Antioxidant extraction based on black rice (Oryza Sativa L. Indica) to prevent free radical

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Abstract. Free radicals are molecules that in their outer orbit have one or more unpaired electrons; they are very labile and very reactive. These molecules have an important role in tissue damage and pathological processes in living organisms. Antioxidants are compounds that can inhibit oxidation reactions by binding free radicals. The antioxidants produced by the human body are very limited, so we need the intake of antioxidants from outside, especially those from food. Research on antioxidant extraction based on black rice (Oryza Sativa L. Indica) to prevent free radicals has been carried out. This research was conducted using the DPPH method to measure the ability to capture free radicals and FRAP methods to measure antioxidant capacity. This study aimed to obtain data on the level of antioxidant activity contained in black rice and its effect to counteract free radicals. The result showed that the ability to capture free radicals contained 25ppm DPPH anthocyanin extract could inhibit 55.00%. While using the FRAP method, the results of black rice anthocyanin extract have a high antioxidant capacity of 824 ± 17.24 µM. It is evident that the DPPH method used to show in antioxidant extracts based on black rice can capture free radicals, while the FRAP method proves that antioxidants in black rice have the capacity to prevent free radicals in the body.

1. Introduction
Free radicals are defined as atoms or molecules that have one or more unpaired electrons [1]. Molecules that lose electrons can become radical and may attract electrons from other molecules that are close to them so that a chain reaction occurs [2]. Free radicals can be formed from the body (endogenous) and outside the body (exogenous). The formation of free radicals can be inhibited by the presence of antioxidants. Antioxidants are chemical compounds that can contribute one or more electrons to free radicals so that free radicals can be suppressed [3]. The human body does not have excess antioxidant reserves, so if there is excessive radical exposure, the body needs exogenous antioxidants. Bioactive compounds in food can act in a variety of biological activities, for example, as antioxidants in the body. The role of food today is not only to meet the nutritional needs and giver of satiety but is also expected to be beneficial for health (functional food). One type of food that is very close to the people of Indonesia is rice. Rice (Oryza sativa) is a staple food in Indonesia and based on
the color of the pericarp there are several kinds of colored rice, such as brown rice, black rice, brown rice. Rice colors such as red, black, and brown contain antioxidant anthocyanins and dyes that are found in the aleuron layer also contain vitamins, especially the group of vitamin B and minerals, especially Fe and Zn. Bioactive compounds that cause pigment in rice are anthocyanin and proanthocyanidin. Hosoda et al. [4] reported that black rice and brown rice contain anthocyanin and proanthocyanidin which are potentially used as sources of antioxidants other than as a source of starch in ruminants. Seawan et al. [5] also stated that pigmented rice has high antioxidant activity potential due to the high content of bioactive compounds. Bioactive compounds in pigmented rice can reduce oxidative stress, prevent cancer, cardiovascular, diabetes complications, and others [6]. One type of antioxidant that has revealed many physiological effects on health is anthocyanin. Anthocyanin is a pigment naturally belonging to the flavonoid group which is responsible for color red, purple and blue in food. The main anthocyanin in black rice is cyanidin-3-glucoside (C3G) which is an important source of anthocyanin in Asia. Other than that, Black rice contains active phytochemicals such as tocopherol, tocotrienol, oryzanols, B complex vitamins, and phenolic compounds [7]. Anthocyanins are a class of flavonoids that are found in many foods that are red, blue, purple and black, but also are water-soluble so they are easily absorbed into the body. The main function as an antioxidant, both inside the cell (intracellular) and outside the cell (extracellular). As an antioxidant anthocyanin can capture radical compounds by donating electrons or transferring hydrogen atoms with hydroxyl groups to free radicals. At this stage, anthocyanin acts as a catcher of ROS at the initiation stage and breaks the chain of propagation in lipid peroxidation. Therefore, it is deemed necessary to conduct research to see the ability of anthocyanin extracts from black rice to counteract free radicals. In this study, extraction was carried out for aleuron from black rice, which is the outer layer of rice containing black pigment, in contrast to previous studies that used bran or even whole grains of black rice.

2. Material and Method
The research material used was black rice from Bantul, Yogyakarta. The farmers' harvested black rice is brought to the UGM Faculty of Agricultural Technology, sorted to be separated with dirt, and dried to a moisture content <13%. Then put in a two-layer sack and stored in a dry room. Anthocyanin extraction is done by maceration using ethanol and citric acid. The selection of this method which is to combine the solvent with the addition of citric acid is expected to maximize the extraction process. Robinson (1995) in Tensiska [8] states that extraction of flavonoid compounds is recommended in an acidic environment, because acid serves to denature the growth of tana man cells, then dissolves anthocyanin pigments so that they can get out of cells, and can prevent flavonoid oxidation. While organic acids which are often used for the extraction of pigments are hydrochloric acid, citric acid, and acetic acid. The flow diagram of the black rice anthocyanin extraction process can be seen in Figure 1.
Black Rice Flour 100 gram

mixed

1 L ethanol 96% + citrate acid 3% 30.0 gram (1:10)

Stirring 400 rpm for ±3 hour at room temperature

Filtration with help of a vacuum pump

Extraction using a rotary evaporator for ±3 hours at a pressure of 305 mmHg - 72 mmHg at 40°C, flowed with nitrogen gas

Viscous black rice extract is stored in a closed container at -20°C

Figure 1. Anthocyanin extraction process flowchart

2.1. DPPH (1,1- diphenyl-2- picrylhydrazyl) method
The antioxidant activity test of black rice extract was carried out by the DPPH method [9]. The crude extract of black rice dissolved in methanol p.a. to obtain concentrations of 25 ppm, 50 ppm, 75 ppm and 100 ppm. Controls using vitamin C are 100 ppm. DPPH solution is prepared by dissolving DPPH crystals in a methanol solvent p.a. with a concentration of 1 mM. The process of making a 1 mM DPPH solution is carried out in low temperatures and protected from sunlight. The extract solution and the BHT antioxidant solution were each taken 4.50 ml and reacted with 500 μl DPPH 1 mM solution in different test tubes. The reaction took place at 37°C for 30 minutes then the absorbance was measured using a UV-VIS spectrophotometer at a wavelength of 517 nm. The absorbance of the blank solution was measured to calculate the percent inhibition. A blank solution was made by reacting to 4.50 ml of methanol solvent with 500 mL of 1 mM DPPH solution in a test tube. The antioxidant activity is expressed in percent inhibition, which is calculated by the formula:

\[
\% \text{ Inhibition} = \frac{\text{absorbance blanks} - \text{absorbance of sample}}{\text{absorbance blanks}} \times 100\%
\]  

2.2. FRAP (Ferric Reducing Antioxidant Power) method
Analysis of total antioxidant activity by the FRAP method, FeSO4.7H2O as standard [10]. In the manufacture of FRAP reagents, 0.1 M acetate buffer solution (pH 3, 6), TPTZ solution (2,4,6-tripyrdyls-triazine) 10 mM in 40 mM HCl and 20 mM FeCl3.6H2O solution are prepared in advance then the solution is mixed in a ratio of 10: 1: 1. A total of 50 μL of sample solution and 150 μL of distilled water were added to the tube which contained 1.5 mL of FRAP reagent. The mixture was incubated for 8 minutes in a dark place at room temperature. The sample absorbance was measured at a wavelength of 594 nm and the results were calculated in Fe + 2 equivalent (Fe + 2mM) using a standard FeSO4.7H2O curve.

3. Result and Discussion

3.1. DPPH
In this study, antioxidant activity was tested using the DPPH method. Vitamin C and vitamin E 100 ppm were used as positive controls. Variations in the levels of extracts used were 25 ppm, 50 ppm, 75 ppm, and 100 ppm anthocyanin. Vitamins C and E are used as positive controls because this type of
antioxidant vitamin is often added to the product. The results of the analysis of antioxidant activity using the DPPH method can be seen in Table 1 and Figure 2. The results showed that the decrease in absorbance occurred as a result of changes in color from purple to yellow, DPPH radical compounds trapped by anthocyanins by donating hydrogen atoms to form DPPH-H complex [11]. The higher the anthocyanin level in black rice extract, the higher the ability of DPPH radical capture will be due to the low black rice extract concentration which will reduce the number of hydrogen atoms, this condition is in accordance with the previous research [12].

The ability to capture DPPH radicals possessed by 25 ppm anthocyanin extract is higher than vitamin C 100 ppm; this condition can occur because anthocyanin extract has the ability to donate hydrogen atoms with the anthocyanin component which majorly has a hydroxyl group in position 3 which is very easy to escape due to enthalpy the energy of the hydroxyl group bond on ring B is the lowest [13]. Antioxidant activity in this study showed that the inhibition ability of 25 ppm extract was 55.00% greater than the antioxidant activity using DPPH method with methanol as a solvent in a previous study by Wanti et al. [14] which was 46.20%.

Table 1. Antioxidant activity of black rice extract using DPPH method

| C(ppm) | A  | % Inhibition |
|--------|----|--------------|
| 25     | 0.44 | 55.00       |
| 50     | 0.27 | 60.86       |
| 75     | 0.18 | 73.91       |
| 100    | 0.15 | 76.81       |
| Vitamin C (100) | 0.66 | 4.30       |

Figure 2. Ability to inhibit free radical by black rice anthocyanin extract

3.2. FRAP

In this study, testing the antioxidant capacity of black rice extract using the FRAP method. The results of black rice anthocyanin extract have a high antioxidant capacity with 3 replications of analysis of 824 μM. Determination of antioxidant activity by FRAP test measures the ability of antioxidants to reduce Fe + 3 in Fe3+ -TPTZ complexes into Fe + 2-TPTZ by donating electrons. The high antioxidant activity in the RR watering treatment is related to the high content of the phenolic compounds and the two flavonoids which are capable of reducing Fe + 3 [15].

Measurement of antioxidant activity can be done by several methods including CUPRAC, DPPH, and FRAP. The FRAP method [16] uses the Fe (TPTZ) 23+ 2,4,6-tripiridyl-triazine besilligan as a
reagent. The blue Fe complex (TPTZ) $^{2+}$ will function as an oxidizing agent and will be reduced to Fe (TPTZ) $^{2+}$ which is yellow with the following reaction:

$$\text{Fe(TPTZ)}^{2+} + \text{AROH} \rightarrow \text{Fe(TPTZ)}^{2+} + \text{H}^+ + \text{AR}$$

So this shows that anthocyanin extract from black rice is a good antioxidant and can prevent free radicals in the body.

| Table 2. Antioxidant activity of black rice extract using FRAP method |
|---------------------------------------------------------------|
| Sample | Concentration of Antioxidant (µM or µmol/L) | Concentration of Antioxidant (µmol/100 gram) |
| Extract | 820.667 ± 17,243 | 820.667 ± 17,243 |

4. Conclusion
Based on research that has been done, the conclusions can be drawn that it is evident the DPPH method used to show in anti-oxidant extracts based on black rice can capture free radicals. The ability to capture DPPH radicals possessed by 25 ppm anthocyanin extract is higher than vitamin C 100 ppm, while the FRAP method proves that antioxidants in black rice have the capacity to prevent free radicals in the body.

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