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Antioxidant Activity of ‘Broken Skin’ Purple Rice, ‘Skinned’ Purple Rice, and Purple Rice Stem Organically Cultivated in Indonesia

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***Abstract*—This research has been carried out to determine the activity of purple rice stem, ‘broken skin’ purple rice and ‘skinned’ purple rice. Preparation of materials includes the collection of purple rice from ‘Tuah Sakato’ farmer group that cultivates the rice using organic methods. The extraction method used is maceration extraction with n-hexane methanol. The rotary evaporator extracts each solvent variation from the solvent. Concentrated extracts from each solvent were tested for their antioxidant activity using the DPPH method (2,2-diphenyl-1-picrylhydrazyl) at five concentrations within the range of 800 – 6000 ppm, based on the sample varieties. The comparison used is ascorbic acid (vitamin C). Determination of the value of antioxidant activity had used the IC_{50}. The results showed that the highest antioxidant activity was possessed by purple rice stems, as evidenced by the IC_{50} value of 1.474 ppm, followed by ‘broken skin’ purple rice with IC_{50} of 4.130 ppm, and the lowest was ‘skinned’ purple rice with IC_{50} of 98.95 ppm. Purple rice stems have the highest antioxidant activity since the antioxidant compound still intact without any further process. Then, ‘broken skin’ purple rice has higher antioxidant activity than ‘skinned’ purple rice because the former still has epidermis that contains high antioxidants. High enough antioxidant activity makes this purple rice an alternative food source that can ward off free radicals causing degenerative diseases due to unhealthy modern lifestyles.**

***Keywords*— antioxidants; ‘broken skin’ purple rice; ‘skinned’ purple rice; straw.**

I. INTRODUCTION

Purple rice (Oryza sativa L.) is grown in Northern Thailand and has been widely recognized as a potential cereal grain containing high amounts of bioactive compounds, which is usually located in the bran layer [1]. Two main anthocyanins found in the purple rice layer and the *aleuron* layer are cyanidin 3-o-glucoside and peonidin-3-o-glucoside. Phenolic acid is also found in the outer layers of purple rice [2]. Bioactive compounds of purple rice show high antioxidant activity due to the presence of antioxidant compounds [3]. The process of extraction and identification of the antioxidant activity and phenolic compounds from numerous pigmented rice cultivars can be seen from previous reports [4], [5].

Previous studies have identified the chemical composition and biological activity of purple rice. Several studies have observed the enriched purple-black pigment, anthocyanin, in purple rice for potential use in the nutraceutical or functional food formulations within the treatment of antioxidant and anti-inflammatory properties [6], [7]. Other beneficial components have been identified in purple rice, including polyphenols, flavonoids, vitamin E, phytic acid, and c-oryzanol [8], [9]. Several studies have examined other properties such as physical, texture, or sensory of purple rice, or compared these properties with white rice [10]–[12].

Purple rice with pigmented granules has long been a unique and traditional food for dessert and medical purposes in many cultures [13]. Currently, these benefits have been widely recognized by the cosmetic and medicine industries; This leads to increased demand from the Asian, US, and Europe [14]–[16]. Dietary antioxidants have the property of protection from free radicals of the aging process and disease progression [17].

Rice (Oryza sativa L.) is a grain type of crop which is selected as a staple food or a source of carbohydrates in developing countries [18]. Generally, there are various varieties of rice, such as white rice, red rice, black rice, and purple rice. Brown rice has low gluten, so brown rice can be used as a substitute for white rice for those on a sugar diet. Red rice has more functional and nutritional values compared to white rice because of the presence of antioxidant components. Then, another benefit of ‘broken skin’ rice is the potential to reduce blood glucose in diabetics. Information about the studies of purple rice in Indonesia is very little [19], [20].

The main content in rice is carbohydrates with little fiber so that it affects the glycemic index of rice. The glycemic index is related to the response of an increase in blood glucose after consuming these foods. Brown rice has a lower Glycemic Index (IG), which is around 50-55 (moderate IG) compared to white rice, which is around 56-78 (high IG). The index of high glycemic shows the improvement of type 2 DM risk on the Japanese men [21].

Health risks can be minimized by adopting a healthy lifestyle, such as eating foods rich in bioactive compounds. Bioactive compounds in food can act in a variety of biological activities, for example, as antioxidants in the body. Today, the role of food is not only to meet the nutritional needs but also to be functional food. Rice is a type of food that is very close to the Indonesian people. Rice has bioactive compounds in antioxidants forms, such as *phenolic acids, flavonoids, tocopherols, tocotrienols, anthocyanins, proanthocyanidins*, γ-oryzanol, and phytic acids [22]. The pigment produced by rice is influenced by the bioactive compounds. Black rice has the potential as a functional food in Indonesia [23].

Bioactive compounds that cause pigment in rice are *anthocyanin* and *proanthocyanidin*, which are potentially used as sources of antioxidants other than as a source of starch in ruminants [24]. Pigmented rice has high antioxidant activity potential due to the high content of bioactive compounds [25]. Bioactive compounds in pigmented rice can reduce oxidative stress, prevent cancer, cardiovascular, diabetes complications, and others [26].

The most phenolic compounds are found in the epidermis layer, so that rubbing (skinned) red rice will reduce the phenolic compound content. Therefore, red rice is generally consumed without going through the process of rubbing, which is in the form of 'broken skin' rice so that the peel is still attached to the endosperm. Red rice peel is rich in anthocyanin, fiber, fatty acids, vitamins, minerals, and phytic acid [8]. Phytic acid (myoinositol Hexa-phosphoric acid, IP6) has become the significant phosphorus storage compound in the seeds and grains of cereal; it is more than 70% of total phosphorus. Phytic acid also can bind several multivalent metal ions, especially the elements of zinc, calcium, and iron. Binding can produce very insoluble salts, causing low bioavailability (uptake) of minerals [27]. Therefore, phytic acid is associated as an anti-nutritional agent.

Pigmented rice may have beneficial effects on humans and animal health [28], [29]. Thus, purple rice has received attention from food producers in Indonesia in forms such as malt, flour, bread, ice cream, and wine [30]. However, its antioxidant concentrations and bioactivity vary with genotypes despite having the same skin color [31]. Purple rice that differs in antioxidant properties also differs in their chemical content, such as anthocyanin [22]. Anthocyanins will decrease by about 80% after cooking with an electric rice cooker while phenolic compounds fall by 54% [32]. Compared to cooking methods, cooking risotto retains more anthocyanins and other phenolic compounds than as a boiling point. Most of the water is absorbed by specific types of seeds with high porosity in the previous method [33]. Some studies show that measuring the texture attributes of rice cooked by sensory and instrumental methods is important because of the increasing popularity of rice and its products [12].

The anthocyanin stability is not only influenced by the heating temperature in the processing process, but is also influenced by intrinsic and extrinsic factors in the product, such as pH, storage temperature, chemical structure, and anthocyanin concentration contained, the presence of light, oxygen, enzymes, proteins, and metal ions [34]. To find out the stability of anthocyanin, initial data on anthocyanin levels from the initial ingredients containing these substances are needed. Therefore, this study is a preliminary study that aims to obtain the largest total of anthocyanin data with extraction methods that have several condition variables. These variables are the solvent variable, the addition of hydrochloric acid, and the condition of rice. Due to the growing popularity of purple rice and limited research to physical and sensory properties, the purpose of this study is directed to study the antioxidant activity of purple rice stems, 'broken skin' purple rice, and 'skinned' purple rice.

II. Materials and Method

The sample used in this study is purple rice obtained from the 'Tuah Sakato 1' farmer group that was cultivated with a gas sludge with full organic conditions. The varieties of purple rice are purple rice stems, 'broken skin' purple rice, and 'skinned' purple rice. The research was carried out from October to December 2018; it was in the Central Laboratory of LLDikti-X on the Khatib Sulaiman Padang. 'Broken skin' and 'skinned' purple rice were mashed using a blender. While purple rice stems were chopped by using a machete, then it is mashed by using a blender. Purple rice antioxidant testing was begun with extraction activities. Extraction was performed by the maceration method using methanol as a solvent in the form of (whole) and smooth, and at a temperature of 25^{o}C. Extraction is done by the maceration method at 25^{o}C. Then proceed with thickening the extract using a rotary evaporator.

A. Instrument and Material

The instruments in this research are UV-Vis spectrophotometer (Shimadzu), analytical scales (Mettler toledo), and rotary evaporator (Butchi). The materials used in this research are purple rice stems (straw), 'broken skin' purple rice and 'skinned' purple rice, ethanol (Merck), methanol (Merck), hydrochloric acid (Merck), potassium chloride (Merck), distilled water, potassium acetate (Merck).

B. Sample Acquisition

Preparation of materials includes the collection of purple rice from 'Tuah Sakato' farmer group that cultivates the rice using organic methods. The proof is complemented by organic certificate ownership.

C. Sample Preparation

The sample used in the form of 50 grams of purple rice is not mixed with flour and rice straw in fine form using a blender to obtain a fine powder. Then maceration was carried out at 25^{o}C using 300 mL of 96% ethanol solution for 3x24 hours. The extract obtained was separated from the solvent using filtration, and the filtrate was taken and then concentrated using a rotary evaporator. The concentrated extract was inserted into an aluminum-coated vial and stored at 4^{o}C.

The concentrated extract was then tested for its antioxidant activity by the DPPH (2,2-diphenyl-1-

picrylhydrazyl) method at five concentrations within the range of 800 – 6000 ppm. The comparison used is ascorbic acid (vitamin C). Determination of the value of antioxidant activity had used the IC_{50}.

D. DPPH Method Test of Antioxidant Activity

1) Determination of Maximum Wavelength: Put 96% ethanol as much as 3 mL into the cuvette, then add a 1 mL of 0.2 M DPPH solution. Furthermore, a maximum λ at a wavelength range of 500-600 nm was sought and measurement results are recorded for later use.

2) Measurement of Antioxidant Activity in Samples: Absorbance control of DPPH solution with a concentration of 0.2 mM was taken as much as 1 mL, then put into a test tube, and 96% ethanol was added. The test tube was closed with aluminum foil and then incubated at 37°C for 30 minutes. After that, the solution was put into a cuvette, and absorbance was measured using UV-Vis Spectro at the maximum λ obtained in the previous stage.

The crude extract sample was dissolved in 96% ethanol with a concentration of 1000 ppm, 1200 ppm, 1400 ppm, 1600 ppm, and 1800 ppm. Prepare test tubes for each crude extract and filled with 3 mL of extract and added 0.2 mM DPPH as much as 1 mL (ratio of DPPH: extract solution dissolved to a certain concentration of 1: 3). After that, the solution was covered with aluminum foil and incubated at 37°C for 30 minutes, then put in a cuvette to the full and absorbed using UV-Vis spectrophotometry at the maximum λ that had been obtained previously. Comparative ascorbic acid (vitamin C) was treated as a sample. Absorbance data obtained from each extract concentration was calculated as a percent (%) of antioxidant activity. The percentage of antioxidant activity was calculated using the formula:

$$\%\ Antioxidant\ activity = \frac{control\ abs - treatment\ abs}{control\ abs} \times 100\% \qquad (1)$$

Caption:
Control abs = Absorbance of 0.2 mM DPPH solution
Treatment abs = Absorbance of purple rice ethanol extract of stems, 'broken skin' or 'skinned.' Or a comparative raw material of vitamin C

III. Result and Discussion

The method of extraction in this study is maceration. The maceration method was chosen because the compound contained in rice is anthocyanin, which is not resistant to heating, and this method can extract large amounts of samples. Besides, the maceration method is the easiest extraction method but requires a large amount of solvent.

Ethanol is a polar solvent that is often used to extract a compound or can be called a universal solvent. In this study, 96% ethanol solvent was used because the physical and chemical properties of anthocyanin were seen from the solubility of anthocyanin in polar solvents such as methanol, acetone, or chloroform more often with water and acidified with hydrochloric acid or formic acid. Anthocyanin is stable at pH 3-5 and temperature 50°C has a molecular weight of 207.08 g/mol and molecular formula $C_{15}H_{11}O$ [37]. Maceration is done at a temperature below 50°C to avoid anthocyanin damage in the sample.

A. Antioxidant Activity of Purple Rice Stems

The results show that the higher concentration of purple rice stems extract will cause a higher percentage of inhibition. Percentage of inhibition is an indicator that shows the ability of a compound as an antioxidant that plays a role in inhibiting free radical compounds, which in this method is DPPH [35]. The higher the percentage of inhibition, the better the ability of these compounds to inhibit free radicals. In Figure 1, the highest percentage of inhibition was obtained in the concentration of purple rice stems with a concentration of 1800 ppm, which reached 81.56%. The linear regression equation obtained is y = 0.0774x - 63.436 with a coefficient of determination (R2) reaching 0.966 which shows the effect of the concentration of five standards of purple rice stems on the percentage of inhibition is quite good.

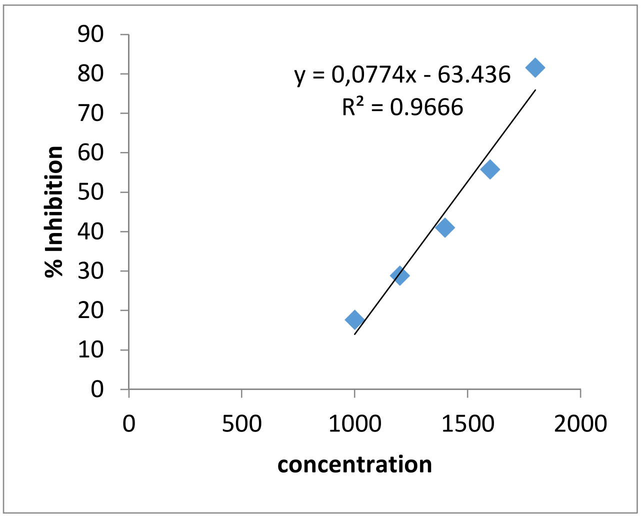

Fig. 1 Linear regression of % inhibition vs concentration for purple rice stems

By using the linear regression equation, the IC_{50} value can be calculated by inputting a percentage value of 50% inhibition. So that the IC_{50} value obtained for purple rice stems is 1.473 ppm. This means it takes a purple rice stems to extract with a concentration of 1.473 ppm to counteract 50% of DPPH free radicals present in the sample solution.

B. Antioxidant Activity of 'Broken Skin' Purple Rice

Figure 2 shows that the concentration of 'broken skin' purple rice extract was proportional to the percentage of inhibition. As mentioned before, the percentage of inhibition is a parameter that shows the ability of a compound as an antioxidant that plays a role in inhibiting free radical compounds, which in this method is DPPH. The higher the percentage of inhibition, the better the ability of these compounds to inhibit free radicals. The results that can be observed Figure 2 are the highest percentages of inhibition obtained in the concentration of 'broken skin' purple rice with a concentration of 6000 ppm, which reached 67.64%. The linear regression equation obtained is y = 0.0084x + 16.966 and the coefficient of determination (R2) reaches 0.997 which indicates the magnitude of the influence of the concentration of standard of 'broken skin' purple rice to the percentage of inhibition.

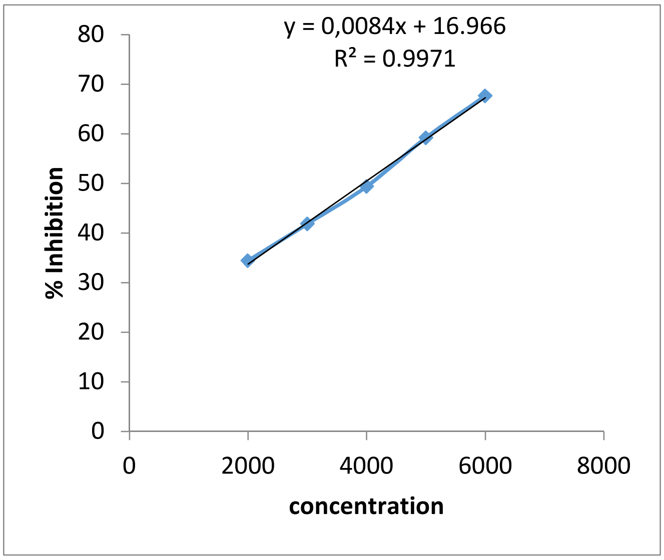

Fig. 2 Linear regression of % inhibition vs concentration for 'broken skin' purple rice

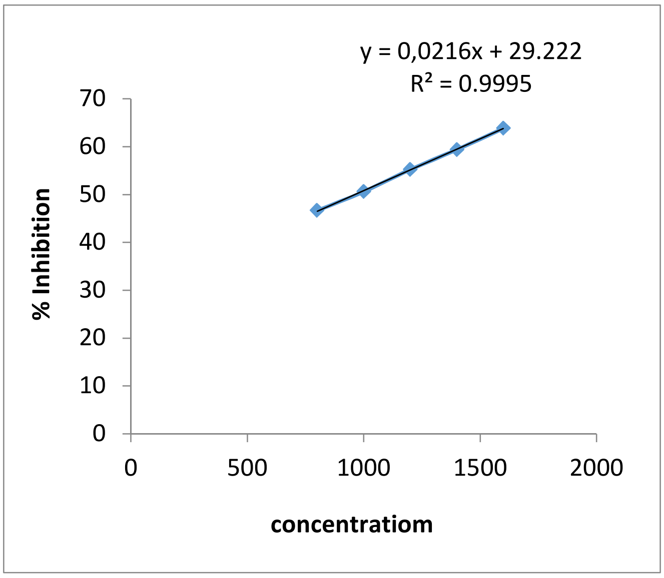

Fig. 3 Linear regression of % inhibition vs concentration for "skinned" purple rice

By using the linear regression equation, the IC_{50} value can be calculated by entering a percentage value of 50% inhibition so that the IC_{50} value for skinned purple rice was 4.130 ppm. This means it takes a purple rice extract of 'broken skin' with a concentration of 4.130 ppm to counteract 50% of the DPPH free radicals present in the sample solution.

C. Antioxidant activity of 'skinned' purple rice

From Figure 3, it can be observed that the concentration of 'skinned' purple rice extract is proportional to the percentage of inhibition or antioxidant activity. In Figure 3, it can be observed that the highest percentage of inhibition was obtained in the concentration of 'skinned' purple rice with a concentration of 1600 ppm, which reached 63.85%. The linear regression equation obtained is $y = 0.0216x + 29.222$, and the coefficient of determination (R2) reaches 0.999, which indicates the magnitude of the influence of 'skinned' purple standard concentrations to the antioxidant activity.

By using the linear regression equation, the IC_{50} value can be determined by entering a percentage value of 50% inhibition. As a result, the IC_{50} value for the 'skinned' purple rice is 98.95 ppm. This means it takes 'skinned' purple rice extract with a concentration of 98.95 ppm to counteract 50% of the DPPH free radicals present in the sample solution. Antioxidant testing was also carried out on vitamin C as a positive control and comparison. Vitamin C validate the method by comparing the results with previous studies. Vitamin C was used as a comparison because vitamin C is a commonly consumed antioxidant [36]. The relationship between vitamin C concentration and percentage of inhibition can be observed in Figure 4.

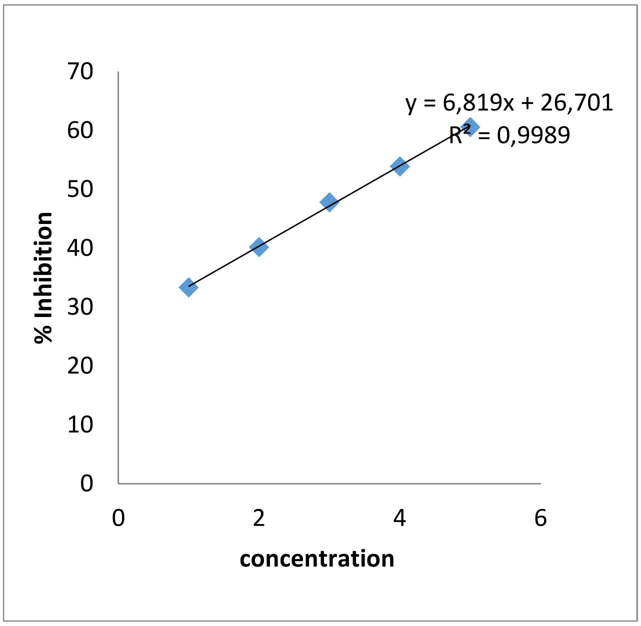

Fig. 4 Linear regression of % inhibition vs concentration for vitamin C

By using the linear regression equation, the IC_{50} value can be calculated by entering a percentage value of 50% inhibition. So, the IC_{50} values for vitamin C of 0.737 ppm. This means it takes vitamin C with a concentration of 0.737 ppm to ward off 50% of DPPH free radicals present in the sample solution.

In the DPPH method, the effectiveness of a sample in counteracting free radicals is called IC_{50}. IC_{50} is the concentration of the samples that can reduce 50% DPPH free radicals. The smaller the IC_{50} value, the greater the percentage of inhibition or antioxidant activity of the sample. IC_{50} values of the extract of purple rice stems, 'broken skin' purple rice, 'skinned' purple rice and vitamin C can be observed in Table I.

TABLE I
IC_{50} VALUES OF PURPLE RICE STEMS, 'BROKEN SKIN' PURPLE RICE, 'SKINNED' PURPLE RICE, AND VITAMIN C

Sample	**IC_{50} (ppm)**
Extract of purple rice stems	1.473
Extract of 'broken skin' purple rice	4.130
Extract of 'skinned' purple rice	98.95
Vitamin C (ascorbic acid)	0.737

The compound has different measurements. A very strong activity of antioxidant when the IC_{50} value is less than 50 ppm. A strong activity of antioxidant when the IC_{50} value is around 50-100 ppm. A moderate activity of antioxidants when the IC_{50} value is around 101-150 ppm. And a weak activity of antioxidant if the IC_{50} value is 151-200 ppm [37]. Based on table I, it can be analyzed that purple rice stems extract has the highest antioxidant activity and is classified as very strong when compared to the other two samples. Purple rice stems have the highest antioxidant activity because the extract of this sample has not gone through further processing so that its antioxidant content is still maintained.

In general, the antioxidant compounds in rice were classified into six groups: *phenolic acids*, *flavonoids*, *anthocyanins* and *proanthocyanidins*, *tocopherols*, and *tocotrienols (vitamin E)*, γ-*oryzanol*, and *phytic acid*. The first three groups are referred to collectively as phenolic compounds [22]. However, the antioxidants in this purple rice have not been determined yet. Thus, further studies about this variety of rice is necessary.

IV. Conclusion

Based on the explanation above, it can be concluded that the highest antioxidant activity is purple rice stems, as evidenced by IC_{50} value of 1.474 ppm, followed by 'broken skin' purple rice with IC_{50} of 4.130 ppm, and the lowest is 'skinned' purple rice with IC_{50} of 98.95 ppm. The lower antioxidant activity of 'skinned' purple rice is due to the epidermis, which contains high antioxidant, has been released from the rice. High enough antioxidant activity makes this purple rice an alternative food source that can ward off free radicals causing degenerative diseases due to unhealthy modern lifestyles. However, further analysis needs to be performed on these processed products based on purple rice.

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