



# Bioactivities of organic riceberry broken rice and crude riceberry rice oil

Vijitra Luang-In, Manatchanok Yotchaisarn,  
Issaraporn Somboonwatthanakul, Sirirat Deeseenthum

Natural Antioxidant Innovation Research Unit, Department of Biotechnology,  
Faculty of Technology, Mahasarakham University, Khamriang, Kantarawichai,  
Mahasarakham 44150 Thailand

## Corresponding Author:

Vijitra Luang-In,  
Department of Biotechnology,  
Faculty of Technology,  
Mahasarakham University,  
Khamriang, Kantarawichai,  
Mahasarakham 44150  
Thailand.  
Tel: + 66 43754085 ext 1833.  
Fax: + 66 43754086.  
E-mail: vijitra.l@msu.ac.th

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## ABSTRACT

**Introduction:** Riceberry broken rice (RBR) and crude riceberry rice oil (CRO) are inevitably generated from organic rice process in Thailand for several tons per year with low value. The aims of this work were to determine gamma-oryzanol content, fatty acid content, antioxidant activities, total phenolic content (TPC), total flavonoid content (TFC), and tyrosinase-stimulating activity of the extracts from RBR and CRO. **Materials and Methods:** Different organic solvents were used to extract bioactive compounds from RBR and ethanol was used for CRO. Extracts were analyzed for gamma-oryzanol content by high-performance liquid chromatography and fatty acid content by gas chromatography-mass spectrometry. Their bioactivities were tested using 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP), 2,2'-Azino-di(3-ethyl-benzthiazoline sulfonic (ABTS) assays. TPC and TFC assays were performed, and tyrosinase-stimulating activities were also determined. **Results:** Gamma-oryzanol content in ethanolic CRO (234 mg/100 g) extract was much higher than that in aqueous RBR extract (52.5 mg/100 g). The four isomers of gamma-oryzanol including 24-methylene campesteryl ferulate (51.3%) > campesteryl ferulate (26.0%) > cycloartanyl ferulate (13.1%) >  $\beta$ -sitosteryl ferulate (9.6%) were found in CRO as well as RBR; however in lesser content. Seven fatty acids in the descending order: 41% oleic acid >30% linoleic acid >19% hexadecanoic acid >8% 10-octadecenoic acid >1% 9-octadecenoic acid > 0.6% pentadecanoic acid >0.4% stearic acid were found in CRO and similarly in RBR. Moreover, RBR extract exhibited antioxidant activities at 6.36 mg Trolox Equivalent Antioxidant Capacity (TEAC)/g (DPPH), 1.33 mg FeSO<sub>4</sub>/g (FRAP), 5.82 mg TEAC/g (ABTS) with TPC of 9.94 mg gallic acid equivalent(GAE)/g, and TFC of 67.2 mg catechin equivalent (CE)/g. Interestingly, aqueous RBR extract and ethanolic CRO exhibited 45% and 26% tyrosinase-stimulating activity, respectively. **Conclusion:** These bioactive compounds and bioactivities in CRO and RBR have a potential to promote hair growth and hair melanogenesis and thus proved useful as cosmetic ingredients in hair product development.

**Keywords:** Antioxidant, broken rice, flavonoid, riceberry rice, tyrosinase

## INTRODUCTION

Riceberry rice is one of the most popular rice cultivars in Thailand with high antioxidant properties offered to the consumers.<sup>[1]</sup> During the process of polished riceberry rice production, broken rice is inevitably generated at 20–30% of riceberry rice product yield (approximately 1,200–1,800 tons per harvest season). These huge amounts of riceberry broken rice (RBR) and crude riceberry rice oil (CRO) are sold by Thai farmers as raw materials for animal feeds or ingredients for puree, snacks, Chinese noodles and hair serum component, respectively, at very low price. In the

previous reports, broken rice extract was shown to exhibit chemopreventive effect against cancer cells and also wound healing effects.<sup>[2-5]</sup> Modern lifestyle has been often considered as one of the primary causes for oxidative stress, and free radicals lead to melanocyte apoptosis in the aging human hair follicle<sup>[6]</sup> and inhibition of methionine sulfoxide repair (MSR).<sup>[7]</sup> Thus, the search for antioxidant natural ingredients for melanogenesis stimulation has been ongoing. Reportedly, broken rice extract has been shown to exhibit anti-hair fall and anti-gray hair abilities due to its vitamin B3 that supports hair strength and gamma-oryzanol that stimulates melanogenesis,

respectively. Rice bran extracted with supercritical CO<sub>2</sub> showed the potential to stimulate hair growth similar to 3% minoxidil, standard hair stimulant chemical. The bioactive compounds include linoleic acid, policosanol, gamma-oryzanol, and gamma-tocotrienol. Results on mRNA expression of cell growth factor via real-time reverse transcriptase-polymerase chain reaction in C57BL/6 mice displayed that both linoleic acid and gamma-oryzanol induced hair follicle formation and also gene expression of vascular endothelial growth factor (VEGF), insulin-like growth factor-1 (IGF-1), and keratinocyte growth factor (KGF) involving in hair growth.<sup>[8]</sup> In addition, the same research group carried out the double-blind, randomized experiment on male volunteers for 16 weeks to evaluate the safety and efficacy of rice bran extracted with supercritical CO<sub>2</sub> on hair fall symptoms in the volunteers.<sup>[9]</sup> The results showed that rice bran extract was able to significantly increase hair density and hair growth in the volunteers without any side effects.

Several factors can adversely affect hair follicles or weaken and make them sensitive to the stimulation of androgens. Recent research has revealed that androgens hampers hair growth through the release of transforming growth factor (TGF) β1. Androgens were induced by the generation of reactive oxygen species leading to release of TGF β1, implying benefit of antioxidants in hair growth.<sup>[10]</sup> The previous study on humans showed that a large majority of female subjects supplemented with omega 3 and 6 and antioxidants for 6 months reported a reduction in hair loss (89.9% of patients), as well as an improvement in hair diameter (86.1%) and hair density (87.3%).<sup>[11]</sup> In addition, the entire gray hair follicles were found to accumulate hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) in millimolar concentrations, and lack catalase and MSR protein expression in association with functional loss of MSR.<sup>[7]</sup> Consequently, methionine sulfoxide formation of methionine residues (Met), including Met 374 in the active site of tyrosinase, the key enzyme in melanogenesis, limits enzyme functionality, which eventually leads to loss of hair color.<sup>[12]</sup>

Tyrosinase or TYR (EC 1.14.18.1) is a copper-containing enzyme, which is ubiquitous in plants and animals. It is known to be a key enzyme for melanogenesis that can be stimulated by a variety of paracrine cytokines<sup>[13]</sup> through diverse signaling pathways by inducing the expression and activation of pigment-related proteins such as microphthalmia-associated transcription factor (MITF), TYR, tyrosine-related protein-1 (TRP-1), and TRP-2.<sup>[13]</sup> Although three enzymes (TYR, TRP-1, and TRP-2) are involved in the melanogenesis pathway, only TYR is exclusively necessary for melanogenesis. Melanin is polyphenol-like biopolymer with a complex structure and colors varying from yellow to black, which was formed through a series of oxidative reactions involving the amino acid tyrosine in the presence of TYR.<sup>[14]</sup> Fortunately, human body possesses endogenous defense mechanisms, such as antioxidative enzymes and nonenzymatic antioxidative molecules that protect it from free radicals by reducing and neutralizing them. However, with age, the production of free radicals increases, while the endogenous defense mechanisms decrease. This imbalance leads to the progressive damage of cellular and molecular structures of hair follicles, presumably resulting in the aging phenotype, i.e., gray hair.<sup>[14]</sup>

Thus far, little is known about bioactivities of organic BRB and CRO. Therefore, the aims of this research were to study bioactive compounds, antioxidant activities, total phenolic content (TPC), total flavonoid content (TFC) as sources of antioxidants and tyrosinase-stimulating activity of RBR and CRO extracts for the potential application as a hair growth stimulant or/and hair pigmenting agent in hair care products.

## MATERIALS AND METHODS

### Agricultural Materials

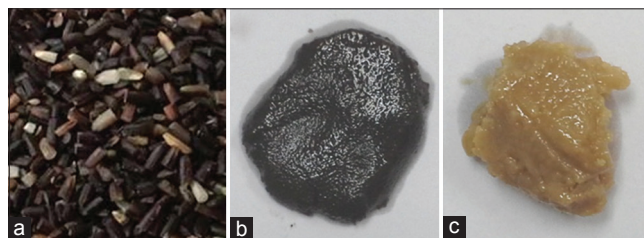
Organic RBR, CRO, and crude jasmine rice oil (CJO) [Figure 1] were obtained from The E-san Community Enterprise Agrarian Network, Roi Et Province, Thailand. Experiments were conducted at Natural Antioxidant Innovation Research Unit, Department of Biotechnology, Faculty of Technology, Mahasarakham University, Thailand.

### Extraction of RBR, CRO, and CJO

RBR was ground to powder using a grinder machine and sieved through 0.5 mm sieve. The powder (200 g) was mixed with four different solvents (1 L); absolute ethanol (RCI Labscan, Thailand), absolute ethanol and deionized water mixture (1:1), deionized water and ethyl acetate (RCI Labscan, Thailand) for 48 h at 200 rpm in a shaker incubator at 45°C. The mixtures were filtered through Whatman no. 1 paper using a vacuum pump to obtain only the supernatants which were then evaporated using a rotatory evaporator at 42°C. The concentrated extracts (1,000 mg/mL) were weighed and dissolved in deionized water. CRO or CJO (200 g) was mixed with absolute ethanol and extracted as the same method mentioned above till the concentrated oil was obtained. The extract solutions were stored at -20°C until use.

### Lipid Extraction and Fatty Acid Analysis

Lipids in organic RBR, CRO and CJO extracts were extracted according to the previous method.<sup>[15]</sup> Approximately 5 g of well-ground samples was extracted with 50.0 mL of chloroform-methanol (2:1, v/v) (RCI Labscan, Thailand) containing 10 mg/L of butylated hydroxytoluene (Sigma-Aldrich, Dorset, UK) and 0.1 mg/mL of nanodecanoic acid (C19:0, Sigma-Aldrich, Dorset, UK) as an internal standard. Then, the samples were stored in a fume hood overnight. Each sample was filtered and transferred into a separate funnel and added with 15 mL of 0.9% sodium chloride. The samples were shaken well to allow the phases to separate; the lower phase was then evaporated and transferred into a 10-mL volumetric flask. Fatty acid methyl



**Figure 1:** Physical appearance of riceberry rice by-products. (a) Riceberry broken rice (RBR) (b) crude riceberry rice oil (CRO) and (c) crude jasmine rice oil (CJO)

esters (FAMES) of the total lipid extract were prepared by transesterification in  $\text{H}_2\text{SO}_4$  (0.9 M in methanol). Before injection into the gas chromatograph, the FAMES were filtered by Sep-pak silica column (Alltech Associates, Inc., Deerfield, IL, US). Samples (1 mL) were analysed quantitatively using a Shimadzu model GC-2014 system (Shimadzu, Tokyo, Japan) fitted with flame ionization detection eluted with  $\text{H}_2$  at  $30 \pm 1$  mL/min, with a split ratio of 1:17. A fused silica capillary column ( $30 \text{ m} \times 0.25 \text{ mm}$ ,  $25 \mu\text{m}$  film thickness; DB-Wax, US) was used. The injector and detector were maintained at  $250^\circ\text{C}$ . Nitrogen was used as a carrier gas and temperature programming was from  $150^\circ\text{C}$  (hold 5 min) to  $230^\circ\text{C}$  at  $15^\circ\text{C}/\text{min}$ , then to  $170^\circ\text{C}$  (hold 10 min) at  $10^\circ\text{C}/\text{min}$ , then to  $200^\circ\text{C}$  (hold 3 min) at  $5^\circ\text{C}/\text{min}$  and then to  $230^\circ\text{C}$  (hold 2 min) at  $15^\circ\text{C}/\text{min}$ . Fatty acids were identified by comparison with standard fatty acids as the previous method.<sup>[16]</sup> Lipid content, fatty acid composition and concentration were calculated as the following formula.

Fatty acid composition (%) = (area under each peak/total areas of all fatty acids appeared in the chromatogram)  $\times 100$

### Gamma-Oryzanol Analysis

This was carried out as the previous method.<sup>[17]</sup> RBR powder (1 g), CRO or CJO (1 g) was mixed with 80% ethanol (8 mL) for 24 h at 200 rpm in a shaker incubator at  $25^\circ\text{C}$ . The mixture was then centrifuged at 6,440 g for 10 min and filtered through  $0.45 \mu\text{m}$  nylon membrane. The supernatant was analysed using high-performance liquid chromatography (HPLC)-20 AD (Shimadzu, Tokyo, Japan) equipped with SPD-M20 a diode array detector, C18 reverse phase ( $4.6 \text{ mm} \times 150 \text{ mm} \times 5 \mu\text{m}$ ) column (Phenomenex, CA, US) with three mobile phase solutions; A: Acetonitrile (RCI Labscan, Thailand), B: Methanol and C: Water under gradient elution system; A:B:C (60:35:5) for 5 min and then changed to isocratic elution system; A:B 60:40 for 5 min and 22:78 at a flow rate of 1.0 mL/min for 60 min. The injection volume was  $20 \mu\text{L}$ . Gamma-oryzanol was identified under ultraviolet-visible diode array at the wavelength of 295 nm by comparing the retention time to the authentic standard gamma-oryzanol (Sigma-Aldrich, Dorset, UK) and quantified using an external calibration curve of the standard.

### Scavenging of 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) Free Radical

The effect of DPPH radical (Sigma-Aldrich, Dorset, UK) was evaluated by the previous methods<sup>[18,19]</sup> with a slight modification. The assay mixture contained  $300 \mu\text{L}$  of 1.0 mM DPPH radical solution, 2.4 mL of 99% ethanol, and  $300 \mu\text{L}$  of 20 mg/mL extract solution. The solution was rapidly mixed, and scavenging capacity was measured by monitoring the decrease in absorbance at 517 nm using an M965+ microplate reader (Metertech; Taipei, Taiwan). The standard Trolox (Sigma-Aldrich, Dorset, UK) was used as a positive control and to construct a calibration curve. The results were expressed as Trolox Equivalent Antioxidant Capacity (TEAC) mg/g extract for triplications.

### Ferric Reducing Antioxidant Power Assay (FRAP)

The antioxidant activity of each extract was evaluated using FRAP assay with some modifications to the previous method.<sup>[20]</sup>

FRAP reagent was prepared in 300 mM acetate buffer pH 3.6 (by adding acetic acid), 10 mM 2,4,6-Tris(2-pyridyl)-s-triazine solution (Sigma-Aldrich, Dorset, UK) in 40 mM HCl (Sigma-Aldrich, Dorset, UK) and 20 mM iron (III) chloride solution (Sigma-Aldrich, Dorset, UK) in the proportion of 1:1:10 (v/v), respectively. A total of  $100 \mu\text{L}$  extract solution (20 mg/mL) was added to  $3,000 \mu\text{L}$  of the FRAP reagent, mixed well and incubated for 15 min at room temperature. The absorbance was measured at 595 nm using a spectrophotometer and iron (II) sulfate ( $\text{FeSO}_4$ ) (Sigma-Aldrich, Dorset, UK) was used as a standard. The results were expressed as  $\text{FeSO}_4$  mg/g extract for triplications.

### 2,2'-Azino-di(3-Ethyl-Benzthiazoline Sulfonic (ABTS)<sup>•+</sup> Scavenging Assay

The ABTS<sup>•+</sup> acid radical cation scavenging assay was carried out with some modifications to the previously reported method.<sup>[21]</sup> A total of  $1000 \mu\text{L}$  extract (20 mg/mL) was added to  $1000 \mu\text{L}$  of ABTS<sup>•+</sup> (Sigma-Aldrich, Dorset, UK) solution and mixed well. The absorbance was measured at 734 nm after 15 min incubation at room temperature. The control included only water and ABTS<sup>•+</sup> solution whereas the blank contained only water. The standard Trolox (Sigma-Aldrich, Dorset, UK) was used as a positive control and to construct a calibration curve. The results were expressed as TEAC mg/g extract for triplications.

### Determination of TPC

Extract solutions (20 mg/mL) were analyzed spectrophotometrically for the content of TPC using a modified Folin-Ciocalteu colorimetric method.<sup>[22]</sup> All extracts ( $20 \mu\text{L}$ ) were mixed with  $100 \mu\text{L}$  of Folin-Ciocalteu reagent (Sigma-Aldrich, Dorset, UK). The samples were mixed well and then allowed to stand for 1 min before  $20 \mu\text{L}$  of a 7.5% (w/v) sodium bicarbonate aqueous solution was added. Samples were allowed to stand for 30 min at room temperature before measurement at 760 nm versus the blank using a microplate reader in comparison with the standards prepared similarly with known gallic acid (Sigma-Aldrich, Dorset, UK) concentrations. All values were expressed as gallic acid equivalent (GAE) mg/g extract for triplications.

### Determination of TFC

TFC was determined by using a colorimetric method described previously<sup>[23]</sup> with slight modifications. In brief,  $25 \mu\text{L}$  of the extract solution (20 mg/mL) was mixed with  $7.5 \mu\text{L}$  of 7% sodium nitrite (Sigma-Aldrich, Dorset, UK) and distilled water ( $12.5 \mu\text{L}$ ) followed by addition of 10%  $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$  solution ( $15 \mu\text{L}$ ). The mixture was well mixed and allowed to stand for 30 min at room temperature before 0.5 mL of 1 M NaOH was added. The mixture was brought to 2.5 mL with distilled water and mixed well. The absorbance was measured immediately against the blank at 510 nm in comparison with the standards prepared similarly with known (+)-catechin (Sigma-Aldrich, Dorset, UK) concentrations. The results were expressed as catechin equivalent (CE) mg/g extract for triplicate.

### Tyrosinase Stimulating Activity

This was performed according to the previous report.<sup>[24]</sup> Ten microliters of 20 mg/mL extract solution were mixed with  $20 \mu\text{L}$

of 1000 U/mL mushroom tyrosinase (Sigma-Aldrich, Dorset, UK) in 110  $\mu$ L of phosphate buffer pH 6.8 in a microplate and incubated at 37°C for 30 min at room temperature. Ten microliters of 10 mM levodopa (L-DOPA) (Sigma-Aldrich, Dorset, UK) was added to the mixture and incubated at 37°C for 40 min at room temperature. Absorbance at 490 nm was recorded using a microplate reader with buffer mixed with L-DOPA and tyrosinase as a control. Experiments were performed in triplicates. Tyrosinase stimulation (%) as the mean of triplications was calculated from the below equation:

$$\% \text{ Stimulation} = \frac{(A-B)}{B} \times 100$$

Where,

A is absorbance of the extract or the standard

B is absorbance of the control without added the extract or standard.

## Statistical Analysis

Measurements were obtained in triplicates as means  $\pm$  standard deviation (SD). Statistical analysis was performed using One-way analysis of variance and Duncan Multiple's Range Test by the software SPSS package version 19.0 (IBM, Armonk, NY, US) at  $P < 0.05$ .

## RESULTS AND DISCUSSION

### HPLC Analysis of Gamma-Oryzanol

It was found that RBR extract contained a low amount of gamma-oryzanol at 52.5 mg/100 g sample based on HPLC analysis. On the other hand, CRO extract and CJO extract showed four to nine times higher amount of gamma-oryzanol at 234 mg/100 g sample and 442 mg/100 g sample, respectively [Table 1]. HPLC chromatogram of the authentic gamma-oryzanol standard displaying four isomers in the order: 24-methylene cycloartanyl ferulate (39.9%) > cycloartanyl ferulate (32.1%) > campesteryl ferulate (19.1%) >  $\beta$ -sitosteryl ferulate (8.9) [Table 1 and Figure 2a] indicating distinct proportions of gamma-oryzanol isomers from organic by-product extracts. The amount of each isomer in RBR extract was in the order: Cycloartanyl ferulate (56.3%) > campesteryl

ferulate (27.5%) > 24-methylene cycloartanyl ferulate (16.2%) >  $\beta$ -sitosteryl ferulate (below limit of detection by HPLC) [Table 1 and Figure 2b]. In contrast, the amount of each isomer in CJO extract was in the order: 24-methylene campesteryl ferulate (59.7%) > campesteryl ferulate (13.6%) > cycloartanyl ferulate (13.5%) >  $\beta$ -sitosteryl ferulate (13.2%) [Table 1 and Figure 2c]. The result was similar to that of CRO extract in the following order: 24-methylene campesteryl ferulate (51.3%) > campesteryl ferulate (26.0%) > cycloartanyl ferulate (13.1%) >  $\beta$ -sitosteryl ferulate (9.6%) [Table 1 and Figure 2d]. The four isomers of gamma-oryzanol from organic by-product extracts in this work were similar to those previously reported.<sup>[25,26]</sup> The gamma-oryzanol was found to be able to stimulate melanogenesis and induce hair follicle formation and enhance new hair generation, and thus our RBR and CRO extracts hold the potential for the use in hair treatment products. However, these amounts were much lower than that found in rice bran oil from India (1,730 mg/100 g sample).<sup>[26]</sup>

### Gas Chromatography–Mass Spectrometry (GC-MS) Analysis of Fatty Acids

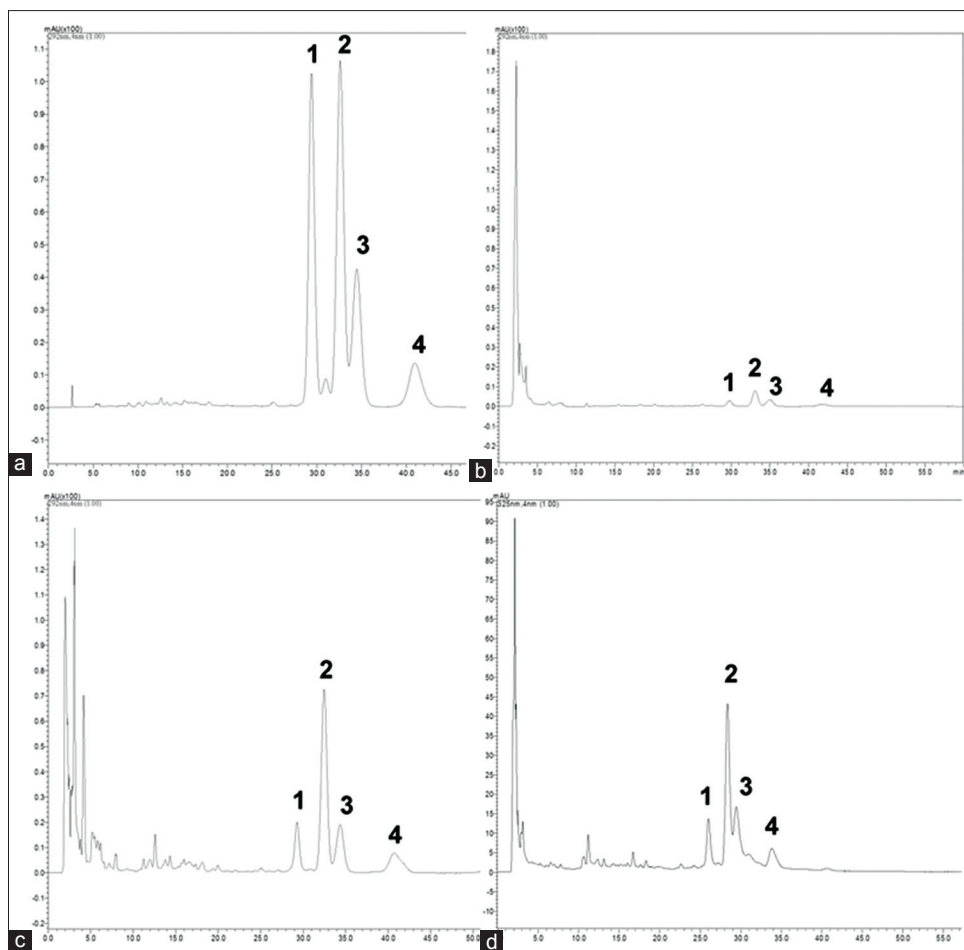
Recent reports showed that rice bran extracted by supercritical CO<sub>2</sub> method had the potential in promoting hair growth similar to 3% minoxidil.<sup>[8]</sup> Linoleic acid and gamma-oryzanol were able to generate hair follicles and induce gene expression of VEGF, IGF-1, and KGF involved in hair growth in C57BL/6 rats.<sup>[8]</sup> Thus, fatty acids in CJO, CRO and RBR extracts were determined by GC-MS analysis to evaluate their potential use as a hair growth promoting cosmetics ingredients. The results showed that all three extracts displayed seven types of fatty acids; (1) pentadecanoic acid (C15:0) at 9.41 min, (2) 9-octadecenoic acid (C18:1) at 13.3 min, (3) 10-octadecenoic acid (C18:1) at 14.4 min, (4) hexadecanoic acid (C16:0) at 23.6 min, (5) stearic acid (C18:0) at 29.4 min, (6) oleic acid (C18:1) at 30.1 min, and (7) linoleic acid (C18:2) at 31.6 min [Figure 3]. Similar trends in fatty acid compositions were found in both CJO and CRO extract in the following order; 42–43% oleic acid >30–31% linoleic acid 19–22% hexadecanoic acid >1–8% 10-octadecenoic acid >0.4–5% stearic acid >0.6–1.4% 9-octadecenoic acid >0.5–0.6% pentadecanoic acid [Table 2].

**Table 1:** Compositions of seven fatty acids of the extracts by GC-MS analysis

Samples	Compositions of four isomers of gamma-oryzanol							
	(A=Content g/100 g; B=relative %)							
	1. Cycloartanyl ferulate		2. 24-methylene cycloartanyl ferulate		3. Campesteryl ferulate		4. $\beta$ -sitosteryl ferulate	
A	B	A	B	A	B	A	B	
Standard Gamma-oryzanol	32.1 <sup>Ba</sup>	32.1 <sup>Ba</sup>	39.9 <sup>Ca</sup>	39.9 <sup>Ca</sup>	19.1 <sup>Bb</sup>	19.1 <sup>Bb</sup>	8.9 <sup>Cc</sup>	8.9 <sup>Cc</sup>
RBR extract	8.5 <sup>Cc</sup>	16.2 <sup>Cc</sup>	29.5 <sup>Ca</sup>	56.3 <sup>Ca</sup>	14.4 <sup>Bb</sup>	27.5 <sup>Bb</sup>	<LOD*	<LOD*
CRO extract	30.6 <sup>Bc</sup>	13.1 <sup>Bc</sup>	120 <sup>Ba</sup>	51.3 <sup>Ba</sup>	60.9 <sup>Ab</sup>	26.0 <sup>Ab</sup>	22.5 <sup>Bc</sup>	9.6 <sup>Bc</sup>
CJO extract	56.6 <sup>Ab</sup>	13.5 <sup>Ab</sup>	250 <sup>Aa</sup>	59.7 <sup>Aa</sup>	56.9 <sup>Ab</sup>	13.6 <sup>Ab</sup>	55.1 <sup>Ab</sup>	13.2 <sup>Ab</sup>

Values were means of triplicates. Superscripts with upper-case letters indicate statistically significant difference in the column and lower-case letters in the row ( $P < 0.05$ ). RBR: Riceberry broken rice, CJO: Crude jasmine rice oil, CRO: Crude riceberry rice oil. % relative = (content of isomer/total content of isomers)  $\times 100\%$ . \* <LOD means the values were lower than the limit of detection, HPLC: High-performance liquid chromatography





**Figure 2:** High-performance liquid chromatography analysis of gamma-oryzanol. (a) Authentic standard of gamma-oryzanol, (b) RBR extract, (c) CJO extract and (d) CRO extract. Four peaks of isomers at 29.4 min cycloartanyl ferulate, 1; 32.6 min 24-methylene cycloartanyl ferulate, 2; 34.5 min campepesteryl ferulate, 3; 40.7 min  $\beta$ -sitosteryl ferulate, 4

Nevertheless, RBR extract showed a different trend in the following order; 47% 10-octadecenoic acid >18% oleic acid >16% 9-octadecenoic acid >12% linoleic acid >8% pentadecanoic acid > hexadecanoic acid and stearic acid (below limit of detection by GC-MS) [Table 2]. In contrast, the previous finding of riceberry rice bran oil extract was shown to contain different fatty acid content of 39.54% oleic acid >30.93% linoleic acid >22.33% palmitic acid >2.01% stearic acid > 0.78% myristic acid.<sup>[27]</sup> This indicated that different by-products of riceberry rice have different fatty acid compositions.

### Antioxidant Activities

The results showed that RBR extract from water exhibited the highest DPPH-scavenging activity at 6.36 mg TEAC/g extract followed by RBR extract from ethanol, ethanol mixed with distilled water and ethyl acetate at 5.07, 4.52 and 3.80 mg TEAC/g extract, respectively [Figure 4]. On the other hand, the CRO extract showed the lowest DPPH-scavenging activity at 1.71 mg TEAC/g extract indicating that DPPH scavenging molecules are likely to have hydrophilic properties and thus more favourably dissolved in more polar solvents. Similarly, FRAP assay results showed that RBR extract from ethanol exhibited the highest FRAP value at 1.33 mg  $\text{FeSO}_4$ /g extract followed by that

from water, ethanol mixed with water and ethyl acetate at 1.19, 0.55 and 0.26 mg  $\text{FeSO}_4$ /g extract. However, the CRO extract showed the lowest FRAP value at 0.18 mg  $\text{FeSO}_4$ /g extract suggesting that antioxidants capable of reducing ferric ion ( $\text{Fe}^{3+}$ ) to ferrous ion ( $\text{Fe}^{2+}$ ) are likely to be polar molecules. Interestingly, ABTS<sup>+</sup> antioxidant assay results demonstrated similar ABTS<sup>+</sup> antioxidant activities from RBR extract from all four solvents at 5.09–5.82 mg TEAC/g extract while CRO extract showed the lowest ABTS<sup>+</sup> antioxidant activity at 3.37 mg TEAC/g extract. Recent finding showed that ethanolic riceberry rice extract (50 mg/mL) had an effective FRAP value at  $1.643.89 \pm 6.03 \text{ FeSO}_4 \mu\text{g/mL}^{[26]}$  which was higher than our FRAP values.

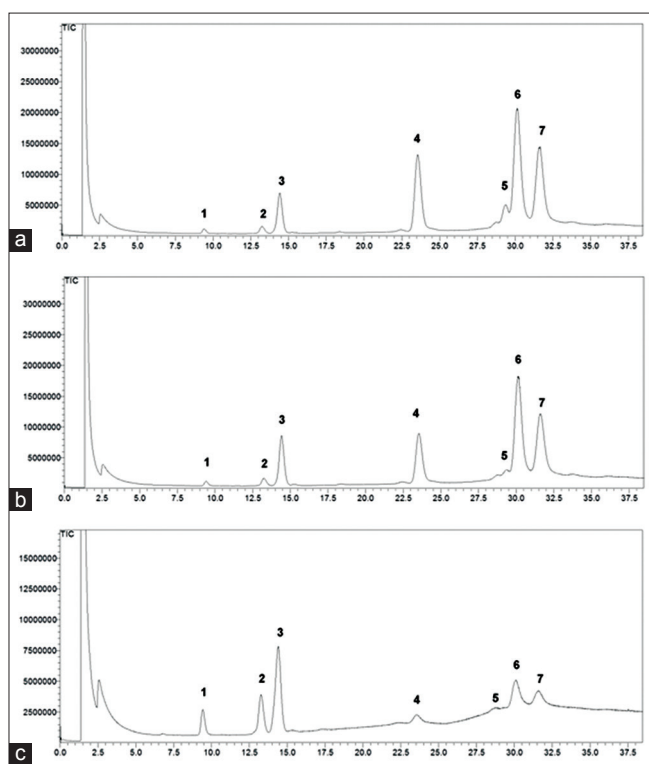
### TPC and TFC

The results showed that the TPC of RBR extract from ethanol was highest at 9.94 mg GAE/g extract followed by that from ethanol mixed with water, water and ethyl acetate at 8.91, 7.24 and 0.51 mg GAE/g extract [Figure 5]. Interestingly, the CRO extract showed rather high 7.11 mg GAE/g extract. In addition, the TFC of RBR extract from ethanol was highest at 67.2 mg CE/g extract followed by that from ethanol mixed with water, water and ethyl acetate at 45.4, 25.7 and 16.0 mg CE/g extract. Interestingly, the CRO extract showed rather

**Table 2:** Compositions of seven fatty acids by GC-MS analysis

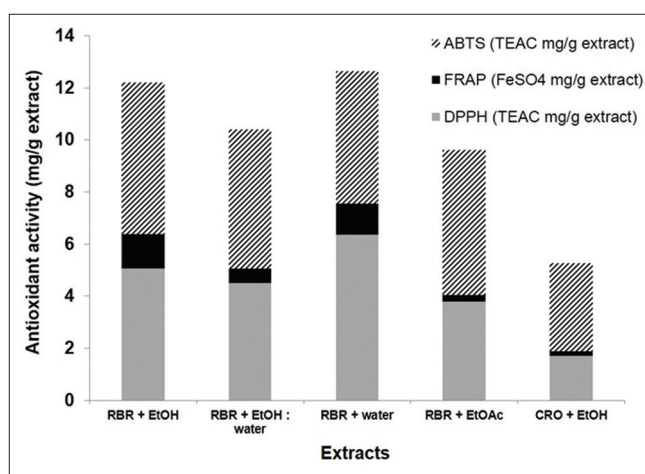
$t_R$ (min)	Fatty acid	Fatty acid composition (%)		
		CJO	CRO	RBR
9.4	Pentadecanoic acid C15:0	0.49±0.35 <sup>Eb</sup>	0.60±0.06 <sup>Eb</sup>	8.20±0.49 <sup>Ca</sup>
13.3	9-Octadecenoic acid C18:1	0.57±0.40 <sup>Eb</sup>	1.38±0.28 <sup>Eb</sup>	15.59±0.78 <sup>Ba</sup>
14.4	10-Octadecenoic acid C18:1	1.21±0.86 <sup>Ec</sup>	7.75±10.96 <sup>Db</sup>	46.81±3.67 <sup>Aa</sup>
23.6	Hexadecanoic acid C16:0	21.67±5.22 <sup>Ca</sup>	18.58±1.5 <sup>Ca</sup>	<LOD*
29.4	Stearic acid C18:0	5.12±3.62 <sup>Ba</sup>	0.41±0.57 <sup>Eb</sup>	<LOD*
30.1	Oleic acid C18:1	43.33±1.26 <sup>Aa</sup>	41.61±4.17 <sup>Aa</sup>	17.88±0.24 <sup>Bb</sup>
31.6	Linoleic acid C18:2	31.29±2.26 <sup>Ba</sup>	29.65±2.33 <sup>Ba</sup>	11.49±0.72 <sup>Bb</sup>
	Total	100	100	100

Values were means of triplicates. Superscripts with upper-case letters indicate statistically significant difference in the column and lower-case letters in the row ( $P < 0.05$ ).  $t_R$ : Retention time, RBR: Riceberry broken rice, CJO: Crude jasmine rice oil, CRO: Crude riceberry rice oil. \* <LOD means the values were lower than the limit of detection. GC-MS: Gas chromatography–mass spectrometry



**Figure 3:** Gas chromatography–mass spectrometry analysis of fatty acids. (a) CJO extract, (b) crude riceberry rice oil extract and (c) RBR extract. Seven peaks of fatty acids at 9.41 min pentadecanoic acid, 1; 13.3 min 9-octadecenoic acid, 2; 14.4 min 10-octadecenoic acid, 3; 23.6 min hexadecanoic acid, 4; 29.4 min stearic acid, 5; 30.1 min oleic acid, 6; 31.6 min linoleic acid, 7.

high TFC at 40.2 mg CE/g extract [Figure 5]. Our TPC results in ethanol, water and ethanol mixed with water (7.24–9.94 mg GAE/g) were approximately three-fold higher than those (2.2–3.2 mg GAE/g) found in the previous report<sup>[29]</sup> using rice brans of various rice varieties in Thailand. Moreover, our TPC results were forty-fold higher than TPC (0.20 mg GAE/g) of Temukut (brewer's rice extract in Malaysia).<sup>[4]</sup> In comparison with the previous finding, our TPC results were ten-fold higher than those (0.121–0.127 mg GAE/g) of KDML105 jasmine broken rice extract.<sup>[30]</sup> This suggested that the pigmented RBR is richer



**Figure 4:** Antioxidant activities of the extracts. Values were means of triplicates. RBR - riceberry broken rice, EtOH - ethanol, EtOAc - ethyl acetate, CRO - crude riceberry rice oil

in phenolic compounds than jasmine broken rice. In addition, TFC results (16.0–67.2 CE mg/g) reported here were a thousand-fold higher than the previously reported TFC values (0.033–0.034 mg QE/g).<sup>[30]</sup> In contrast, the recent report<sup>[31]</sup> found that 70:30 ethanol-water at 6 h of total extraction time for riceberry rice extract was the best extraction condition to obtain the highest of TPC, ABTS<sup>•+</sup> and FRAP antioxidant activities at 53.354 ± 1.373 mg GAE/g extract, 0.015 ± 0.0003 mg/ml and 2,765.766 ± 39.844 mg TEAC/g extract, respectively. The previously reported TPC, ABTS<sup>•+</sup> and FRAP results were higher than ours indicating that extraction solvent combination and extraction time may play a key part in extracting bioactive compounds from rice by-products. These optimal extraction conditions will be further studied when a large scale of bioactive compound extraction is required for industrial purposes.

### Tyrosinase Stimulating Activity

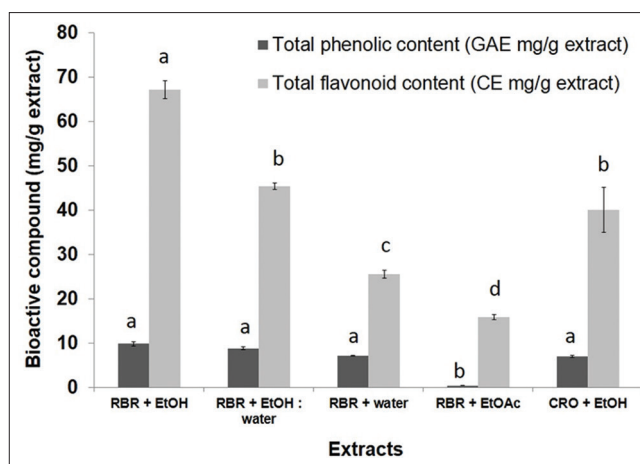
The results showed that RBR extract from ethanol exhibited the highest tyrosinase-stimulating activity at 45% followed by RBR extract from distilled water and ethanol mixed with distilled water at 40% and 34%, respectively [Figure 6].

However, the RBR extract from ethyl acetate showed the least tyrosinase-stimulating activity at 23% similar to 26% found in CRO extract. The results indicated that bioactive compounds with tyrosinase-stimulating activity must have hydrophilic property since RBR extraction by ethanol or distilled water resulted in high tyrosinase-stimulating activity. On the other hand, RBR extract by ethyl acetate and CRO extracts exhibited low tyrosinase-stimulating activity suggesting that hydrophobic compounds are less likely to stimulate tyrosinase. Previously, ethanolic riceberry rice extract (50 mg/ml) had the highest tyrosinase stimulating activity at  $91.8 \pm 0.10\%$  which was approximately two-fold higher than our ethanolic RBR extract. This may possibly due to higher concentration of extract (50 mg/ml) used in their work.<sup>[28]</sup> Tyrosinase-stimulating activities of the extracts [Figure 4] were correlated with antioxidant activities of the extracts [Figure 5]. For example, RBR ethanolic extract and RBR water extract with the two highest antioxidant activities exhibited the two highest tyrosinase-stimulating activities. Similarly, tyrosinase-stimulating activities of the extracts were correlated with the TPC and TFC [Figure 6], except for RBR water extract. For example, RBR ethanolic extract with the highest TPC and TFC combined exhibited the highest tyrosinase-stimulating activity while RBR ethylacetate extract with the lowest TPC and TFC combined exhibited the lowest tyrosinase-stimulating activity. The previous reports support the link between antioxidants and melanogenesis. The occurrence of active oxygen species, including lipid peroxide, free radicals and hydrogen peroxide was found to inhibit melanogenesis in melanoma cells.<sup>[32,33]</sup> However, antioxidants were able to induce melanin deposition through its eliminating and reducing action on active oxygen species in melanin-producing cells.<sup>[32,33]</sup>

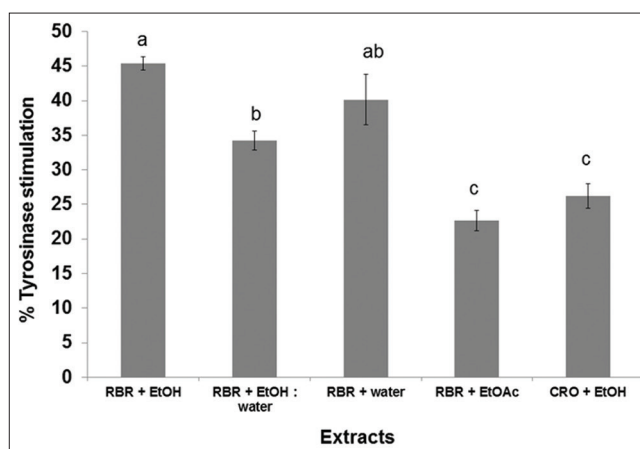
In addition, previous findings also support the link between phenolic/flavonoid compounds and melanogenesis stimulation. The active component of Chinese herbal extracts (*Fallopia multiflora*) mainly containing 2, 3, 5, 4'-Tetrahydroxystilbene 2-O- $\beta$ -D-glucoside, gallic acid (a phenolic compound), and physcion (a phenolic compound) were found to show significant increased activity for mushroom tyrosinase (8.1~34.7% of that in control) and melanogenesis in mouse melanocytes (18~53% of that in control).<sup>[15]</sup> Quercetin (3, 3', 4', 5, 7-pentahydroxyflavone), one of the representative flavonoids present in many vegetables and fruits, was found to stimulate melanin synthesis in dose-dependent manner in hair follicle tissues from the buccal region of C3H/HeN Jel mice than control tissue. Additionally, the expression of tyrosinase protein and TRP-2 were significantly enhanced, thereby enhancing melanin producibility in hair follicle tissues.<sup>[34]</sup> Our results indicated that high antioxidant activities, high TPC and high TFC enhanced tyrosinase stimulating activity, and thus may lead to stimulated melanogenesis preventing grey hair from occurring.

## CONCLUSIONS

This is the first report to show that RBR and CRO extracts from organic rice process in Thailand can be used in the development of value-added hair care products. These extracts contained gamma-oryzanol, fatty acid content, phenolics, flavonoids with antioxidant activity and tyrosinase-stimulating activity. These can be applied as potentially active ingredients



**Figure 5:** Total phenolic content and total flavonoid content of the extract. Values were means  $\pm$  SD of triplicates. RBR - riceberry broken rice, EtOH - ethanol, EtOAc - ethyl acetate, CRO - crude riceberry rice oil. Lower case letters indicate statistically significant difference among bars in the same type of content ( $P < 0.05$ )



**Figure 6:** Tyrosinase-stimulating activities of the extracts. Values were means  $\pm$  SD of triplicates. RBR - riceberry broken rice, EtOH - ethanol, EtOAc - ethyl acetate, CRO - crude riceberry rice oil. Lower case letters indicate statistically significant difference among bars ( $P < 0.05$ )

in cosmetics to promote hair growth or/and hair pigmentation. Hopefully, Thai farmers can make use of these RBR and CRO extracts to increase their incomes by producing more value-added products out of them.

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