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DETERMINATION OF ANTOSIANIN TOTAL LEVELS AND ANTIOXIDANT ACTIVITIES IN BLACK GLUTINOUS RICE EXTRACT AND FERMENTED BLACK GLUTINOUS RICE EXTRACT

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ABSTRACT— Black glutinous rice and Fermented Black Glutinous Rice contain compounds that act as antioxidants, namely anthocyanin compounds. The purpose of this study was to determine the total anthocyanin concentration and antioxidant activity contained in black glutinous rice and Fermented Black Glutinous Rice. Samples of black glutinous rice and Fermented Black Glutinous Rice were extracted using methanol which was acidified with 1% HCL, then the samples were dried using freeze dry. The first study was conducted to identify anthocyanins by using color reagents, then determine the total anthocyanin based on the differential pH method and determine the antioxidant activity with the DPPH method to obtain IC50 values. Based on the research, the total anthocyanin obtained in black glutinous rice was 4,2582 mg/100 gram and antioxidant activity was 14.654,67 ppm, whereas the total anthocyanin in Fermented Black Glutinous Rice was 8,0989 mg/100 gram and antioxidant activity was 11.837,68 ppm. From these results, it can be concluded that Fermented Black Glutinous Rice has greater anthocyanin and antioxidant activity than black glutinous rice.

KEYWORDS: Black Gkutinous Rice, Fermented Black Glutinous Rice, extraction, anthocyanin antioxidants.

1. INTRODUCTION

Free radicals are atoms or molecules that are very unstable (having one or more electrons without a pair), to obtain stable conditions this compound reacts with molecules around it so that unpaired electrons can be paired (Younget al, 2014). Free radical compounds arise due to various complex chemical processes in the body, which are byproducts of the oxidation or burning of cells that take place during breathing, cell metabolism, excessive exercise, inflammation or when the body is exposed to environmental pollution such as motor vehicle fumes, cigarette smoke, pollutants, and solar radiation or UV exposure, low radiation, electromagnetic rays, and so on. These conditions cause these compounds to be very reactive and can damage tissue (Maulida and Zulkarnaen, 2010). Damaged tissue causes various kinds of degenerative diseases such as asterosclerosis, coronary heart disease (CHD), and diabetes mellitus, which we all know is a bad impact on health (Rohmatussolihat, 2009). Because of the influence of free radicals that are not good for the health of the body, the body needs an important component that can ward off free radical attacks. An important component that is able to save the cells of the human body from the danger of free radicals is antioxidants. Antioxidants are electron-giving compounds (donor electrons) or reductants that are able to

capture foreign compounds that are free radicals (compounds that have unpaired electrons) (Winarsi, 2007). These antioxidants work by donating hydrogen atoms to radicals has a hydroxyl group to form a harmless molecule that is water (Youngson, 2005). Antioxidants can prevent health problems by reducing free radicals that cause damage to cell components that result in the emergence of various degenerative chronic diseases such as cancer, atherosclerosis, and cataracts. Antioxidants can be enzymatic antioxidants such as superoxide dismutase or SOD, catalase, and glutathione peroxidase, and non-enzymatic antioxidants such as vitamins A, C, E, β-carotene, flavonoids, isoflavins, flavones, anthocyanins, catechins, and isokatekin. Anthocyanin is a good compound for health because it has antioxidant activity (Abdel-Aal et al., 2006). Anthocyanin is a natural pigment that is responsible for the presence of red, blue, purple to black in some types of fruits and vegetables. This compound is a derivative of polyhydroxy or polymethoxy from 2-phenylbenzopyrilum and most are in plants in the form of glycosides or bound to sugar molecules. The sugar components that are usually found are glucose, galactose, ramnosa, arabinosa, and xylose (Rezqy et al., 2017). One source of anthocyanin that has the potential to be developed in Indonesia is colored rice. Currently there are several types of rice that are rich in anthocyanin, such as black rice, red rice, black glutinous rice (Oryza sativa L. cv Kam Doi Saked), and others (Suhartatik et al, 2013). The use of anthocyanin in black glutinous rice as a natural coloring agent for food has not been developed. Black glutinous rice is rice which is very rare and has a blackish purple color, this is because aleuron and endospermia produce anthocyanin with high intensity so that the deep purple approaches black. Black Glutinous rice is usually used as a basic ingredient for making snacks or snacks, such as brownies, rengginang, black Glutinous rice porridge, and Fermented Black Glutinous Rice. Fermented Black Glutinous Rice e is a traditional food that is consumed by many Indonesian people that is produced from the fermentation process (Prakosa and Santosa, 2010). Glutinous rice both, white Glutinous and rice black Glutinous rice has a good texture because the amylopectin content is high, especially in the type of Fermented. Glutinous Rice from fluffier Glutinous rice.

Suhartatik et al (2013) in their study stated that the highest levels of anthocyanin are contained in Setail black rice, which is 11.23 mg / g sample with a pH of 1.0 extraction solution. Research conducted by Yustina (2007) states that the total anthocyanin on Fermented Black Glutinous Rice is 1,747 mg / 100 g and has an oxidant activity of 84.583%. In both studies anthocyanin levels were tested using the pH differential method. The difference with previous research lies in Black Glutinous rice and Fermented Black Glutinous Rice used. Anthocyanin analysis and antioxidant activity on difference with previous research lies in black Glutinous rice and and black Glutinous rice using UV-VIS spectrophotometer. The reason for using the UV-VIS spectrophotometry method is because the liquid and color preparations can be determined by ultraviolet spectrophotometry. In addition to analyzing the levels of anthocyanin is done by comparing the absorbance of samples at pH 1 and pH 4.5 where the absorbance value is obtained from measurements using a UV-VIS spectrophotometer. Anthocyanins are compounds that have chromophore groups, compounds that have chromophore groups can be measured using a UV-VIS spectrophotometer. UV-VIS spectrophotometry method also has many advantages that is easier, faster and specific for the analysis of test substances. Based on the above matters, a study was conducted on Determination of Total Anthocyanin Levels and Antioxidant Activity in Black Glutinous Rice Extract and Fermented Black Glutinous Rice Extract using the UV-Vis Spectrophotometry Method. With this research, it is expected to provide information and education to the public about anthocyanin levels found in black Glutinous rice and Fermented Black Glutinous Rice. General Purpose is knowing the total levels of anthocyanin and antioxidant activity in black Glutinous rice extract and Fermented Black Glutinous Riceextract. The aim of this research are to find out anthocyanin levels in black glutinous rice extract and Fermented Black Glutinous Rice extract using a differential pH

method and Fermented Black Glutinous Rice extract using the DPPH method expressed by IC50 values.

2. Subjects and Methods

This type of research used in this study is descriptive because this study aims to determine the total levels of anthocyanin and antioxidant activity in black Glutinous rice and Fermented Black Glutinous Rice using the UV-VIS Spectrophotometry method. Research Design is Non-Experimental. The population in this study is Black Glutinous rice and difference with previous research lies in Black Glutinous rice and sold in the Cililin area. The sample in this study was Fermented Black Glutinous Rice and black Glutinous rice obtained from one of the traders in the Cililin area. Research and data analysis were carried out at the Integrated Laboratory of Health Polytechnic Bandung and the Laboratory of Pharmacy Health Polytechnic in Bandung. Research and data analysis were conducted in March-May 2019. The type of data used in this study is primary data. The data was obtained from sample analysis in the laboratory.

3. Results

3.1 Research Results Identification of Anthocyanins by Color Reagents

Anthocyanin testing using color reagents was carried out using HCl 2N and NaOH 2 N. Color identification results are shown in Table 1 and Table 2

Table 1 Results of Color Identification in Black Glutinous Rice Extract

Reagents	**Before Heating**	**After heating**
HCl	Red	Red
NaOH	Red	The color red fade away

Table 2 Results of Color Identification in Fermented Black Glutinous Rice Extract

Reagents	**Before Warm up**	**Before Warm up**
HCl	Red	Red
NaOH	Red	The color red fade away

3.2 Research Results Total Anthocyanin Black Glutinous Rice Extract

Total anthocyanin testing was carried out on black glutinous rice extract using the pH difference method. Data on the measurement of absorbance values at wavelengths of 510 nm and 700 nm at pH 1 and pH 4.5 are shown in Table 3.

Table 3 Test Results for Total Anthocyanin Black Glutinous Rice Extract

Absorbance	**pH 1**	**pH 4.5**
510	1.071	0.076
700	0.931	0.038

3.3 Research Results of Quercetin Antioxidant Activity

Testing the antioxidant activity of quercetin is used as a positive control. The measurement results of absorbance values at 516 nm wavelength are shown in Table 4. In testing the antioxidant activity in quercetin, IC50 values 9.915 ppm were obtained.

Table 4 Results of Quercetin Antioxidant Activity Analysis

Concentration	Quercetin Absorbance	% inhibition	IC 50 Value (ppm)
0	0.774	0	
5	0.530	31.5245	
10	0.298	61.4987	9.915
15	0.135	82.5581	
20	0.071	90.8269	
25	0.062	91.9897	

Antioxidant activity is expressed by an Inhibition Concentration of 50% (IC50), which is a concentration that causes a 50% loss of DPPH activity (Molyneux, 2004).

3.4 Research Results of Antioxidant Activity of Black Glutinous Rice Extract

Testing the antioxidant activity of quercetin is used as a positive control. The measurement results of absorbance values at 516 nm wavelength are shown in Table 5. In testing the antioxidant activity in quercetin, IC50 values of 14,654.67 ppm were obtained.

Table 5 Results of Antioxidant Activity Analysis of Black Glutinous Rice Extract

Concentration	Quercetin Absorbance	% inhibition	IC 50 Value (ppm)
0	0.608	0.000	
2000	0.531	12.664	
4000	0.497	18.257	14,654.67
6000	0.431	29.112	
8000	0.390	35.855	
10000	0.373	38.651	

Antioxidant activity is expressed by an Inhibition Concentration of 50% (IC50), which is a concentration that causes a 50% loss of DPPH activity (Molyneux, 2004).

3.5 Research Results of Antioxidant Activity of Fermented Black Glutinous Rice Extract

Testing the antioxidant activity of quercetin is used as a positive control. The results of measurements of absorbance at a wavelength of 516 nm are shown in Table 6. In testing the antioxidant activity of Fermented Back Glutinous Rice extract ICIC values of 11,837.68 ppm were obtained.

Table 6 Results of Antioxidant Activity Analysis of Fermented Black Glutinous Rice Extract

Concentration	Quercetin Absorbance	% inhibition	IC 50 Value

			(ppm)
0	0.608	0.000	
2000	0.518	14.803	
4000	0.491	19.243	11,837.68
6000	0.434	28.618	
8000	0.380	37.500	
10000	0.346	43.092	

Antioxidant activity is expressed by an Inhibition Concentration of 50% (IC50), which is a concentration that causes a 50% loss of DPPH activity (Molyneux, 2004).

4. Discussion

Black Glutinous Rice and Fermented Black Glutinous Rice have a variety of potential, one of them in this study is as a food ingredient that contains anthocyanin compounds that are good for health because it has antioxidant activity. The sample of black Glutinous rice and Fermented Black Glutinous Rpe used in this study came from one of the sellers in the Cililin area, which in the Cililin area was a village built by the Poltekkes Kemmenkes Bandung. The black Glutinous rice and Fermented Black Glutinous Rice obtained were made into thick extract. The production of thick black glutinous rice extract and Fermented Black Glutinous Rice was done by maceration, namely by soaking black glutinous rice and Fermented Black Glutinous Rice in a liquid finder. The maceration method is used because can reduce the risk of damage to active substances, use simple equipment, efficient in terms of time, and good for compounds that are not heat resistant. Extraction solvent used is a mixture of methanol and 1% HCl with a ratio of 85:15 to 100 grams of sample. Methanol solvent is used because it has several advantages, methanol can extract compounds better than other solvents other than that anthocyanin is soluble in polar solvents. Lack of methanol as a solvent that is toxic. Ethanol solvent is not used because when it is used to extract anthocyanin in rice the amount of anthocyanin extracted is small (Vina, 2018). In addition, methanol is more polar compared to ethanol because it has a smaller number of C atoms. In this study water solvents were not used because water is a good medium for bacterial growth so it was feared that during the extraction process it was overgrown with bacteria, so the solvent used was methanol as a solvent. Addition of 1% HCl solution is done to provide an acidic atmosphere because anthocyanin is more stable in an acidic atmosphere. HCl was chosen because it can denaturate plant cell membranes and dissolve anthocyanin compounds out of cells.

Maceration extraction is carried out for 3 x 24 hours, this is done so that the extraction of the pigment can be extracted perfectly. Every 24 hours the extraction solvent is replaced with a new solvent to avoid saturated solvents which no longer attract compounds. When the maceration process is stirred. The purpose of stirring is to homogenize the solution during the immersion process and speed up contact between the sample and the solvent. Extracted maceration results are filtered using filter paper. The maserate obtained was concentrated using a water bath with a temperature of 40 ° C, this was done to concentrate the extract and also separate the solvent with the active compound in black glutinous rice and Fermented Black Glutinous Rice. The viscous extract obtained was dried with a freeze dryer at -40 ° C. The freeze dryer / freeze drying process is carried out because it has the advantage of being safer against the risk of degradation of compounds in the extract. After getting the dried extract, then the yield is calculated, the yield obtained on black glutinous rice extract is 11.664% while the yield on Fermented Black Glutinous Rice extract is

3.1073%. Anthocyanin identification is done by using HCl and NaOH color reagents. The black Glutinous rice and Fermented Black Glutinous Rice showed the results of a red solution if the extract was added to HCl while when added NaOH showed the results of the red solution which faded over time. This is because anthocyanin has greater stability under acidic conditions, whereas in neutral and alkaline anthocyanin solutions are unstable. Anthocyanin testing was carried out on black Glutinous rice extract and Fermented Black Glutinous Rice using the pH difference method. Data from the measurement of absorbance values at wavelengths of 510 nm and 700 nm at pH 1 and pH 4.5. The pH 1 solution is made because at pH 1 anthocyanin is in the form of cavity flavilium which is the most stable and most colorful condition, whereas at pH 4.5 anthocyanin is in the form of carbinol in which carbinol is a colorless compound. So the higher the pH value, the color of anthocyanin becoming increasingly pale and finally colorless. The stability of anthocyanin is influenced by several factors including temperature, changes in pH, light and oxygen, as well as other factors such as metal ions. In addition, the analysis process on the sample is not directly carried out when the dry extract is ready, so that the conditions and the storage period of the sample cause the anthocyanin compounds to degrade. High processing temperature will also cause anthocyanin compounds to degrade because anthocyanin compounds are very vulnerable to the heating process (Niendyah, 2004; Febrianti et al, 2014).

Factors that can affect the color of anthocyanins are changes in pH, heating, pigment concentration, the presence of a mixture with other compounds, the number of hydroxy groups and methoxy also affect the color of anthocyanin. The acidic nature will cause the color of anthocyanin to be red, while the nature of the base will cause the color of anthocyanin to turn blue. Besides that the dominant hydroxy group causes the color to tend to be blue and relatively unstable, while the dominant methoxy group causes the color to be red and relatively more stable. While the heating process can cause anthocyanin compounds to degrade. Anthocyanin degradation can cause changes in the structure of anthocyanin into ketone products. The formation of ketone products causes a reduction in the number of anthocyanin phenolic hydroxyl groups that act as donors of hydrogen to free radicals thereby reducing its ability to reduce free radicals. If anthocyanin is degraded, it can cause changes in the structure of anthocyanin into ketone products which can reduce its ability to reduce free radicals so that their antioxidant activity decreases (Febrianti et al, 2014). Measurement data obtained total anthocyanin levels in black glutinous rice extract of 4.2582 mg / 100 grams. While the total levels of anthocyanin in Fermented Black Glutinous Rice extract of 8.0989 mg / 100 grams. In a previous study conducted by Suhartatik and Yustina stated that Fermented Black Glutinous Rice was 1,747 mg / 100 grams. The difference in the total value of anthocyanin is due to the type of blackGlutinous rice and Fermented Black Glutinous Rice used differently. The total value of anthocyanin can be obtained in small amounts due to the small extark yield obtained in addition to that because black glutinous rice extract and Fermented Black Glutinous Rice are degraded by light so that the anthocyanin pigment is fading. The level of anthocyanin in Fermented Black Glutinous Rice is greater than the level of anthocyanin in blackGlutinous rice this is because the Fermented Black Glutinous Rice undergoes several reaction processes at the time of manufacture such as fermentation. Fermented food usually has a higher nutritional value than its ingredients, this is caused by microbial activity breaking down complex components into simpler substances so that it is easier to digest, causing the total anthocyanin on the Fermented Black Glutinous Rice to be greater than the total anthocyanin on rice.

Antioxidant testing was carried out on dried extracts of black Glutinous rice and Fermented Black Glutinous Rice using the DPPH-Spectrophotometer method. The purpose of this method is to determine the antioxidant activity (IC50) in an extract. Antioxidant activity is expressed by 50% inhibition concentration

(IC50), which is the concentration of the sample which causes a loss of 50% of DPPH activity (Molyneux, 2004). In determining the antioxidant activity by DPPH method which is measured by UV-Vis spectrophotometry, where the principle is a fading reaction or decay of color from purple to yellow color incubated for 30 minutes. Antioxidant activity was measured by three measurements with each of 5 concentration variations and then reacted with DPPH, incubated for 30 minutes then measured using a UV-vis spectrophotometer at a wavelength of 516 nm. The reduction of antioxidant free radicals is carried out within 30 minutes because the reaction between the antioxidant reduction and DPPH is best at 30 minutes if more or less it will affect the results when tested using a spectrophotometer. Antioxidant activity was measured by counting the number of measurements of DPPH purple light intensity which was proportional to the reduction in DPPH concentration. The damping is produced by the reaction of the picrihidrazil molecule with hydrogen atoms released by the molecular component of the sample to form the diphenyl picri compound of hydrogen and cause the DPPH to decay from purple to yellow. This method was chosen because this method is simple, easy, and fast. The samples used in this test are black Glutinous rice extract, Fermented Black Glutinous Rice extract and quercetin positive control. In table 4.3, the profile of quercetin antioxidant activity is made to find out how much the antioxidant activity of quercetin is by looking at the results between the concentration (µg / ml) and absorbance. The higher the concentration of the solution used, the smaller the absorbance produced. The stronger antioxidant activity of a compound is characterized by a change in purple to yellow. Quercetin is used as a positive control because it is a proven standard of antioxidant activity (Nina and Liani, 2014).

In the study, quercetin is used because quercetin is a powerful antioxidant activity. Vitamin C is not used as a comparison Antioxidant activity was measured by three measurements with each of 5 concentration variations and then reacted with DPPH, incubated for 30 minutes then measured using a UV-vis spectrophotometer at a wavelength of 516 nm. The reduction of antioxidant free radicals is carried out within 30 minutes because the reaction between the antioxidant reduction and DPPH is best at 30 minutes if more or less it will affect the results when tested using a spectrophotometer. Antioxidant activity was measured by counting the number of measurements of DPPH purple light intensity which was proportional to the reduction in DPPH concentration. The damping is produced by the reaction of the picrihidrazil molecule with hydrogen atoms released by the molecular component of the sample to form the diphenyl picri compound of hydrogen and causes the DPPH to decay from purple to yellow. This method was chosen because this method is simple, easy, and fast. The samples used in this test are black Glutinous rice extract, Fermented Black Glutinous Rice extract and quercetin positive control. In table 4.3, the profile of quercetin antioxidant activity is made to find out how much the antioxidant activity of quercetin is by looking at the results between the concentration (µg / ml) and absorbance. The higher the concentration of the solution is used, the smaller the absorbance produced. The stronger antioxidant activity of a compound is characterized by a change in purple to yellow. Quercetin is used as a positive control because it is a proven standard of antioxidant activity (Nina and Liani, 2014). In the study, quercetin is used because quercetin is a powerful antioxidant activity. Vitamin C is not used as a comparison. Based on research data it can be seen that the higher the concentration of black glutinous rice extract and Fermented Black Glutinous Rice extract added, the greater the percentage of inhibition produced, because in black glutinous rice contains compounds that act as antioxidants that have the potential to ward off or inhibit free radicals DPPH solution added to the sample is marked by changing the color of the extract, so that if the percentage of black glutinous rice extract and Fermented Black Glutinous Rice added is greater, the content of antioxidant compounds will also be greater and the inhibition of the extract against free radical activity will also increase due to the number of electrons from the extract Black glutinous rice taken by free radicals which will cause a greater

percentage of inhibition. This is in accordance with research conducted by Andriyani (2012) that the percentage of extract inhibition of free radical activity increases with increasing extract concentration. The added DPPH solution can react with compounds that can donate hydrogen atoms, due to the presence of unpaired electrons, DPPH gives a strong absorption at 516-517 nm, when the electrons become paired by the presence of free radical scavengers, the absorbance decreases stoichiometry according to the number of electrons in the electron was taken. According to Ariyanto (2006), the strength level of antioxidant compounds using the DPPH method can be classified according to the IC50 value, where the smaller the IC50 value means the higher the antioxidant activity. The antioxidant activity of black glutinous rice extract and Fermented Black Glutinous Rice extract has a weak intensity because IC50 is between> 150 μg / mL. This can occur because the decrease in antioxidant activity is influenced by several factors such as light and heat. Heat or light can trigger pre oxidation. In other words, the sample has donated its H atom to form hydroperoxide, so the reducing power to DPPH is lower. According to Kurniasih (2011), a substance has antioxidant properties if the IC50 value is less than 200 μg / mL. If the IC50 value obtained is between 200-2000 μg / mL, then the substance is less active but still has the potential as an antioxidant. From the IC50 value it can be seen that quercetin has a strong antioxidant activity compared to black Glutinous rice extract and Fermented Black Glutinous Rice extract this is because quercetin is a pure compound so that its antioxidant activity is strong. The antioxidant activity of Fermented Black Glutinous Rice is greater than the antioxidant activity of black Glutinous rice even though the antioxidant intensity is weak, this is because the Fermented Black Glutinous Rice contains total anthocyanin levels greater than the total anthocyanin levels in black glutinous rice, which is anthocyanin which has compounds antioxidant activity. In addition, Fermented Black Glutinous Rice has also undergone a fermentation process which will have a higher nutritional value than its ingredients.

5. Conclusions

Total anthocyanin in Fermented Black Glutinous Rice extract 8.0989 mg / 100 gram was greater than the total anthocyanin in black glutinous rice 4.2582 mg / 100 gram. Antioxidant activity research show that black glutinous rice and Fermented Black Glutinous Rice contain antioxidants. The value of antioxidant activity on black glutinous rice is 14,654.67 ppm, while the value of antioxidant activity on black glutinous terrace is 11,837.68 ppm.

6. Suggestions

Further research needs to be done on Black Glutinous Rice and Black Glutinous Fermented Black Glutinous Rice using different extraction solvents so that the effect of the type of solvent on antioxidant content and total anthocyanin will be known. Further research needs to be done on black Glutinous rice and Fermented Black Glutinous Rice using HPLC so that it will be known the types of anthocyanin contained in black Glutinous rice and Fermented Black Glutinous Rice

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