INTRODUCTION

Antioxidant is a compound which could inhibit or prevent oxidation process caused by free radical [1]. Synthetic antioxidants such as Butyl Hydroxy Anisol and Butyl Hydroxy Toluene are very effective in preventing oxidation process. However, synthetic antioxidant can cause disruption on the liver and affects the function of enzymes in the liver [2]. The concern about the side effects of synthetic antioxidant makes natural antioxidants a viable alternative.

Rice is a staple food consumed by most Asian countries, especially in Indonesia. Color pigments on the outer layer of rice or aleurone from black rice have been reported to contain anthocyanin compounds.

Anthocyanin is one of the secondary metabolite compounds that belong to the class of flavonoids that have many uses and are found in many types of plants. Anthocyanin compounds acting as antioxidants and free radical catchers, thereby contributing to the prevention of degenerative diseases [4].

Adrian Baitairiza’s research (2014) used DPPH-free radical damping method to conduct antioxidant activity test in vitro on extract of black rice (Oryza sativa L), and the result showed that black rice extract has IC50 value of 41.5 bpj [5]. Therefore, this study aimed at making functional antioxidant drinks from dry extracts of black rice that could quench thirst, healthy, practical, and consumable by the community.

Functional drinks are beverages that contain elements of nutrients or non-nutrients and if consumed could provide beneficial effect on health. Functional drinks are a type of food or food product that has functional features and plays a role in protection, prevention, treatment of disease, improve body functions, and prevent aging [6].

The aim of this study was to obtain data on antioxidant activity from ethanol condensed extract 70%, dry extract of black rice, and black rice water extract. To produce a functional beverage that has antioxidant activity from dry extract of black rice ethanol 70% and water extract.

MATERIALS AND METHODS

Materials
Black rice (O. sativa L.), sodium benzoate, ethanol 70%, distilled water, chloroform, 95% ethanol, 30% ammonia, hydrochloric acid, Mayer reagent, Dragendorff’s reagent, concentrated hydrochloric acid, Mg, amyl alcohol, iron (III) chloride 1%, sodium hydroxide 1 N, ethers, acetic acid anhydrous, concentrated sulfuric acid, 10% ammonia, alcohol, sulfuric acid P, 10% nitric acid, sodium acetate P, potato dextrose agar hatchery, nutrient agar, methanol, DPPH.

Equipment
Glasswares, blender, rotary vacuum, microanalytic scale, micropipette, incubator, ultraviolet-visible (Shimadzu 1700) spectrophotometer, atomic absorption spectrophotometer, cuvette, porcelain crucible, shallow dish, weighing bottle, desiccator, kiln, oven, Petri dish, filter paper, ash-free filter paper, freeze dryer.

Steps of research
Preparation of black rice extract
Black rice extract was made by kinetic maceration using 70% ethanol. As much as 2000 g of black rice was macerated with 70% ethanol and 4 L of water until all the simplifications were submerged, then the simplicia was soaked for 24 h. The filtrate was then filtered and collected by repeating maceration process until it was perfectly extracted, the extract was collected together and then thickened with...
vacuum rotary evaporator, and thick extract was obtained. Thick extract was undergone freeze-drying process. One sachet of functional drink formula was made of 4 g of dried extract of black rice, 1 g of red ginger powder, and 5 mg of sodium benzoate.

**Phytochemical screening**

The phytochemical analysis of black rice extract has been performed to find the presence of major secondary metabolites such as flavonoids, tannins, saponins, steroid, and triterpenoid.

**Extract quality parameter determination**

Specific parameters determination
Organoleptic inspection, determination of dissolved compounds in water, determination of dissolved compounds in ethanol.

Non-specific parameters determination
Examination loss on drying, water content, total ash, acid not soluble ash content, residual solvents, heavy metal contaminants (Pb and Cd), and examination of microbial contamination.

**Antioxidant activity test**

About 70% ethanol condensed extract, dried extract, dried extract (pure water solvent), and functional beverage of black rice.

Into each of the test solvent and the comparative aqueous solution (positive control) was added 1 ml of 0.4 mM DPPH solution and methanol proanalysis up to 5.0 mL, then homogenized. The DPPH solution was added to the blank solution, test solution, and comparative solution (positive control), then immediately
incubated for 30 min at 37°C, then uptake test was done at 516.5 nm wavelength.

Antioxidant activity calculation
Formula for calculating free radical immersion (%)

\[ \text{Inhibition} = \frac{\text{blanc absorption} - \text{sample absorption}}{\text{blanc absorption}} \times 100\% \]

Next, \( \text{IC}_{50} \) calculation is using linear regression \( y = a + bx \). \( \text{IC}_{50} \) stated in x.

\( \text{IC}_{50} \) (inhibition concentration 50) is antioxidant concentration (mg/L) which could inhibit 50% free radical.

RESULTS AND DISCUSSION

Preparation of 70% ethanol extract black rice

Phytochemical screening

Secondary metabolite content test was done against powder and ethanol extract 70% black rice. The results of phytochemical screening of powders and extracts can be seen in Table 2 and in Appendix 10.

Extract quality parameter determination

Specific parameter

a. Extract identification

Extract identification result can be seen in Table 3.

b. Organoleptic

Organoleptic extract result can be seen in Table 4.

Making of black rice dry extract

Dry powder making was done with freeze-drying method which was carried out in “Herbarium Bogoriense” Botanical Field of Biology Research Center-LIPI, Cibinong.

Functional drink formula

Antioxidant test result from 5 g of functional drinks shows antioxidant activity of \( \text{IC}_{50} \) 46.36 bpj. Based on this result, the functional drinks were made of 4 g of dried extract of black rice, 1 g of red ginger powder, and 5 mg of sodium benzoate.

Antioxidant activity test

Based on the data above, the antioxidant activity test on ethanol extract 70% of black rice, dry black rice extract, dry extract of black rice (pure...
Table 3: Identity determination result of 70% ethanol extract black rice

| No. | Extract identity | Test result |
|-----|-----------------|-------------|
| 1   | Extract name    | O. sativa fructus extractum spissum |
| 2   | Latin name of extract | O. sativa L. |
| 3   | Part of plant used | Fructus |
| 4   | Indonesian name of the plant | Beras hitam |

Table 4: Result of extract organoleptic test

| No. | Organoleptik | Test result |
|-----|--------------|-------------|
| 1   | Texture      | Thick extract |
| 2   | Color        | Dark purple |
| 3   | Smell        | Specific aroma |
| 4   | Taste        | Bitter |

Table 5: Antioxidant activity test result

| Sample                                      | IC_{50} Value (bpj) |
|---------------------------------------------|---------------------|
| BP Vitamin C                                | 2.2464 |
| Ethanol extract 70% black rice              | 37.5587 |
| Black rice dry extract                      | 231.0101 |
| Black rice dry extract (pure water solvent) | 44.7412 |
| Functional drink black rice dry extract     | 46.3594 |

Table 6: Organoleptic test result on functional drink

| No. | Organoleptic | Test result |
|-----|--------------|-------------|
| 1   | Shape        | Powder |
| 2   | Color        | Black |
| 3   | Odor         | Distinctive aroma |

Table 7: Microbe contamination test result

| No. | Parameter | Result              | BPOM requirement (colony/g sample) |
|-----|-----------|---------------------|-----------------------------------|
| 1   | Total plat account (ALT) | 0.310×10^3 | ≤3×10^3 |
| 2   | AKK       | 0.6997×10^2 | ≤1×10^2 |

AKK: Angka Kapang Khamir

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