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Research Article

DRY EXTRACT OF BLACK RICE *(ORYZA SATIVA* L.*)* AS ANTIOXIDANT IN THE FORM OF FUNCTIONAL DRINK

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ABSTRACT

Objective: Based on previous research, black rice (*Oryza sativa L.*) has been proven to have antioxidant activity which is classified in the very strong category, approximately 41.5 bpj. this The aim of this study was to produce antioxidant functional drink.

Methods: Simplicia powder prepared from black rice *(O. sativa L.)* sample by extracting with 70% ethanol solvent using kinetic maceration, followed by phytochemical screening, determination of extract quality, drying extract with freeze-drying method, then dry extract obtained used to produce functional drinks on which evaluation and antioxidant activity test carried out. The antioxidant activity test used in this research was DPPH-free radical scavenging method.

Results: Screening result showed that both simplicia and extract contain flavonoid compounds, saponins, tannins, steroids, and triterpenoids. Result from dried extract quality determination shown to be thick consistency, has blackish-purple color, has specific aroma and bitter, water-soluble extract content of 59.04%; ethanol-soluble extract content of 69.77%; moisture content of 7.65%; loss on drying 9.40%; total ash content of 5.17%; acid-insoluble ash content of 0.84%; water-soluble ash content 4.15%; and residual solvent 0.44%. Pb and Cd metal contamination were 1.1994 mg/kg and 0.0905 mg/kg, respectively. Total plate count microbial contamination TNTC and the number of yeast and mold found to be 0.0387×10^3 colony/g. Evaluation test of functional drinks with powder type and found to be blackish-purple, and specific aroma, and total plate count microbial contamination was 0.310×10^3 colony/g, and the number of yeast and mold found was 0.6997×10^2 colony/g. Result of IC_{50} antioxidant activity of viscous extract was 37.5587 bpj; dried extract was 231.0101 bpj; dried extract (distilled water) was 44.7412 bpj; and functional drinks were 46.3594 bpj.

Conclusion: Thus, results suggest that dried black rice's extract and functional drink produced found to have very strong antioxidant activity.

Keywords: Black rice, Antioxidant, Functional drinks, DPPH.

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INTRODUCTION

Antioxidant is a compound which could inhibit or prevent oxidation process caused by free radical [1]. Synthetic antioxidants such as Butil Hidroksi Anisol and Butil Hidroksi 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 IC_{50} 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 pa, 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

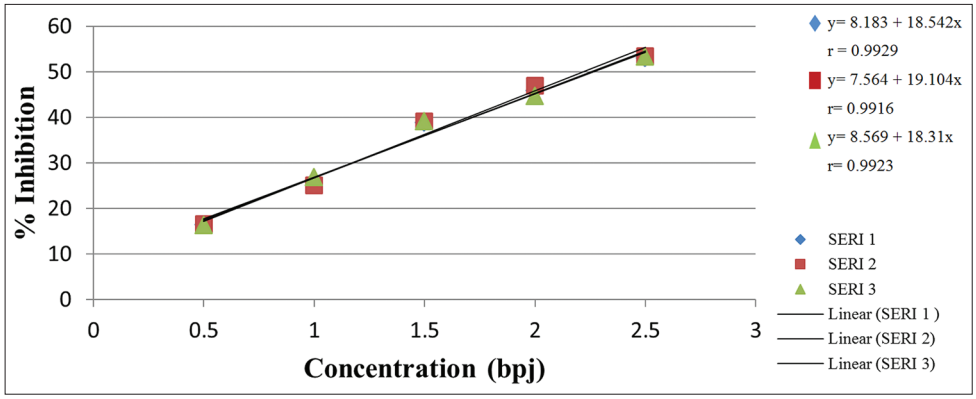

Fig. 1: Relation between concentration (x) and % inhibition (y) on BP Vitamin C

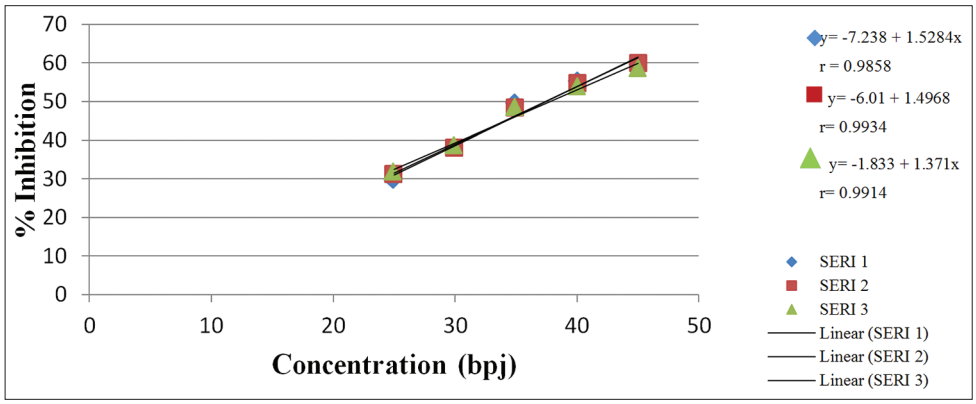

Fig. 2: Relation between concentration (x) and % inhibition (y) on thick 70% ethanol extract black rice

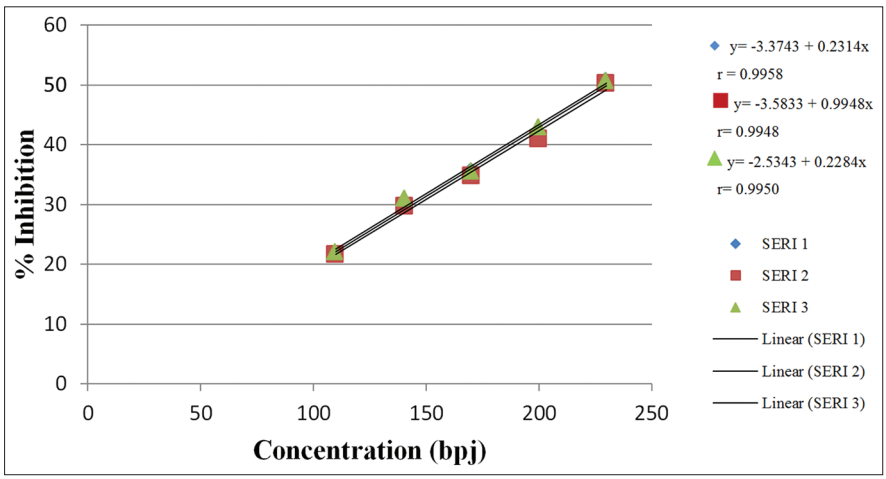

Fig. 3: Relation between concentration (x) and % inhibition (y) on dry black rice extract

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

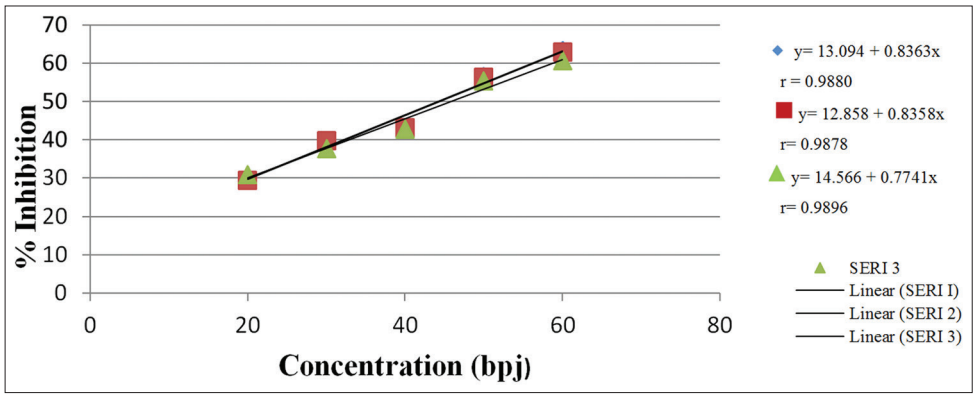

Fig. 4: Relation between concentration (x) and % inhibition (y) on dry black rice extract (pure water solvent)

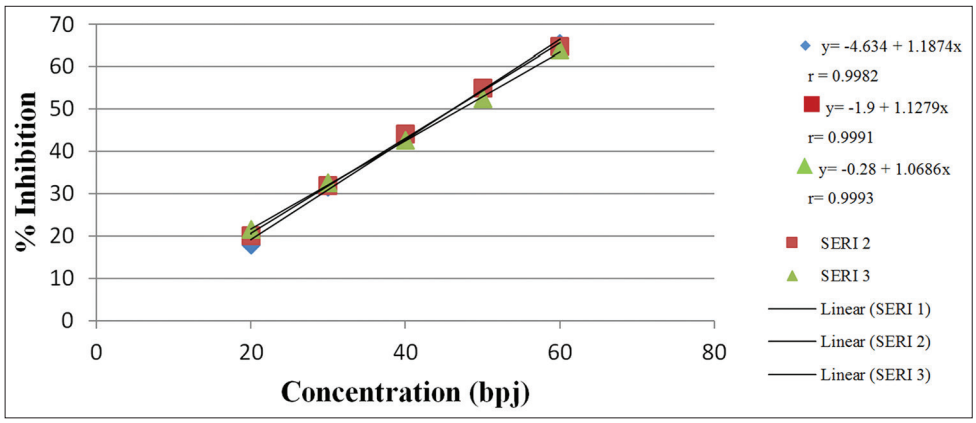

Fig. 5: Relation between concentration (x) and % inhibition (y) on functional drink black rice extract

Table 1: DER native and black rice 70% and ethanol extract immersion

Material	Simplicia (g)	Extract amount (g)	DER native	Rendement (%)
Black rice	2009.3	83.2631	24.1319	4.14

Table 2: Phytochemical filtering result for powder and 70% ethanol extract black rice

No	Phytochemical filtering	Powder	Extract
1	Alkaloid	-	-
2	Flavonoid	+	+
3	Saponine	+	+
4	Tanin galat	+	+
	Tanin catecuat	+	+
5	Quinone	-	-
6	Steroid	+	+
7	Triterpenoid	+	+
8	Olive Oil	-	-
9	Cumarin	-	-

+: Positive reaction, -: Negative reaction

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 (%)

$$\text{Inhibition} = \frac{\text{blanc absorption-sample absorbtion}}{\text{blanc bsorption}} \times 100\%$$

Next, IC_{50} calculation is using linear regression y = a+bx. IC_{50} stated in x.

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 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

O. sativa: Oryza sativa

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 (red ginger powder addition and sodium benzoic)	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	$\leq3\times10^3$
2	AKK	0.6997×10^2	$\leq1\times10^2$

AKK: Angka Kapang Khamir

water solvent), and functional beverage of dry extract of black rice each yield $I_{c5}0$ value of 37.5587 bpj, 231.0101 bpj, 44.7412 bpj, and 46.3594 bpj. This suggests that the test substance has antioxidant activity. The presence of antioxidant activity is due to a class of secondary metabolite compounds such as flavonoids contained in ethanol extract 70% black rice. Flavonoid compounds can inhibit oxidation by giving H atoms that bind free radicals to produce more stable compounds.

The antioxidant activity of ethanol viscous extract of 70% black rice was weaker than the comparative standard of Vitamin C with IC_{50} value of 37.5587 bpj but still showed very strong antioxidant properties. The results of black rice antioxidant activity in this study were stronger than the previous study which showed IC_{50} value of 41.5 bpj. This is due to the fact that this used ethanol 70% as a seeker so that flavonoid compounds can be perfectly seared.

The antioxidant activity of dry extract of black rice has a weaker IC_{50} compared to the ethanol condensed extract of 70% black rice, which is 231.0101 bpj. This may be due to the addition of maltodextrin filler in the freeze-drying process so that maltodextrin coated the viscous extract and caused the test sample was not extracted perfectly. The reason for selecting maltodextrin as a filler in the freeze-drying process is it is a safe, relatively inexpensive, easy-to-use, more often used filler in the food industry than other fillers.

In the test of functional drink made from dry extract of black rice (using pure water as solvent of test substance) with IC_{50} equal to 44.7412 bpj, red ginger (as aroma and flavor enhancer) and sodium benzoate as preserver were added. After this, we obtained IC_{50} value of 46.3594 bpj which shows very strong antioxidant properties. This decrease in antioxidant activity is due to the addition of red ginger and sodium benzoate. Red ginger cannot act as antioxidant because it was made not in extract form, so the compounds that have activity as an antioxidant is not extracted during antioxidant activity testing.

Functional drink evaluation

Organoleptic

Organoleptic test result on functional drink dry black rice extract can be seen in Table 6.

Organoleptic examination is one of the specific parameters determined using the five senses and aims to provide early recognition as well as signify the hallmark of functional beverage of dried extract of black rice in form, color, and odor. Organoleptic examination results showed a dry extract of black rice in the form of powder, black with distinctive aroma.

Microbe contamination

Microbe contamination test result on functional drink dry black rice extract can be seen in Table 7.

Testing of microbial contamination is done to find out the number of microorganisms contained in functional drinks of black dry rice extract, which in excess amount can be harmful to health. Functional drinks of dried extracts of black rice obtained should not contain pathogenic or non-pathogenic microbes beyond predetermined limits.

The results of the contamination test shown in Table 7 still meet the requirements stipulated by the Food and Drug Supervisory Agency (BPOM) on the Determination of the Maximum Limit of Microbial and Chemical Feeding in Foods which are for ALT $\leq3\times10^3$ and for AKK $\leq1\times10^2$. The presence of microbes in the extract can occur during the processing of drinks that are less hygienic, but the amount of contamination contained in the drink is still within the maximum limit of microbial contamination.

CONCLUSION

1. About 70% ethanol condensed extract and dry extract of black rice with maltodextrin filler had antioxidant activity of 37.55 bpj and 231.38 bpj, respectively.
2. Functional drinks extract of dried black rice with the addition of red ginger is a very powerful antioxidant because it has an IC_{50} value of 46.36 bpj.

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