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Antioxidant and phytochemical content of commercial brown rice (Ecobrown) and white rice (Jasmine, Jati Super Special and Manggo Thai) for potential cosmetic rice powder raw materials

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Abstract

Cosmetic rice powder or "*bedak sejuk*" has long being used as part of traditional skincare routine. The powdered rice extract is applied on the face, usually at night and will be rinsed off the next morning to obtain smooth and clear skin complexion. Usually, the regular rice available in one's kitchen is used to make the cosmetic rice powder but different grades of rice (based on the price) will give different effects to the skin. This study aims to investigate and compare the bioactivities and phytochemical composition of different grades of rice available in the market. The rice sample used in this study was Ecobrown rice, Jasmine White rice, Jati Super Special Tempatan rice and Manggo Thai rice. All rice samples were extracted using distilled water by maceration process. The bioactivities; antioxidant capacity was measured using DPPH assay. Phytochemical content was determined as total phenolic and flavonoid contents (TPC and TFC). High Performance Liquid Chromatography (HPLC) was also used to determine phenolic content expressed as gallic acid equivalent (GAE). The result showed that brown rice had significantly higher antioxidants with IC50 values of 6.4 mg/mL in DPPH assay. The observed activities were corresponding with total phenolic content, where EcoBrown rice has a significantly higher amount of phenolic content ($p \leq 0.05$) compared to other samples. Meanwhile, almost similar TFC was observed in all rice samples with Jati Super Special. The analysis of phenolic content was correlated with HPLC analysis, where brown rice was shown to have the highest amount of gallic acid content. In conclusion, brown rice can be considered as a better choice to produce "*bedak sejuk*".

1. INTRODUCTION

Rice belongs to the genus Oryza and it is a semiaquatic annual grass. Rice (*Oryza sativa*) is cultivated around the world and is the staple food for almost 40% of the world population (Vijay & Roy, 2018). Other than that, rice extract has long being used in the traditional skincare routine in many cultures to obtain smooth skin complexion (Marto *et al.*, 2018). In the Malay traditional cosmetic practice, the rice extract powder is being used as a mask, usually applied at night to prevent skin breakouts and to obtain fairer skin and even skin tone (Dzulfakar, Tan, & Tasirin, 2015). Although there are varieties of skincare and cosmetics products available in the market nowadays, the

use of "*bedak sejuk*" is still relevant and getting high demand from consumers who prefer natural cosmetics products that are free from any harsh chemicals. Moreover, rice extracts have been shown to be non-irritant (Marto *et al.*, 2018).

Rice bran contains a rich amount of antioxidant components such as phenolic acids and flavonoids that can be beneficial to the skin, particularly for anti-aging defence (Kanlayavattanakul, Lourith, & Chaikul, 2016). Meanwhile, black or red rice cultivars are known to contain a significantly high amount of procyanidin or anthocyanin content (Tamprasit *et al.*, 2019; Vichit, Saewan, & Prinyarux, 2018). Procyanidins possess antioxidants and matrix metalloproteinase inhibitory activities which play a

significant role in skin anti-aging defence (Santos-Buelga & Scalbert, 2000). Meanwhile, flavonoids such as tricin, apigenin, isorhamnetin, myricetin and luteolin in rice have been shown to possess antioxidant, anti-inflammatory, anti-microbial and as well as anti-cancer (Singh *et al.*, 2017). The nutritional benefits (phytochemical content) of rice is the main factor to determine rice quality, other than physical appearance and aroma (Custodio *et al.*, 2019).

Similarly, the quality of cosmetic rice powder is enhanced with better choice of raw materials (rice). Technological advances to produce quality bioactive substances such as rice stem cells allow innovative development of rice-based cosmetic products. For example, incorporation of red rice stem cell extract in creams has shown significant improvement in skin whitening, skin moisture and skin elasticity compared to placebo group (Vichit, Saewan, & Prinyarux, 2018). This technology allows production of quality bioactive without any issues of seasonal harvesting, growing rate, microorganism contamination and pollution (Schürch, Blum, & Zülli, 2008). Thus, more options of raw materials can be used to develop cosmetic rice powder. In this present study, two types of rice (brown and white rice) available in the market were used to investigate and compare the antioxidant and phytochemical content of each of the rice samples. Though studies on brown and white rice are extended, very few are focusing on the commercial rice source present in the market. This study will benefit the cosmetic industry and consumer to better choose raw materials that can be used for cosmetic rice powder.

2. MATERIALS AND METHODS
2.1. Sample preparation
Rice sample was purchased from a local supermarket in Jeli, Kelantan. The rice sample (250 g) was ground into powder using a miller and stored in a closed container at room temperature until further used.

2.2. Sample Extraction
Rice samples were extracted according to Batubara *et al.*, (2017) with a slight modification. The powdered rice sample was weighed (5 g) and macerated with 25 ml of distilled water (1:5 w/v) in a closed container. The container was shaken rigorously for about 2-5 minutes and the mixture was let to sit for 24 hours. Lastly, the mixture was filtered using a muslin cloth and the filtrate was collected. The filtrate was placed in an oven at 40 °C until dried extract powder was obtained.

2.3. Antioxidant activity
2.3.1. DPPH assay
Previous method was followed with modifications (Suwannalert & Rattanachitthawat, 2011). The DPPH

working solution (0.04 mg/ml) was prepared with 95 % ethanol with an absorbance of 0.95 ± 0.01 unit at 540 nm. The rice extract (120 μl) was mixed with the DPPH working solution (1080 μl) and the absorbance of the mixture was immediately measured spectrophotometrically at a wavelength of 540 nm. Ascorbic acid was used as standard. Total antioxidant activity of the rice extract was expressed as mg ascorbic acid/g sample, obtained from the calibration curve of standard ascorbic acid at concentrations ranging from 0, 25, 50, 75, 100 mg/ml. DPPH radical scavenging activity (%) calculated using following formula:

$$\text{Scavenging activity (\%)} = [(\text{Absorbance}_{control} - \text{Absorbance}_{sample}) / \text{Absorbance}_{control}] \times 100 \quad (1)$$

2.4. Phytochemical Content
2.4.1. Total Phenolic Content (TPC)
Previous method was followed with a slight modification (Moko *et al.*, 2014). The total phenolic content of rice was determined using Follin-Ciocalteu reagent. 100 μl of 0, 5, 10, 15, 20 mg/ml rice extract was mixed with 1.5 ml 10% sodium carbonate solution and then 3 ml of 10% of Follin-Ciocalteu reagent was added. The final mixture was kept in the dark at ambient condition for 2 hours to complete the reaction. The absorbance was measured spectrophotometrically at 765 nm. All measurements were determined in triplicate and the data were expressed as mg Gallic Acid Equivalent (GAE) per g of rice extract. The concentrations of gallic acid that was used as standard were 0,5,10,15, and 20 mg/ml.

2.4.2. Total Flavonoid Content (TFC)
Total flavonoid content was determined based on the previous method with a slight modification (Pengkumsri, 2015). 150 μL of 5% sodium nitrite was mixed with 2 mL of distilled water and 500 μL of 0, 5, 10, 15, 20 mg/ml of rice extracts or quercetin (positive control) at different concentrations. The mixture was incubated at room temperature for 5 min and later added with 150 μL of 10% aluminium chloride hexahydrate solution. The mixture was further incubated for 6 min at RT. 1 mL of 1 M sodium hydroxide was added and the total volume was made up to 5 mL using deionized water. The mixture was incubated at room temperature for 10 min after appropriate mixing. After incubation, absorbance was measured at 510 nm and the total flavonoid content was denoted as mg quercetin equivalent (mg QE) per g of extract.

2.5. High Performance Liquid Chromatography (HPLC) Analysis
HPLC analysis was performed according to Tian, Nakamura & Kayahara (2004) modifications. The HPLC system used consist of an SCL-10Avp system controller,

two LC-6AD solvent delivery units, a CTO10Avp column oven, an SPD-M10Avp UV–vis photodiode array detector, and a Multi-PDA Class-VP workstation (Shimadzu, Kyoto, Japan). Separations were conducted with a Waters Cosmosil® 5C18-MS-II reversed-phase column (150 mm × 4.6 mm i.d., Milford, MA, USA). The gradient elution was performed with a mobile phase 50:50. Working solutions were prepared by diluting the rice extract to get the concentration of 100 mg/mL. A washing period of 8 min with 80% solvent B and reequilibration period of 15 min with 5% solvent B were used between individual runs. Chromatography was performed at 38 ºC with a flow rate of 0.8 ml/min and injection volume of 5 μl. The standard used in this analysis was gallic acid with concentration range of 3 - 9 mg/mL in methanol. The concentration of gallic acid in the sample was based on the peak area under the curve.

2.6. Statistical Analysis

Data were analysed using Analysis of Variance (ANOVA) and $p \leq 0.05$ was considered as significant.

3. RESULT AND DISCUSSION

3.1 Antioxidant activity

Free radicals are ubiquitous in all living things. The DPPH assay measured the ability of the rice extract to neutralize DPPH radical, which was observable from the colour changes of the DPPH solution from purple to clear. The IC50 value of 6.4 mg/ml showed that brown rice (EcoBrown) was the most potent antioxidant as compared to the other rice extract samples. Higher potency indicates that a compound or an extract is able to scavenge 50% of the free radicals at lower concentration, thus requiring a small amount (Moko *et al.*, 2014). Ecobrown rice has significantly higher AAE with 374.4 ± 40.6 mg/g sample compared to the other rice extract samples as shown in Table 1. A study on phenolics and antioxidant capacity of brown rice in China showed that brown rice contained higher amounts of phenolics, specifically ferulic and *p*-coumaric acids in free and bound forms that were responsible for the measured free radical scavenging activities (Ye *et al.*, 2016). This data supports this present study where the same trend of antioxidant and phenolic content was observed. Antioxidants such as phenolics and flavonoids have been used as active ingredients in many cosmeceuticals products to either prevent skin aging or to enhance skin appearance (Ganceviciene *et al.*, 2012). These compounds have the ability to scavenge free radicals that can harm the skin. In this study, Ecobrown had

significantly high AAE compared with other rice extract suggesting brown rice extract contains higher amounts of antioxidants that can benefit the skin; thus, better could be a better option for "*bedak sejuk*" production.

Table 1: The IC50 values and ascorbic acid equivalent (AAE), mg AA/g sample determined using DPPH assay. * Different alphabets indicate significant differences at $p \leq 0.05$.

Sample	IC50 (mg/ml)	AAE (mg AA/g sample)
Brown rice (EcoBrown)	6.4	374.4 ± 40.6 [a]
White rice (Jasmine)	21.1	139.9 ± 27.9 [b]
White rice (Jati Super Special Tempatan)	39.8	81.7 ± 51.9 [b]
White rice (Manggo Thai)	36.8	83.6 ± 32.3 [b]

3.2 Total Phenolic Content and Total Flavonoid

Table 2 shows that brown rice extract has a significantly higher amount of TPC (p≤ 0.05). The results suggest that the antioxidant activity exhibited by brown rice could be attributed to the phenolic compound present. This present data is supported by a previous study by Goffman and Bergman (2004) that found the pericarp colour of light brown has a lower number of phenolic acids compared to other darker coloured rice. The high levels of phenolic compounds in germinated brown rice are due to the dismantling of the cell wall during germination resulting in high freeform phenolics (Tian, Nakamura, & Kayahara, 2004). Oki *et al.*, (2002) also found that the total phenolic in pigmented rice is higher than non-pigmented rice. Similarly, Vichapong *et al.*, (2010) also found that the total phenolic, total flavonoid and antioxidant activity in brown rice and pigmented form of rice were higher than non-pigmented and polished-rice. Ravichanthiran *et al.*, (2018) states that brown rice contains many types of phenolic acids that are well known to have antioxidant activities. However, an almost similar amount of TFC was observed in all rice extract samples except for Jati Super Special (white rice) that has higher TFC. This result agrees with previous study by Shen *et al.*, (2009) that showed several white rice also contained high amounts of flavonoids and phenolics content rather than pigmented or red rice. Ecobrown was predicted to contain higher flavonoids content than white rice because flavonoids are responsible for the coloration in coloured rice. However, almost similar flavonoids content measured may be due to degradation of the flavonoids during milling processing causing the TFC to be similar to those of white rice.

Table 2: TPC and TFC content of rice extract samples expressed as Gallic Acid Equivalent (GAE) and Quercetin Equivalent (QE) respectively. Different alphabets indicate significant differences at $p \leq 0.05$.

Sample	TPC (GAE mg/ g sample)	TFC (QE mg/ g sample)
Brown rice (EcoBrown)	526.4 ± 122.1[a]	50.3 ± 7.2[a]
White rice (Jasmine)	153.9 ± 16.7[b]	39.8 ± 4.7[a]
White rice (Jati Super Special Tempatan)	138.1 ± 44.3[b]	74.3 ± 13.7[a]
White rice (Manggo Thai)	101.6 ± 15.1[b]	30.8 ± 6.0[a]

3.3 High Performance Liquid Chromatography (HPLC)

Gallic acid in rice samples were assayed by chromatographic analysis using HPLC-UV. Standard gallic acid at 3,6 and 9 mg/ml showed retention time ranging from 8.327 min – 8.352 min, as shown in Figure 1 A, B and C respectively. The plotted regression line of gallic acid (area *vs.* concentration) showed a good linearity where R = 0.9679 as shown in Figure 1D. This suggests that the concentration of gallic acid is correlated with the area, and therefore was used to estimate the gallic acid content in the rice extract sample.

Table 3 shows the retention time (rt) and area under the peak for each of the rice extract samples. As previously established that the area correlated with the concentration of gallic acid present in a sample, the HPLC analysis indicates that brown rice has the highest amount of phenolic content. This result is in agreement with previous phenolic analysis by FC method (section 3.2). Goffman and Bergman, (2004) also observed that rice genotypes with light colour have lower value of gallic acid compared with the dark colour rice. Some of the most dominant phenolic acids in rice other than gallic are ferulic, protocatechuic and syringic acids (Ding et al., 2019). These compounds have been shown to benefit the skin as potent antioxidants (Panzella, 2020); thus, Ecobrown with the highest gallic acid content is suitable for active ingredients in cosmeceuticals such as "*bedak sejuk*".

Table 3: Table shows the retention time, area and GAE for each rice extract sample.

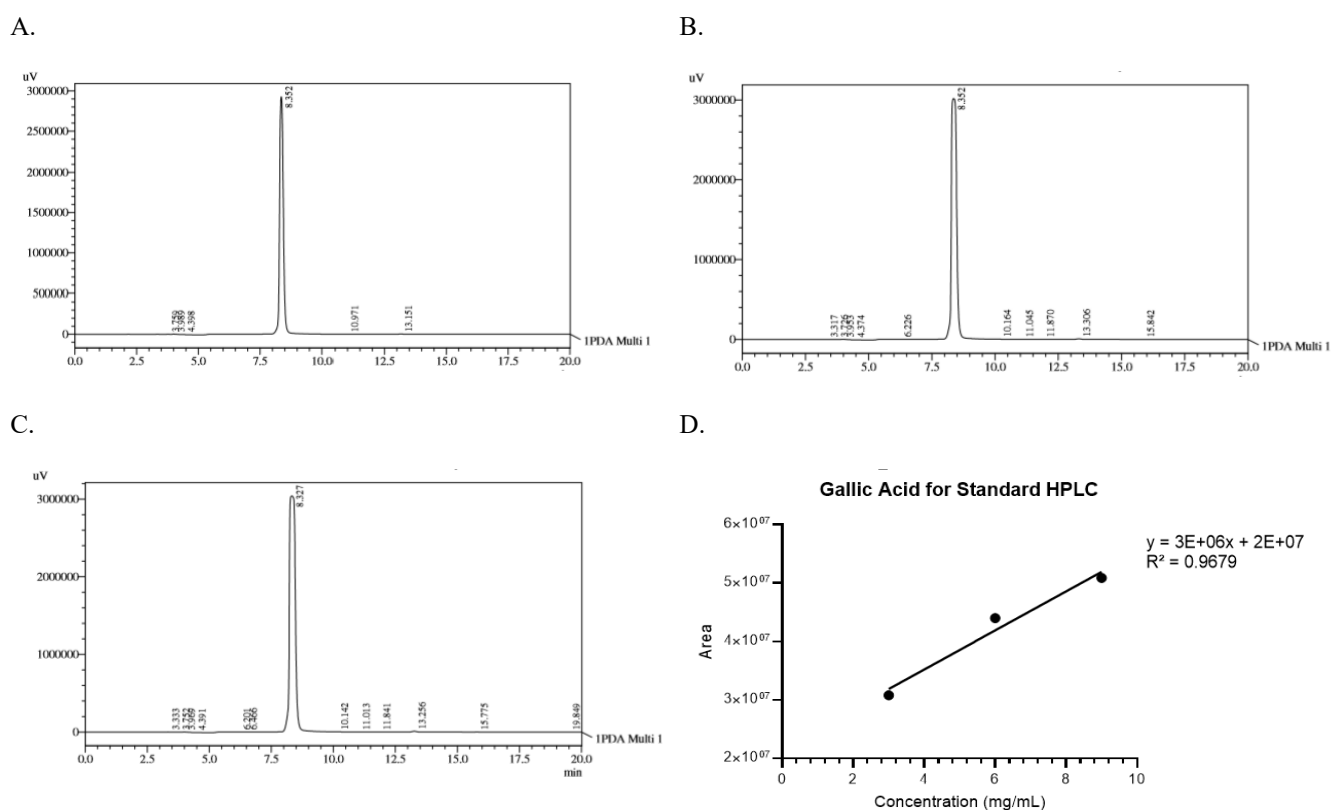| Sample	Retention time	Area
Brown rice (EcoBrown)	8.357	757600
White rice (Jasmine)	8.459	570554
White rice (Jati Super Special Tempatan)	8.460	400074
White rice (Manggo Thai)	8.392	402627

A.

B.

C.

D.

Figure 1: Figure A, B and C show the HPLC chromatogram peaks for standard gallic acid at 3, 6 and 9 mg/mL; while D is the regression line for area *vs.* concentration for the standard.

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4.   CONCLUSION

As a conclusion, brown rice (higher grade/price) has higher antioxidant activity as compared to other rice samples. The antioxidant activity might be attributed to the phenolic content in the rice sample where the brown rice was found to have higher amounts of phenolic. With that, it can be concluded that brown rice can be a better option to be used as a raw material to produce cosmetic rice powder that can give more benefits to the skin.

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