the standard gallic acid (mg GAE/mL).

The aluminum chloride colorimetric method was applied with some modifications for determination of the content of total flavonoids (Chatuphonprasert *et al*., 2012). The reaction mixture contained aluminum chloride, sodium acetate, and an aliquot of the rice extract in a final volume of 200 mL. After incubation, the absorbance of the reaction mixture was measured at a wavelength of 405 nm. The content of total flavonoids was calculated as the weight unit equivalent to the dry weight of the standard quercetin (mg QE/mL).

Scavenging activity of 2,2-azinobis-3-ethylbenzothaiazoline-6-sulphonic acid (ABTS) radical assay

The total antioxidant capacity of the rice extract was determined by using the ABTS radical cation protocol of Bao *et al*. (2005) with some modification. ABTS was dissolved in potassium persulfate to give an absorbance of 0.700 at 700 nm. The solution was mixed with the rice extract at a ratio of 20:1 and allowed to stand for 6 min, before the absorbance was recorded at a wavelength of 700 nm.

Lipid peroxidation assays

A mixture of vitamin C, $FeCl_2$, and the liver homogenate in Tris-HCl buffer was incu bated at 37°C for 1 h. One ml of the mixture was incubated with the serial dilution of rice extract, before TBA solution was added to form a complex with MDA. Then the mixture was boiled at 100°C for 15 min. After allowing to cooling down, the reaction was stopped with n-butanol and subjected for centrifugation at 1,000*g* for 10 min. The upper phase was measured for TBARS using spectrofluorometry with an excitation wavelength of 520 nm and an emission wavelength of 590 nm using MDA as a standard (Jearapong *et al.*, 2014).

Statistics

The results were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's *post hoc* test (SPSS ver. 11.5). $p \leq 0.05$ was considered statistically significant.

3. Results and Discussion

Characterization of the extracts from Thai colored rice cultivars

The extracts from prominent red (Hom-Dang and Hom-Kularb-Dang) and black (Kum-Doi-Saket and Hom-Dum-Sukhothai 2) Thai rice cultivars were characterized for contents of bioactive compounds including total phenolic and total flavonoids contents (Fig. 1). The extracts of the two red rice samples contained more nearly as two-times more total phenolics and total flavonoids than the extracts from the black rice samples; Hom-Dang rice had the highest content of total phenolics (421±7 mg GAE/mL) and total flavonoids (368±53 mg QE/mL).

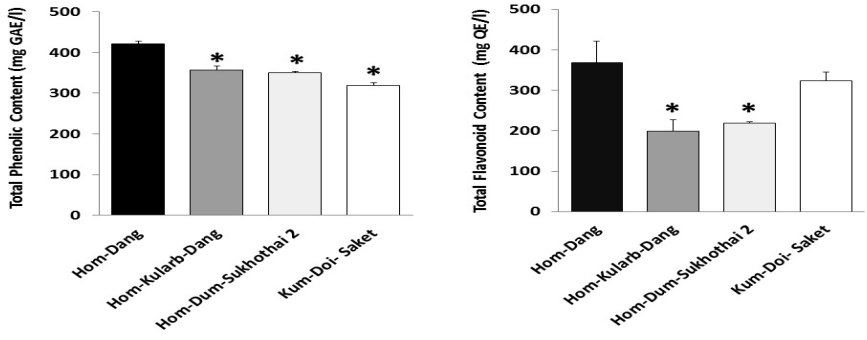

Figure 1. The contents of total phenolic and total flavonoids in the colored rice cultivars

***In vitro* antioxidant potentials of the Thai colored rice cultivars**

ROS underlie oxidative injury of biomo lecules. Ferrous ion catalyzes the production of free radicals and it additionally initiates and reproduces reactions of free radical chain, subsequently leading to continuous formation of free radicals-byproducts (Min and Ahn, 2005). Lipid peroxidation is a harmful reaction involving free radicals, in which MDA is the final product (Jearapong *et al*., 2014). To evaluate the effects of Thai rice cultivars on these processes, the antioxidant capacity of Hom-Dang, Hom-Kularb-Dang, Kum-Doi-Saket, and Hom-Dum-Sukhothai2 cultivars was performed using ABTS radical scav enging and lipid peroxidation assays. The IC_{50} value for reducing the initial radical concentration by 50% was compared to that of vitamin C, as a

strong antioxidant the highest ability to reduce all type of radicals (Padayatty *et al.*, 2003). The Hom-Dang extract showed the lowest IC_{50} (1.1- to 3.2-folds lower than the other rice cultivars) in both radical scavenging assays, namely ABTS and lipid peroxidation activities (Fig. 2). These findings corresponded to the previous report that the colored rice possessed greater antioxidant effect than the white rice (Finocchiaro *et al.*, 2010; Shen *et al*., 2009).

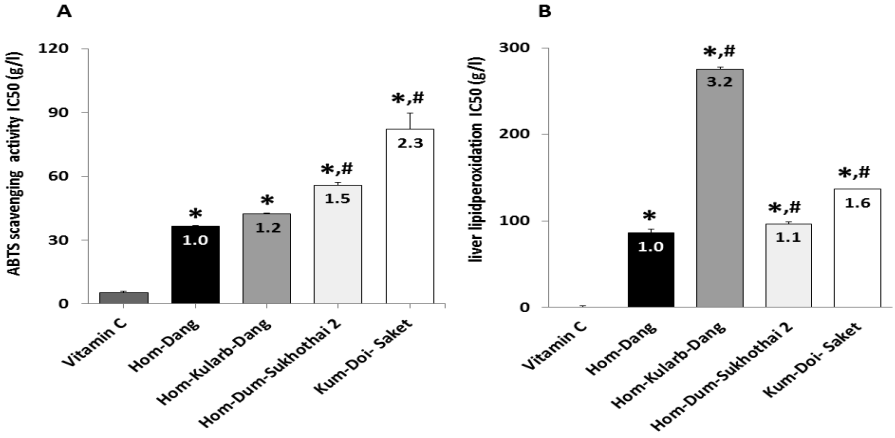

Figure 2. *In vitro* ABTS radical scavenging activity and lipid peroxidation of the colored rice cultivars. Value in each column represents the fold-IC_{50} to the Hom-Dang.

4. Conclusion

In summary, the Hom-Dang rice cultivar extract demonstrated promising *in vitro* antio xidative potentials. Hence, the Hom-Dang rice cultivar might bring ultimate benefit for development as an alternative health supplement, e.g. for the chronic oxidative stress condition.

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