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Antioxidant Activity, Total Phenolic Content and Anti-Tyrosinase Activity of Thai Colored Rice Cultivar Extracts

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Abstract

This study aimed to evaluate the antioxidant activity, total phenolic content and tyrosinase inhibitory activity of Thai colored rice. Two different colored rice cultivars (Sew-Dang and Nor-Prae) were extracted using water and 95% ethanol. Antioxidant activities of rice extracts were tested using three different methods: DPPH, ABTS and FRAP. These analyses revealed that ethanolic extracts produced stronger antioxidant activity than water extracts. The ethanolic extract of Nor-Prae rice grains contained 114.12 mg GAE/g extract, 147.55 mg TE/g extract, and 152.44 mg $FeSO_4$/g extract tested by DPPH, ABTS and FRAP assays respectively which were significantly higher than Sew-Dang rice ($P<0.05$).Total phenolic content was determined using the Folin-Ciocalteu method, and the ethanolic extract had significantly greater levels of phenolics than water extracts ($P<0.05$). Nor-Prae ethanolic extracts were determined to possess the greatest phenolic content, which was 39.18 mg GAE/g extract, relative to other extracts assessed. Tyrosinase inhibitory activities of rice extracts were determined using the dopachrome microplate method. The greatest tyrosinase inhibitory activity was produced by the ethanolic extract of Nor-Prae rice, which inhibited tyrosinase 24.19%, while all water extracts had no affect on the activity of tyrosinase. In summary, the ethanolic extract of Nor-Prae rice had the strongest antioxidant capacity, greatest total phenolic content and the greatest capacity to inhibit tyrosinase activity. This indicated the potential of colored rice as a source of natural antioxidants and tyrosinase inhibitors, which might be used for further cosmetic or pharmaceutical product development.

Introduction

Rice (*Oryza sativa* L.) belongs to the Poaceae family, and is a staple food consumed in Asia and is also the most important export of Thailand. Generally, rice contains nutraceuticals such as vitamin E, vitamin B complex, minerals, fiber and important amino acid (Liu, 2007; Yodmanee et al., 2011). Colored rice is a good source of phytochemical components such as phenolic compounds, anthocyanins and γ- oryzanol, which has been reported to be an efficient antioxidant (Chakuton et al., 2012). Several studies have shown that colored

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rice exhibits greater antioxidant activity and contains more potent antioxidant compounds, including anthocyanins and phenolic compounds, than white rice (Ahuja et. al., 2007; Vichapong et al., 2010; Chakuton et al., 2012). Antioxidant compounds promote health by protecting the cells of the body from damage caused by free radicals and reactive oxygen species. Moreover, previous studies have shown that antioxidant activity correlates well with total phenolic content in rice (Yodmanee et al., 2011; Nakornriab, 2018). The most prominent phenolic compounds present in colored rice have been reported to be anthocyanins (Iqbal et al., 2005; Zhang et al., 2006; Yawadio et al., 2007). Therefore, it is important to determine the antioxidant activity and total levels of phenolic compounds in Thai colored rice cultivars.

Tyrosinase is a copper-containing enzyme involved in the production of melanin. This enzyme catalyzes the oxidation of L-tyrosine to 3-(3,4-dihydroxy-phenyl)-L-alanine (L-DOPA) and oxidation of L-DOPA to produce dopachrome, which is results eventually in the production of melanin pigment (Kim & Uyama, 2005). High levels of production of tyrosinase enzyme leads to a dermatological disorder such as age spots, melanoma, freckles and hyperpigmentation (Costin & Hearing, 2007; Ortonne & Bissett, 2008). Moreover, tyrosinase also is responsible for the enzymatic browning of fruits and vegetables, which results in discoloration. This is an unfavorable characteristic and results in economic and nutritional losses (Martinez & Whitaker, 1995). The identification of tyrosinase inhibitors may be important for the production of cosmetic products as well as the food industry (Wang et al., 2011; Loizzo et al, 2012). Anti-tyrosinase compounds are derived from both synthetic and natural sources such as kojic acid, hydroquinone, arbutin, ellagic acid, and ascorbic acid (Zolghadri et al., 2019). Moreover, several phenolic compounds have been reported to contain antioxidant activity along with anti-tyrosinase activity in various plant species (Rashed et al., 2016; Chatatikun & Chiabchalard, 2017). Although various Thai colored rice cultivars have been reported to have tyrosinase inhibitory activity (Jansom et al., 2010), some varieties are weak tyrosinase inhibitors (Teeranachaideekul et al, 2018). Further, there is no assessment of the bioactive activity and phytochemical components within Sew-Dang and Nor-Prae rice cultivars. Sew-Dang rice (*Oryza sativa* L. cv. Sew-Dang) and Nor-Prae rice (*Oryza sativa* L. cv. Nor-Prae) are glutinous rice cultivars cultivated in Chiang Rai, Thailand. Both rice cultivars have a deep red bran layer (Chaichana, 2019). This study aimed to evaluate antioxidant activity using various antioxidant models. Furthermore, the total phenolic content and tyrosinase enzyme inhibition of water and ethanolic extracts of Thai rice cultivars were also determined.

Materials and Methods

1. Sample preparation

Sew-Dang (*Oryza sativa* L. cv. Sew-Dang) and Nor Prae rice (*Oryza sativa* L. cv. Nor-Prae) were collected from Chiang San district, Chiang Rai, Thailand. The rice grains were dried at 60°C. Dried samples were ground into a fine powder (100 g) and extractions were performed using distilled water and 95% ethanol (1:10 w/v) at room temperature with frequent agitation for 24 h. The mixture was filtrated through Whatman no. 1 filters. Crude rice extracts were obtained from filtrates that were evaporated using a rotary evaporator under reduced pressure and lyophilized via freeze-drying.

2. Antioxidant activity

2.1 DPPH radical scavenging assay

The DPPH radical scavenging ability of rice extracts was determined according to the modified method of Brand–Williams et al. (1995) and Ho et al. (2010). Briefly, 0.5 mL of various concentrations of plant extracts in methanol were added to 1.5 mL of 0.1 mM DPPH in methanol. The mixtures were incubated in the dark at room temperature for 20 min. Absorbance was measured at 517 nm using UV/Visible spectrophotometry (Biochrom Libra S60, UK). The percentage of free radical inhibition provided by the extract was calculated using the following equation:

$$\% \text{ Inhibition} = [(A- (B-C)/A] \times 100 \quad (1)$$

Where A is the absorbance of the control (DPPH solution), B is the absorbance of the tested sample (the plant extract with DPPH solution), and C is the absorbance of the blank sample (the plant extract without DPPH solution).

The IC_{50} value was defined as the concentration of the sample required to scavenge 50% of DPPH radicals. The IC_{50} was obtained from the linear regression of the dose-response curve of % inhibition versus concentration. Then, the antioxidant activity of rice extracts was reported as the gallic acid equivalent

antioxidant capacity per gram extract (mg GAE/g extract), as follows:

$$\text{Antioxidant activity (mg GAE/g extract)} = \frac{(IC_{50}\ \text{gallic acid (mg/mL)})}{(IC_{50}\ \text{rice extract (mg/mL)})} \times 1000 \quad (2)$$

2.2 ABTS radical scavenging assay

The ABTS radical scavenging activity was measured by assessing the color change associated with the formation of an ABTS cation radical ($ABTS^{\bullet+}$), with slight modifications (Re et al., 1999). The $ABTS^{\bullet+}$ was generated via a reaction between 7 mM ABTS and 2.45 mM potassium persulfate ($K_2S_2O_8$). The mixture was incubated in the dark at room temperature for 12-16 h before used. Afterward, the ABTS working solution was diluted with 95% ethanol until the solution produced an absorbance of 0.700 ± 0.02 at 734 nm. To assess experimental replicates, 20 µL of various concentrations of rice extracts were mixed with the $ABTS^{\bullet+}$ working solution and incubated in the dark for 6 min before their absorbance was measured at 734 nm using UV/Visible spectrophotometry (Biochrom Libra S60, UK). Trolox solution was used as a standard. The percentage of free radical inhibition of rice extracts were calculated using the following equation:

$$\%\ \text{Inhibition} = [(A-(B-C)/A] \times 100 \quad (3)$$

Where A is the absorbance of the control (ABTS solution), B is the absorbance of the tested sample (the extracts with ABTS solution), and C is the absorbance of the blank sample (the extract without ABTS solution).

The IC_{50} value was defined as the concentration of the sample required to scavenge 50% of ABTS radicals. The IC_{50} was obtained from the linear regression of the dose-response curve of % inhibition versus concentration. The antioxidant activity of rice extracts was reported as the trolox equivalent antioxidant capacity per gram extract (mg TE/g extract).

$$\text{Antioxidant activity (mg TE/g extract)} = \frac{(IC_{50}\ \text{trolox (mg/mL)})}{(IC_{50}\ \text{rice extract (mg/mL)})} \times 1000 \quad (4)$$

2.3 Ferric reducing antioxidant power (FRAP) assay

Reducing power was determined using a ferric reducing antioxidant power (FRAP) assay described by Benzie & Strain (1996), with some modifications. Briefly, extracts were dissolved in 95% ethanol and 1.0 mg/mL concentrations of extracts were obtained. Then, an aliquot of 500 µL of rice extract was mixed with 1.5 mL FRAP reagent (10 mM TPTZ solution, 20 mM $FeCl_3.6H_2O$, 300 mM acetate buffer, pH 3.6 and deionized water). Next, mixtures were incubated in the dark 15 min and measured at 593 nm using a UV/Visible spectrophotometer (Biochrom Libra S60, UK). Deionized water was used as a blank solution. Reducing power was calculated from a $FeSO_4$ standard curve and expressed as mg $FeSO_4$/g extract.

3. Total phenolic compound content assay

Total phenolic content was determined using the Folin-Ciocalteu method, with some modifications (Chandler & Dodds, 1983). In brief, 0.25 mL rice extract (1 mg/mL) was mixed with 1.25 mL water, 0.25 mL 95% ethanol and 0.125 mL 50% Folin-Ciocalteu reagent. Mixtures were incubated 5 min at room temperature, 0.25 mL 5% Na_2CO_3 was added and mixtures were incubated in the dark for 1 h. The absorbance of each solution was measured at 725 nm using 95% ethanol as a blank. Total phenolic content was calculated from a gallic acid standard curve and expressed as mg gallic acid equivalents (mg GAE/g extract).

4. Tyrosinase inhibitory assay

Tyrosinase inhibitory activity of rice extracts was evaluated using the dopachrome microplate method (Potduang et al., 2008). Rice extracts were diluted with 20% ethanol to a final concentration of 1 mg/mL. Then, 50 µL of each rice extract was mixed with 150 µL 20 mM phosphate buffer (pH 6.8) and 50 µL mushroom tyrosinase (313 units/mL). Mixtures were incubated at 37°C for 10 min. Afterward, 50 µL of 0.34 mM 3,4-Dihydroxy-L-phehylalanine (L-DOPA) was added to each well and incubated at 37°C for an additional 10 min. Absorbance was measured at 492 nm using a microplate reader (M965+, Metertech, Taiwan). Kojic acid (1 mg/mL) was used as a positive control. Percentage tyrosinase inhibition was calculated using the following equation:

$$\text{Tyrosinase inhibition (\%)} = [(A-B)-(C-D)/A-B] \times 100 \quad (5)$$

Where A is the absorbance of the control (L-DOPA, tyrosinase); B is the absorbance of the blank (L-DOPA); C is the absorbance of the sample (rice extract, L-DOPA and tyrosinase) and D is the blank for C (L-DOPA mixed with rice extract).

5. Statistical analysis

The results of three replicates were reported as a mean ± SD. Analysis of variance (ANOVA) was calculated using Duncan's new multiple range test (DMRT). Values of $P < 0.05$ were considered statistically significant.

Results and Discussion

1. Antioxidant activity

In this study, two glutinous colored rice cultivars (Sew-Dang and Nor-Prae) were extracted using water and 95% ethanol. The water and ethanolic extracts of both rice cultivars were tested for their antioxidant activity, total phenolic content, and tyrosinase inhibitory activity. Various methods have been used to analyze the antioxidant capacity in several plant materials, and since they all function based on different reaction mechanisms results of each type of test may differ (Pérez-Jiménez & Saura-Calixto, 2006). Therefore, at least two test methods should be used to produce results that reliably indicate the antioxidant activity of plant samples (Pérez-Jiménez et al., 2008). In this study, three different methods including DPPH, ABTS and FRAP were used to analyze the antioxidant capacity of rice extracts.

The DPPH method has been widely used to provide standard information regarding the antioxidant activity of various plant species. DPPH is a stable nitrogen radical species that is capable of accepting either electron or hydrogen radicals to form a stable diamagnetic molecule. Antioxidants are able to reduce stable radical DPPH to is yellow-colored, non-radical form, DPPH-H through their hydrogen donating capabilities (Cotelle et al., 1996). The results of DPPH radical scavenging activity indicated the concentration of the sample required to scavenge 50% of DPPH radicals (IC_{50}) and was expressed as the gallic acid equivalent antioxidant capacity per gram extract (mg GAE/g extract). The lower the IC_{50} value, the greater antioxidant activity it represented. As shown in Table 1, gallic acid which was used as a standard compound had the lowest IC_{50} value of 0.006 mg/mL. Among colored rice extracts, the ethanolic extract of Nor-Prae rice had the lowest IC_{50} (0.05 mg/mL), followed by the ethanolic extract of Sew-Dang rice (0.11 mg/mL) and the water extract of Nor-Prae rice (0.75 mg/mL). The highest IC_{50} was found in the water extract of Sew-Dang rice (1.39 mg/mL). For antioxidant activity, our results demonstrated that the significantly ($P<0.05$) greatest antioxidant activity (114.12 mg GAE/g extracts) was attributed to the ethanolic extract of Nor-Prae rice, followed by the ethanolic extract of Sew-Dang rice (53.66 mg GAE/g extracts). Water extracts of both types rice were determined to contain radical scavenging activities of 7.63 and 4.11 mg GAE/g extract, respectively, which was significantly lower than activities of ethanolic extracts ($P<0.05$).

Table 1 DPPH radical scavenging activity of water and ethanolic extracts of Sew-Dang and Nor-Prae rice cultivars

Rice cultivars	Extracts	IC_{50} (mg/mL)	DPPH radical scavenging activity (mg GAE/g extract)
Sew-Dang	Water	1.39 ± 0.067^{a}	4.11 ± 0.206^{c}
	Ethanol	0.11 ± 0.004^{c}	53.66 ± 1.996^{b}
Nor-Prae	Water	0.75 ± 0.011^{b}	7.63 ± 0.117^{c}
	Ethanol	0.05 ± 0.022^{d}	114.12 ± 4.569^{a}
Gallic acid	-	0.006 ± 0.000^{d}	-

Remark: The data are given as mean ± standard deviation (SD) of triplicate data. Values followed by different letters in column were significantly different ($P<0.05$).

ABTS is another method that has been widely used to measure the radical scavenging activity of antioxidant compounds. ABTS can be oxidized by potassium persulfate or manganese dioxide, which gives rise to the ABTS cation radical ($ABTS^{\bullet+}$). The ABTS method measures the ability of the sample to donate an electron or hydrogen to $ABTS^{\bullet+}$ (blue-green color) to form ABTS (colorless) (Moon & Shibamoto, 2009; Alam et al., 2013). The $ABTS^{\bullet+}$ radical scavenging activity in term of the extract concentration required to inhibit 50% of initial ABTS radical (IC_{50}) and was expressed as mg trolox equivalent antioxidant activity per gram of extract (mg TE/g extract). As shown in Table 2, ABTS assays revealed that the radical scavenging activities of rice extracts were similar to those of the DPPH assay. Trolox was used as a standard antioxidant compound and gave the lowest IC_{50} with 0.35 mg/mL ($P<0.05$). Furthermore, the result showed that the IC_{50} of colored rice extracts ranged from 2.39-97.75 mg/mL. The lowest IC_{50} among colored rice extracts was found in the ethanolic extract of Nor-Prae rice with IC_{50} value of 2.39 mg/mL followed by the ethanolic extract of Sew-Dang rice with IC_{50} of 5.94 mg/mL. However, both water extracts had higher IC_{50} values with 54.57 mg/mL (Nor-Prae) and 97.75 mg/mL (Sew-Dang). The ABTS radical scavenging activity of the ethanolic extract of Nor-Prae rice was 147.55 mg TE/g extract, which was significantly stronger than the ethanolic extract of Sew-Dang rice, which was 59.23 mg TE/g extract ($P<0.05$). The aqueous extracts of rice types, Nor-Prae and Sew-Dang, exhibited weak antioxidant activity, which was determined to be 6.45 and 3.60 mg TE/g extract, respectively.

Table 2 ABTS radical scavenging activity of water and ethanolic extract of Sew-Dang and Nor-Prae rice cultivars

Rice cultivars	Extracts	IC_{50} (mg/mL)	ABTS radical scavenging activity (mg TE/g extract)
Sew-Dang	Water	97.75 ± 1.329[a]	3.60 ± 0.049[c]
	Ethanol	5.94 ± 0.099[c]	59.23 ± 0.998[b]
Nor-Prae	Water	54.57 ± 1.142[b]	6.45 ± 0.134[c]
	Ethanol	2.39 ± 0.089[d]	147.55 ± 5.543[a]
Trolox	-	0.35 ± 0.002[e]	-

Remark: The data are given as mean ± standard deviation (SD) of triplicate data. Values followed by different letters in column were significantly different (P<0.05).

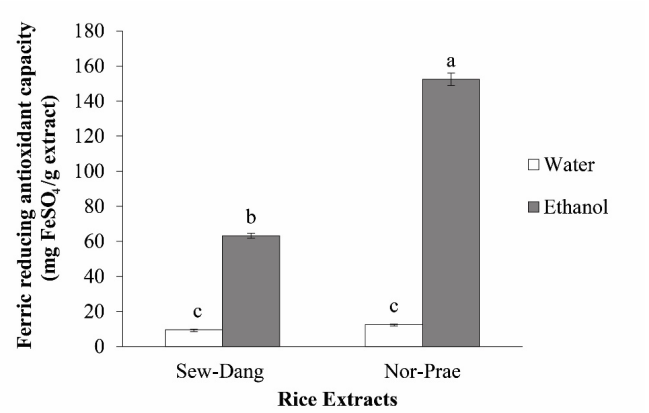

Fig. 1 Ferric reducing antioxidant power of water and ethanolic extracts of Sew-Dang and Nor-Prae rice cultivars. Each bar represents the mean of three replicates and the error bars indicate the standard error of the means. Values labelled with different letters were significantly different (P < 0.05).

Also, a FRAP assay was used to measure the quantity of antioxidants or reductants present within rice extracts. Antioxidants can reduce ferric (III) ions to ferrous (II) ions via a redox-linked colorimetric reaction (Benzie & Strain, 1996; Li et al., 2006). The ferric reducing antioxidant power of colored rice is provided Fig. 1. The ethanolic extracts of Nor-Prae rice had the greatest (P<*0.05*) ferric reducing capacity (152.44 mg $FeSO_4$/g extract), followed by that of the ethanolic extract of Sew-Deng rice, which was determined to be 63.24 mg $FeSO_4$/g extract. Again, water extracts of Nor-Prae and Sew-Dang rice had low levels of ferric reducing capacity, and values determined for the extracts were 12.64 and 9.61 mg $FeSO_4$/g extract, respectively. The antioxidant activities of Sew-Dang and Nor-Prae rice cultivars have not been reported. However, other colored rice cultivars demonstrated good sources of antioxidant compounds. According to the previous report, Vichit and Saewan (2015) revealed that some black and red rice cultivars gave strong antioxidant activity ranging from 0.06 - 1.36 mg AAE/mL for FRAP, IC_{50} 0.10 - 1.12 mg/mL for DPPH and 7.57- 40.48 % for TBARS. A similar result was reported by Moko et al. (2014) that the colored rice had higher antioxidant activity than non-colored varieties. It was shown that the red rice varieties had the highest DPPH radical scavenging ability with 88.29% and also had the lowest IC_{50} with 26.26 µg/mL.

2. Total phenolic compound content assay

Phenolic compounds are commonly found in plants and have been reported to have several biological functions including antibacterial and antioxidant activities (Soobratte et al., 2005). The main phenolics in colored rice cultivars are phenolic acids such as ferulic, coumaric, caffeic, cinnamic and gallic acids (Tian et al., 2005; Zhou et al., 2004). Also, another phenolic in colored rice is mainly anthocyanin, which has strong antioxidant activity (Goufo & Trindade, 2014). Phenolic compounds act as antioxidants, since they are able to scavenge free radicals, to donate hydrogen atoms or electrons and to chelate metal cations (Javamardi et al., 2003). Previous publications have shown that antioxidant activity correlates with phenolic content in several plant species (Velioglu et al., 1998; Zhang et al., 2006; Yawadio et al., 2007; Do et al., 2014). As shown in Fig. 2, investigations of the phenolic content of rice extracts produced similar results as the DPPH, ABTS and FRAP assays. This suggests that the antioxidant activity of colored rice likely depends on total phenolic content. The highest levels of phenols were observed in the ethanolic extract of Nor-Prae rice, followed by the ethanolic extract of Sew-Dang rice in which levels were determined to be 39.18 and 22.41 mg GAE/g extract, respectively. Additionally, water extracts of the both types of rice contained significantly (P<*0.05*) lower levels of phenolics: 4.43 and 2.32 mg GAE/g extract, respectively. These findings are consistent with a previous report by Yodmanee et al. (2011), which showed that the antioxidant capacity of dehusked rice grain extracts were correlated with polyphenol content. Furthermore, Nakornriab (2018) reported that brown rice extracts had the highest total phenolic content and antioxidant activity. The researchers further showed that total phenol content and antioxidant activity was tightly correlated.

Solvents used to extract bioactive compounds of plants have an effect on the resulting biological activities of

extracts. Most phenolic substances range from polar to nonpolar in plants, thus the choice of solvent for extractions is very important for phenolic compounds (Do et al., 2014). A previous study reported that the majority of solvents used for extracting antioxidant compounds are comprised of mixtures of organic solvents including ethanol, methanol, and acetone. Ethanol has previously been shown to be a good solvent for extracting antioxidant compounds from plant materials. The studies also suggested that ethanol is the most suitable nontoxic solvent for extracting the compounds (Dai & Mumper, 2010). In this study, all ethanolic extracts obtained had higher antioxidant capacities than water extracts when any of the three measures of antioxidant capacity were considered. Also, ethanolic extracts had greater phenolic content than water extracts. This is consistent with a previous report, which showed that ethanolic extracts of colored rice (Sang-Yod red rice) produced strong DPPH radical scavenging activity and also had high total phenolic and flavonoid content (Hansakul et al., 2011). This finding suggests solvent used for extraction plays an important role in determining antioxidant activity and phenolic content, which is due to differences in the solubilities of compounds within samples. However, the antioxidant activity and concentration of polyphenol compounds also depend on the cultivars of colored rice used. In the present study, Nor-Prae rice extracts possessed significantly ($P<0.05$) greater antioxidant capacities and had significantly greater levels of total phenolic content than Sew-Dang rice extracts.

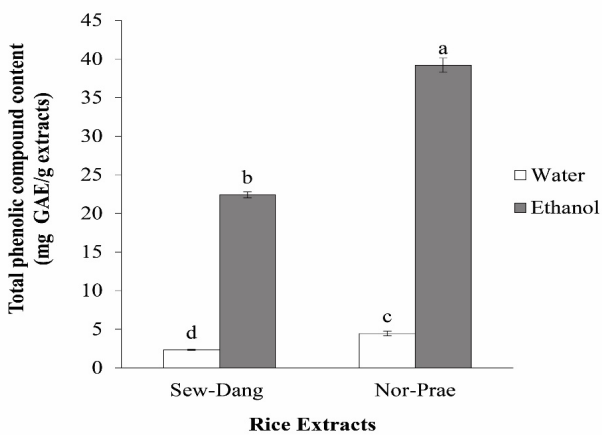

Fig. 2 Total phenolic compound content of water and ethanolic extracts of Sew-Dang and Nor-Prae rice cultivars. Each bar represents the mean of three replicates and error bars indicate the standard error. Values labelled with different letters were significantly different ($P < 0.05$).

3. Tyrosinase inhibitory assay

Tyrosinase is a polyphenol oxidase enzyme that is involved in melanin biosynthesis in organisms. Overproduction of tyrosinase leads to hyperpigmentation in human and animal skin and also affects the fruit and vegetable quality (Chang, 2009). Tyrosinase is a copper-containing enzyme that catalyzes the conversion of L-tyrosine to L-DOPA, and the oxidation of L-DOPA to dopaquinone, which are required for melanin biosynthesis (Kim & Uyama, 2005). Therefore, identification of a tyrosinase inhibitor has the potential to inhibit the process of hyperpigmentation. In our study, the ability of rice extracts to inhibit tyrosinase activity was evaluated using a dopachrome assay, and L-DOPA was used as a substrate of the tyrosinase enzyme. The results of the tyrosinase inhibitory activity assay are shown in Table 3. The tyrosinase inhibitory activity of rice extracts was influenced by rice cultivar and solvents used for extractions. Anti-tyrosinase activity of rice extracts were compared to 1 mg/mL kojic acid (positive control). At this concentration, both rice extracts had a lower inhibitory activities against tyrosinase than kojic acid, which had the significantly ($P<0.05$) highest tyrosinase inhibitory activity assessed, with percentage inhibition of 92.74%. Among rice extracts tested, the ethanolic extracts of Nor-Prae and Sew-Dang rice displayed significant anti-tyrosinase activity while water extracts of both rice cultivars did not affect tyrosinase activity because their activities were so low. The highest level of tyrosinase inhibitory activity was determined to be associated with the ethanolic extract of Nor-Prae rice, which had a percentage inhibition of 24.19%. The ethanolic extract of Sew-Dang rice exhibited a low inhibitory activity against the tyrosinase enzyme, with a percentage inhibition value of 6.45%. This result is consistent with reports of Jansom et al. (2010), who showed that some purple glutinous rice extracts showed strong tyrosinase inhibitory activity. Similar anti-tyrosinase abilities were also reported in red rice (*Oryza nivara*) extracts (Batubara et al., 2017). Several polyphenol compounds found in natural sources have been shown to be effective tyrosinase inhibitors including gallic acid, kaempferol, quercetin, catechin, and rhamnetin (Orhan & Khan, 2014; Lee et al., 2016; Panzella & Napolitano, 2019). Miyazawa et al. (2003) demonstrated that protocatechuic acid methyl ester isolated from black rice bran had strong tyrosinase inhibitory activity.

Table 3 Tyrosinase inhibitory activity of water and ethanolic extracts of Sew-Dang and Nor-Prae rice cultivars

Rice cultivars	Extracts	Tyrosinase Inhibition (%)
Sew-Dang	Water	ND
	Ethanol	6.45 ± 1.40^{c}
Nor-Prae	Water	ND
	Ethanol	24.19 ± 1.40^{b}
Kojic acid	-	92.74 ± 2.42^{a}

Remark: The data are given as mean ± standard deviation (SD) of triplicate data. Values followed by different letters were significantly different ($P<0.05$), ND: Not determined at assayed concentration (consequence of low activity).

Conclusion

The present study indicates that the biological activities of colored rice extracts depend on the type of rice cultivar used and the solvent used for extractions. The ethanolic extracts had more potent antioxidant activity, greater total phenolic content and increased tyrosinase inhibitory activity relative to water extracts. The ethanolic extract of Nor-Prae rice produced the strongest antioxidant activity according to all three methods used to measure antioxidant capacity. Further, it also had the highest total phenolic content and the greatest tyrosinase inhibitory activity of all extracts examined. Therefore, this research suggests that Nor-Prae rice extracts contain potent of antioxidant compounds and tyrosinase inhibitors, which are likely phenolics. The HPLC quantitative analysis and stability test of colored rice extract should be investigated in further works in order to achieve pharmaceutical product development.

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