

Tyrosinase Inhibition and Antioxidant Activities of Riceberry (*Oryza sativa L.*)

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Abstract: Riceberry, deep purple grain (*Oryza sativa L.*), a cross-breed strain from the Jao Hom Nin rice and Khoa Dawk Mali 105. Rice Bran (RB) and Rice Husk (RH) are considered as wastes from cultivation. In this study, the RB and RH were extracted with the 3 different methods namely boiling with water (H₂O), maceration with 95% ethanol (EtOH) and fermentation with *Saccharomyces cerevisiae* var. burgundy (WF). The main phytochemicals found in those extracts were xanthones and flavonoids. For anti-oxidation activity tests, the rice bran extracts from fermentation (RB-WF) extracts gave the highest free radical scavenging activity by DPPH assay (SC₅₀ = 0.25±0.02 mg/mL) and metal chelating activity by Ferrous Ion Chelating (FIC) assay (MC₅₀ = 0.22±0.02 mg/mL) which were comparable to vitamin C and EDTA, respectively (p<0.05). In addition, the rice bran extract from boiling (RB-H₂O) and the rice husk extract from fermentation (RH-WF) shown the highest inhibition of lipid peroxidation by Ferric-Thiocyanate (FTC) which was comparable to vitamin E (p<0.05). Moreover, the RB-WF exhibited the highest tyrosinase inhibition activity by the modified dopachrome method (IC₅₀ = 0.11±0.01 mg/mL). This result suggested that the RB-WF extract might be beneficial to be develop as whitening and antioxidant agents for cosmetics.

Key words: Riceberry, antioxidant, anti-tyrosinase activity, cosmetics, whitening, vitamin

INTRODUCTION

Melanin is a pigment located in eye, hair and skin of animals. It is produced in melanosomes of melanocytes which response to Ultraviolet B (UVB)-irradiation through the process called melanogenesis. Also, it plays a role in protection the skin damages from ultraviolet radiation. However, overproduction of melanins can cause the skin problems such as freckles, melasma and melanoma skin cancer (Pillaiyar *et al.*, 2017). Tyrosinase (EC 1.14.18.1) is a membrane-bound glycoprotein containing with copper ion and rate-limiting enzyme of catalyzing of melanin production with modified of L-tyrosine to L-dihydroxyphenylalanine (L-DOPA) and L-DOPA to L-DOPA quinone by hydroxylation and oxidation (Han *et al.*, 2014).

The overexposure to sunlight (UVA and UVB) can enhancing level of Reactive Oxygen Species (ROS) such as Hydrogen peroxide (H₂O₂), hydroxyl radicals (OH) and superoxide radical(O₂⁻) which play key role in the regulation of oxidative stress and melanogenesis (upregulation of tyrosinase activity) in melanocytes. Ascorbic acid derivatives, tocopherol analogues and reduced-glutathione (GSH) are a ROS scavenger shown

down-regulate melanogenesis through inhibit tyrosinase activity (Yap *et al.*, 2010; Panich, 2011; Kim *et al.*, 2015). Extracts from various plants showed that ROS scavenger and inhibit tyrosinase activity such as leaves of *Ardisia elliptica* Thunb and heartwood of *Artocarpus lakoocha* as well as leaves and tubers of *Hypoxis aurea* Lour which is a potential ingredient for cosmetic products such as anti-wrinkle agents and skin whitening products (Tengamnuay *et al.*, 2006; Boonpisuttinant *et al.*, 2014; Chatatikun and Chiabchalard, 2017). Moreover, black rice and red rice in Indonesia shown the most potent in antioxidant and tyrosinase inhibition, respectively (Batubara *et al.*, 2017).

Riceberry rice, deep purple grain (*Oryza sativa L.*), a cross-breed strain from the Kao Hom Nin rice and Khoa Dawk Mali 105. It is a new valuable rice variety and the most popular brown rice consumption in Thailand (Kongkachuichai *et al.*, 2013). Rice grain and rice bran are highly nutritious, contains polyphenols compounds, anthocyanin and Oryzanol which show that antioxidant activity and can reduce the risk factor of diseases such as cancer, cardiovascular and diabetes (Prangthip *et al.*, 2013; Sirichokworrakit *et al.*, 2015). However, Rice Bran (RB) and Rice Husk (RH) are considered as wastes from

cultivation and rice production process. Previously, they were used for animal's feeding, rice bran oil industries or vegetable cover. The rice waste utilization would be beneficial to a value added and reduction of the amount and removing costs of the wastes from rice milling. The aim of this study has investigated on the antioxidant activity and tyrosinase inhibition activity of extracts of RB and RH in order to evaluate the possible use as a skin-whitening agent for cosmetic and food supplement applications.

MATERIALS AND METHODS

Preparation and extraction: The Rice Bran (RB) and Rice Husk (RH) were collected from Nong Suea District, Pathumthani, Thailand. 100 g of the RB and RH powders were extracted by Boiling (H₂O) with 1 L of distilled water for 2 h) Maceration (EtOH) with 1 L of 95% (v/v) Ethanol at room temperature (25±2°C) with shaking for 48 h and) Fermentation (WF) with *Saccharomyces cerevisiae* var. burgundy for 7 days. After that, the extracts were filtered through Whatman No. 1 filter paper connected with a vacuum pump. The filtrates were dried by a rotary evaporator and freeze-dryer. The crude extracts were kept in glass bottles and stored at 4°C until use. The percentage yields were calculated on the dry weight basis.

Phytochemical analysis: All extracts were examined phytochemicals constituents including alkaloids, flavonoids, anthraquinones, carotenoids, glycosides, tannins and xanthenes as described by Boonpisuttinant *et al.* (2012).

Antioxidant activities Boonpisuttinant *et al.* (2012): Free radical scavenging activity (DPPH assay) briefly, 50 µL of the extracts at the various concentrations and 50 µL of DPPH solution (0.5 mg/mL in ethanol) were put into each well of a 96-well microplate and incubated at 25°C for 30 min. The absorbances were measured by a microplate reader at 515 nm. Ascorbic acid was used as a positive control.

Chelating activity (FIC assay) briefly, 50 µL of the extracts at the various concentrations and 50 µL of ferrozine solution and 50 µL of 1 mg/mL FeCl₂ were added into each well of a 96-well microplate and incubated in the dark at 25°C for 60 min. The absorbances were measured by a microplate reader at 560 nm. EDTA was used as a positive control.

Lipid peroxidation (FTC assay). This experiment was performed using the linoleic acid system as described by Pitija *et al.* (2013). Briefly, 50 µL of the extracts at the various concentrations, 50 µL of 1 mg/mL linoleic acid,

50 µL of 1 mg/mL NH₄SCN solution and 50 µL of 1 mg/mL FeCl₂ were mixed in a 96-well microplate and incubated in the dark at 25°C for 60 min. The absorbances were measured by a microplate reader at 450 nm. α-Tocopherol was used as a positive control. The percentages of the DPPH, FIC and FCT assays were calculated as the following:

$$\text{Inhibition (\%)} = [(A_0 - A_1)/A_0] \times 100 \quad (1)$$

Where:

A₀ = The absorbance of the control

A₁ = The absorbance of the treatments

The concentrations providing 50% scavenging (SC₅₀ mg/mL), 50% chelation (MC₅₀ mg/mL) and 50% peroxidation (LC₅₀ mg/mL) were calculated from the graph plotted between % inhibition and the sample concentrations.

Tyrosinase inhibition activity: Tyrosinase inhibition activity was assayed by the modified dopachrome method using tyrosine as a substrate as previously described (Boonpisuttinant *et al.*, 2014). Briefly, 50 µL of the samples at various concentrations, 50 µL of 0.1 mg/mL L-tyrosine, 50 µL of 0.1 mg/mL mushroom tyrosinase and 50 µL of 0.1 mM phosphate buffer were added in 96-well microplates and incubated at 37°C for 60 min. Kojic acid was used as a positive control. Before and after incubations, the absorbances were measured at 450 nm by a microplate reader. The percentages of tyrosinase inhibition were calculated according to the following Eq. 2:

$$\text{Inhibition activity (\%)} = [(A-B)-(C-D)]/(A-B) \times 100 \quad (2)$$

Where:

A = The absorbance of the blank after incubation

B = The absorbance of the blank before incubation

C = The absorbance of the samples after incubation

D = The absorbance of the samples before incubation

The concentrations providing 50% inhibition (IC₅₀ mg/mL) was calculated from the graph plotted between % inhibition activity and the concentrations.

Statistical analysis: The results were presented as the mean±SD of three independent experiments (n = 3). ANOVA and Tukey's HSD test were used for the analysis of the tested results at the significance level of p<0.05.

RESULTS AND DISCUSSION

The extraction yield, characteristics and photochemical constituents of extracts in the rice bran extracts were shown in Table 1. The yield of Rice bran

Table 1: The extraction yields, characteristics and phytochemical constituent of riceberry rice extract

| Samples | Yields (%) | Characteristics | Phytochemical | | | | | |
|---------------------|------------|--------------------------------|---------------|---------------|-------------|------------|---------|-----------|
| | | | Alkaloids | Anthraquinone | Carotenoids | Flavonoids | Tannins | Xanthenes |
| RB-EtOH | 4.31 | Brown, Viscous, slight odor | - | - | - | - | - | + |
| RH-EtOH | 2.91 | Light brown, viscous, odorless | - | - | - | + | + | + |
| RB-H ₂ O | 5.01 | Light pink, powder, odorless | - | - | - | + | - | + |
| RH-H ₂ O | 3.47 | Light brown, powder, odorless | + | - | - | + | + | + |
| RB-WF | 10.20 | Light pink, powder, sight odor | - | - | - | + | - | + |
| RH-WF | 1.20 | Strong brown, powder, odorless | + | - | - | + | + | - |

RB is Rice Bran, RH is Rice Husk; H₂O was boiling with distil water for 2 h, EtOH was maceration with 95% ethanol for 48 h, WF was fermentation with *S. cerevisiae* var. burgundy for 7 day

Table 2: Antioxidant and tyrosinase inhibition activity of Riceberry rice extract

| Samples | Antioxidant | | |
|---------------------|--|---|---|
| | Freeradical scavenging activity [SC ₅₀ (mg/mL)] | Metalchelating [MC ₅₀ (mg/mL)] | Inhibition of lipid peroxidation [LC ₅₀ (mg/mL)] |
| RB-EtOH | 3.06±0.07 ^d | 3.65±1.06 ^c | 7.42±0.23 ^c |
| RH-EtOH | 2.71±0.22 ^c | 0.17±0.10 ^e | 5.33±0.46 ^b |
| RB-H ₂ O | 3.63±0.29 ^d | 1.30±0.33 ^b | 3.43±0.09 ^a |
| RH-H ₂ O | 5.82±0.20 ^e | 0.72±1.00 ^b | 5.33±0.45 ^b |
| RB-WF | 0.25±0.02 ^b | 0.22±0.02 ^a | 5.41±0.47 ^b |
| RH-WF | 0.43±0.09 ^b | 0.79±0.42 ^b | 3.33±0.20 ^a |
| L-ascorbic acid | 0.05±0.05 ^a | ND | ND |
| EDTA | ND | 0.40±0.61 ^b | ND |
| α-tocopherol | ND | ND | 3.95±0.76 |

*RB is Rice Bran; RH is Rice Husk; H₂O was boiling with distil water for 2 h, EtOH was maceration with 95% ethanol for 48 h, WF was fermentation with *S. cerevisiae* var. burgundy for 7 days. ND is not detected

extracted by fermentation with *S. cerevisiae* (RB-WF) for 7 days showed the highest extraction yield (10.2%) and has been light pink powder as well as Slight sweet odor. The main phytochemical constituents of the riceberry rice extract were flavonoids and xanthenes while anthraquinones carotenoids were not found in all extracts. The nature of medicinal plants, solvents and temperature used in the condition process may affect to the present difference of phytochemicals and may also affect in the biological activity (Manosroi *et al.*, 2012; Selvakumari *et al.*, 2016). This result is consistent with report (Ghasemzadeh *et al.*, 2018), rice bran contenting with flavonoid and phenolic acid compounds such as quercetin apigenin, luteolin, syringic acid, cinnamic acid and p-coumaric acid which have been highest antioxidant activities and exhibiting anti-inflammatory, hypocholesterolemic, anti-cance and anti-diabetic properties (Sivamaruthi *et al.*, 2018). Therefore, the bioactive components in riceberry rice extract might have antioxidant potential and tyrosinase inhibition activity.

In Table 2, all riceberry extracts showed all antioxidant activities. The RB-WF and RH-WF gave the highest free radical Scavenging activity (SC₅₀) but lower than ascorbic acid (0.05±0.05 mg/mL) (p<0.05). Moreover, RH-EtOH and RB-WF showed the highest metal chelating activity (MC₅₀) of 0.17±0.10 and 0.22±0.02 mg/mL which higher than that of EDTA (0.40±0.61 mg/mL) of about 2 times (p<0.05) and RB-H₂O and RH-WF gave the highest lipid peroxidation activity with the LC₅₀ of 3.43±0.09 and 3.33±0.20 mg/mL which were comparable to α-tocopherol

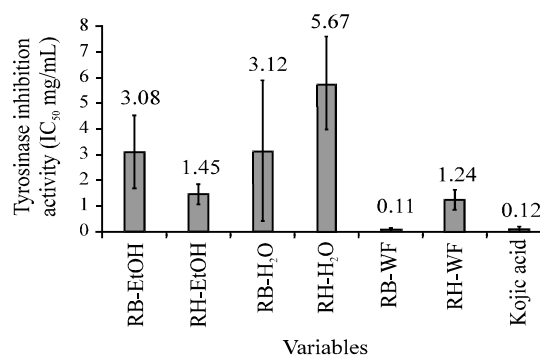


Fig. 1: Tyrosinase inhibition activity of riceberry rice extract. Rice bran; RH is Rice Husk; H₂O was boiling with distil water for 2 h; EtOH was maceration with 95% ethanol for 48 h; WF was fermentation with *S. cerevisiae* var. burgundy for 7 days

(3.95±0.76 mg/mL) (p<0.05). The tyrosinase inhibition activity of the RB-WF xexhibited significantly the highest activities with the IC₅₀ value of 0.11±0.01 mg/mL which was comparable to kojic acid (0.12±0.10 mg/mL) (p<0.05) (Fig. 1). The anti-oxidant and tyrosinase inhibition activities of the extracts might be due to their phytochemical constituents which has been reported for their several bioactivities such as anti-oxidation, tyrosinase inhibition activity, zanti-inflammatory and anti-cancer (Ghasemzadeh *et al.*, 2018; Nguyen *et al.*, 2016, Sivamaruthi *et al.*, 2018).

CONCLUSION

The RB-WF extract demonstrates the highest antioxidant and inhibition of tyrosinase activities might be beneficial to further develop as whitening and antioxidant agents for cosmetic application.

ACKNOWLEDGEMENT

This research was financial supported by Rajamangala University of Technology Thanyaburi (RMUTT), Thailand and The Thailand Research Fund (TRF), Thailand.

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