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RESEARCH ARTICLE

Optimization of Temperature and Time of Ultrasonic-Assisted Extraction Method on Flavonoid and Antioxidant Activity of Brown Rice from Demak, Indonesia

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ABSTRACT

Brown rice contains a number of bioactive components, such as pigments and flavonoids, which act as antioxidant. Plants widely distribute polar flavonoid compounds, which actively function as antioxidant. Antioxidants are substances that can significantly inhibit or prevent the oxidation of the substrate. The body requires antioxidants to defend itself against attacks by free radicals. The goal of this study was to find the best temperature and time for the Ultrasonic-Assisted Extraction (UAE) method to measure the antioxidant activity and flavonoids in brown rice extract using UV-Visible spectrophotometry. The extract was prepared using the UAE method with 96% ethanol solvent at 45°C and 55°C for 20–30 minutes. Data obtained was statistically analyzed using the one-way ANOVA test. The study found that brown rice extracted with 96% ethanol at 45°C for 20 minutes produced 4.089 mg QE (Quercetin Equivalent)/g of total flavonoids. The antioxidant activity was the lowest, with an IC_{50} value of 381.102 ppm. The statistical analysis using the one-way ANOVA test revealed that there were no differences in temperature or time optimization that affected the levels of flavonoids and antioxidants. It can be concluded that the 96% ethanol extract of brown rice has the best flavonoid and antioxidant content. The best treatment was at 45°C and 20 minutes of extraction time.

Keywords: Antioxidant, brown rice, flavonoids, UAE

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Introduction

Rice is an essential food for billions worldwide to consume. Brown rice (*Oryza rufipogon*) has more benefits than white rice [1]. Brown rice contains a number of bioactive components, such as pigments and flavonoid compounds, which act as antioxidants [2]. Flavonoid compounds are phenolic compounds found in many plants. Flavonoids have red, blue, and purple colors [3]. Flavonoids are derived from C6-C7 units (phenyl-propane) that are sourced from phenylalanine and C6 units that are produced from the polyethylene pathway, thus forming a chain arrangement of C6-C3-C6. Flavonoids are composed of several groups: anthocyanins, flavanons, catechins, flavons, and flavonols.

Antioxidants are substances that, in small concentrations, are able to significantly inhibit the oxidation of the substrate. According to the sources obtained, antioxidants can be categorized into two types: synthetic and natural [4]. Synthetic antioxidants that are permitted and commonly used for food are tocopherol [5]. However, fresh vegetables and fruits contain the best natural antioxidants. The body requires antioxidants to defends itself against free radicals. Free radicals are reactive atoms or molecules with unpaired electrons that are unstable and very reactive to withdraw electrons from other molecules in the body to achieve stability. This can cause potential damage to biomolecules by damaging the integrity of lipids, proteins, and DNA, which leads to increased oxidative stress. Oxidative stress is linked to various health concerns, including diabetes mellitus, cardiovascular disease, and even cancer [6].

This research method of extraction using the UAE method that is an ultrasonic wave extraction method with a frequencies ranging from 20 to 2000 kHz. The UAE extraction method has an advantage over other methods [7]. Temperature and time are the key factors influencing extraction efficiency in UAE. The temperature and durations research carried out by Yuliantari *et al*. [8] on the influence of these factors on the flavonoid content and antioxidant activity of soursop leaves (*Annona muricata* L.) using ultrasonic with various temperatures (35°C, 45°C and 55°C) and durations (10, 20, and 30 minutes) using 96% ethanol as the solvent. Their findings, with a yield of 19.14% total 903.90 mg QE/g flavonoid, and the lowest IC_{50} value 258.155 mg/L at 45°C for 20 minutes serving as a valuable reference of this study. Therefore, researchers conducted this study to optimize the temperature and time of the UAE method for flavonoid levels and antioxidant activity in brown rice extract, using UV-Visible spectrophotometry.

Materials and Methods

Materials

1. Plants

The material used in this research was brown rice obtained from a mill in Tegalombo Village, Mlatiharjo, Gajah District, Demak Regency, and identified in the Laboratory Department of Biology at Universitas Ahmad Dahlan Bantul, Indonesia with identification letter No.135/Lab.Bio/B/III/2023.

2. Chemicals

The material used were 96% ethanol (Merck®), magnesium powder (Sigma®), concentrated HCl (Merck®), 10% NaOH solution, HCl 2N solution, ethanol p.a. (Smart-Lab®), 2% aluminum(III) chloride (Merck®), distilled water, quercetin, sodium acetate (Merck®), DPPH (2,2- Diphenyl-2-Picrylhydrazil) (Merck®).

Methods

Fifteen g of brown rice powder were weighed on an analytical balance and transferred to an Erlenmeyer flask. Next, 150 mL of 96% ethanol (1:10 w/v) was added to the flask. Then the mixture was extracted using a combination of temperatures (45°C and 55°C) and times (20 and 30 minutes) in an ultrasonic bath with a frequency of 47 kHz. After extraction, the solution was filtered with Whatman paper No. 1. Then, the obtained filtrate concentrated using a rotary vacuum evaporator.

1. Determination of Total Flavonoid Levels

Ten mg of brown rice extract were weighed and transferred into a 10 mL volumetric flask. Ethanol p.a. was added to the mark, resulting a solution with a concentration of 1000 ppm. One mL of this 1000 ppm was transferred into a 10 mL volumetric flask. Then 0.1 mL of 10% aluminum(III) chloride, 0.1 mL of 1 M sodium acetate solution, and ethanol were added to the mark of the flask. The mixture was shaken until homogeneous. The sample solution was incubated at room temperature according to the operating time results. Then the absorbance was measured at the maximum wavelength and replicated three times. For each absorbance value obtained, concentration was calculated using a linear equation from the standard curve of quercetin that had been measured [9].

2. Determination of Antioxidant Activity

Measurement of free radical scavenging activity used DPPH. A 1000 ppm extract solution was made by weighing 25 mg of extract, placing it in a 25 mL volumetric flask, then ethanol p.a. was added to the mark. Next, a series concentrations of 100, 200, 300, 400, and 500 ppm were made. A total of 1 mL of 0.4 mM DPPH solution was added to each sample solution concentration up to the limit mark. Absorption was measured by UV-Vis spectrophotometers at a wavelength of 50 nm.

Result

The result of phytochemical screening can be seen in **Table 1**.

Table 1. Phytochemical screening results

Secondary Method	Quantitative Test	Phytochemical Screening			
		Temperature 45^{0}C		Temperature 55^{0}C	
		20 Minutes	30 Minutes	20 Miutes	30 Minutes
Flavonoid	Wilstater test	Orange (+)	Orange (+)	Orange (+)	Orange (+)
	Bate-Smith test	Dark red (+)	Dark red (+)	Dark red (+)	Dark red (+)
	10% NaOH test	Dark brown (+)	Dark brown (+)	Dark brown (+)	Dark brown (+)

Determination of Flavonoid Levels

The result of flavonoid content of 96% ethanol extract of brown rice and the result of quercetin DPPH reduction by 96% ethanol extract can be seen in **Tables 2** and **3**.

Table 2. Flavonoid content of 96% ethanol extract of brown rice

Repetition	Flavonoid Content (mg QE/g)			
	Temperature 45^{0}C		Temperature 55^{0}C	
	20 Minutes	30 Minutes	20 Minutes	30 Minutes
1	2.346	1.715	2.184	2.200
2	4.461	3.230	4.161	3.807
3	5.461	5.292	5.169	5.423
Average ± SD	4.089± 1.590	3.412± 1.795	3.838± 1.518	3.810± 1.611

Table 3. Result of quercetin DPPH reduction by 96% ethanol extract of brown rice, with control absorbance 0.778

Value	Repetition	Quercetin	Quercetin DPPH Reduction (ppm)			
			Temperature 45^{0}C		Temperature 55^{0}C	
			20 Minutes	20 Minutes	20 Minutes	20 Minutes
IC_{50} (ppm)	RI	5.948	381.767	394.371	295.046	404.093
	RII	6.115	377.653	392.552	446.297	421.324
	RIII	5.848	383.887	433.097	423.712	400.765
Average		5.970	381.102	406.673	388.351	408.744

Discussion

Phytochemical screening tests were carried out on each 96% ethanol extract of brown rice to determine the presence of flavonoid compounds. The results presented in **Table 1**, confirmed that the 96% ethanol extract of brown rice contained flavonoids.

Determination of the total flavonoid content of 96% ethanol extract of brown rice was carried out using the $AlCl_3$ reagent reaction and using quercetin as a standard. The results of determination of the total flavonoid content of 96% ethanol extract of brown rice presented in **Table 2**.

Determination of the total flavonoid content of 96% ethanol extract was carried out using the $AlCl_3$ colorimetric method. As ilustrated in **Figure 1**, the principle of this method is that aluminum chloride forms a complex compound with the keto group on the C-4 atom and the hydroxyl group on the C-3 or C-5 atom from the flavon and flavonol groups [10].

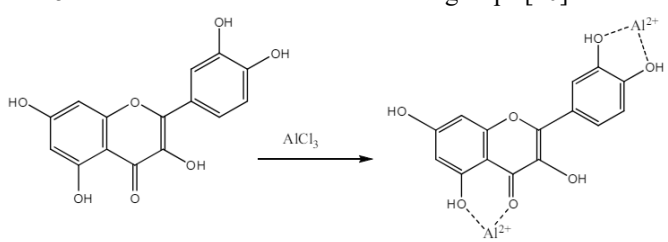

Figure 1. Formation of the quercetin-$AlCl_3$ complex compund

The result of total flavonoids obtained from three replications. The highest total flavonoids of 96% ethanol extract of brown rice was achieved from the extraction temperature of 45°C for 20 minutes with 4.089 mg QE/g of total flavonoids.

This study observed that increasing the extraction temperature and time did not significantly affect the total flavonoids content, even at 45°C extraction for 30 minutes it decreased. These findings align with Yuliantari *et al.* [8] research on soursop leaves extract using ultrasonics, where the optimal conditions was at a temperature of 45°C for 20 minutes produced 903.90 mg QE/g of total flavonoids. According to Winata and Yunianta [11] the longer extraction times, the quantity of extracted material also increases because the opportunity between material and solvent to meet is greater so the results will increase until the solution saturation point reached. Bioactive components such as flavonoids are not resistant in high temperatures above 50°C, so they have structural changes and produce inferior extracts. Very low temperature and short extraction time will produce low yield [12]. Ibrahim *et al.* [13] reported that the increasing of the temperature and extraction time needs to be considered, too high extraction temperatures and too long extraction times that exceed the optimum time limit are able to cause the loss of compounds in the solution due to evaporation, and if the extraction temperature is too low, not all active compounds are extracted from the material so that bring out the low levels of active compounds obtained.

The antioxidant activity of 96% ethanol extract of brown rice can be determined from IC_{50} value. Here, calculation of percent inhibition and IC_{50} of free antiradicals by brown rice extract and quercetin. Percent inhibition refers to the material's ability to inhibit free radical activity which is related to the concentration of a sample. The IC_{50} value is a parameter used to interpret the results of DPPH. Lower IC_{50} value of the sample indicate greater antioxidant capacity [14].

Based on the results in **Table 3**, it shows that the quercetin antioxidant activity test as a comparison has an IC_{50} value of 19.809 ppm. The IC_{50} value of brown rice extract at 45°C for 20 and 30 minutes were 381.102 and 406.673 ppm and the IC_{50} value of brown rice extract at 55°C for 20 and 30 minutes were 388.351 and 408.744 ppm, respectively. The IC_{50} values indicate that the 96% ethanol extract of brown rice has weak antioxidant activity, as extracts with IC_{50} values > 200 ppm are generally categorized as weak antioxidants.

The results revealed the brown rice extract with the lowest IC_{50} value was obtained using extraction temperature of 45°C for 20 minutes, which was 381.102 ppm. This is influenced by the total levels of flavonoids extracted from brown rice, the higher total flavonoids content, leading to stronger antioxidant activity. Perwiratami *et al.* [15] support this corelation, that higher total flavonoid contents leads to stronger antioxidants capacity. However, the IC_{50} value of brown rice extract reminds higher compared to soursop leaves extract as reported by Yuliantari *et al.* [8] where the IC_{50} value is 258.102 ppm.

Conclusion

Based on the results of research and data analysis carried out in this study, it can be concluded that the 96% ethanol extract of brown rice has the highest levels of total flavonoids and antioxidant activity obtained from the extraction temperature treatment of 45°C for 20 minutes.

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