Research Article

Antioxidant Activity of Hotong (Setaria italica) Seeds from Buru and its Implications for Biochemistry Learning

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Abstract.
Hotong Buru seeds (*Setaria italica*) are a local wisdom food ingredient in South Buru Regency, Maluku Province, Indonesia. These hotong seeds are widely consumed by the people of South Buru as a staple food substitute for rice. Hotong Buru seeds contain bioactive components that have antioxidant properties, including tannins and Vitamin E. Tannins are polyphenols, one of the anti-nutrients found in food ingredients. These components are mostly contained in the epidermis. This research aims to analyze the antioxidant activity of Hotong Buru (*Setaria italica*) seeds and their implication for Biochemistry Learning. Antioxidant analysis was conducted using 1,1-dephenyl-2-picryl-hydrazyl (DPPH) as a free radical. Descriptive analysis was used to describe the chemical composition and quality of Hotong Buru seeds in tables and graphs. Inferential analysis was done using one way ANOVA technique to analyze the effect of food quality. All data were presented descriptively, and antioxidant activity was calculated using the SPSS Program. The results showed that Hotong seeds from Buru Island positively contain secondary metabolite compounds such as alkaloids, flavonoids, terpenoids, steroids, tannins, and saponins. The content of these secondary metabolites can act as antioxidants for the human body. Antioxidants are very important to delay or inhibit damage to molecular compounds. In addition, the results of this study are implicated in biology learning, especially biochemistry, food science, and nutrition courses. The phytochemical test showed that there were antioxidants in Hotong Buru seeds ingredients, there are alkaloids, flavonoids, steroids, tannins, terpenoids, and saponins. The implications of research results for biology learning have been made clear in the form of practical instructions that have been applied to students of the Pattimura University Biology Education Study Program.

Keywords: Hotong buru, antioxidants, culinary ingredients, biochemistry learning

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Published: 11 November 2024

Publishing services provided by Knowledge E

Selection and Peer-review under the responsibility of the 8th Isedu Conference Committee.

1. Introduction

Free radicals can come from outside the body, such as pollution, dust, alcohol, and cigarette smoke, or from within the body, where they are continuously produced because of normal metabolism [1]. Antioxidants can delay or inhibit cell damage

because their molecular compounds are stable enough to finance electrons to reactive free radicals and neutralize them, thereby reducing their capacity to damage [2]. So far, antioxidant information from hotong seeds from South Buru is not available, so it is necessary to conduct research to determine the antioxidant content (chemical composition) of hotong buru culinary from South Buru Regency. These results can be one of the asdvantages of culinary products made from hotong seeds [3,4,5].Various Hotong Buru products have been used as culinary ingredients or ready-to-eat foods made from Hotong Buru (±350 types of culinary ingredients) and have received the Muri Record in 2016. In this culinary ingredient product, many additional food ingredients need to be analyzed. Antioxidants work by donating one electron to oxidant compounds so that their activity can be inhibited. The main cause of oxidative damage in the body is oxidant compounds, both in the form of free radicals and other reactive oxygen compounds that are oxidizing. Oxidative damage occurs when the body has low levels of oxidants and cannot compensate for the reactivity of oxidant compounds. the amount of hydrogen absorbed [6].

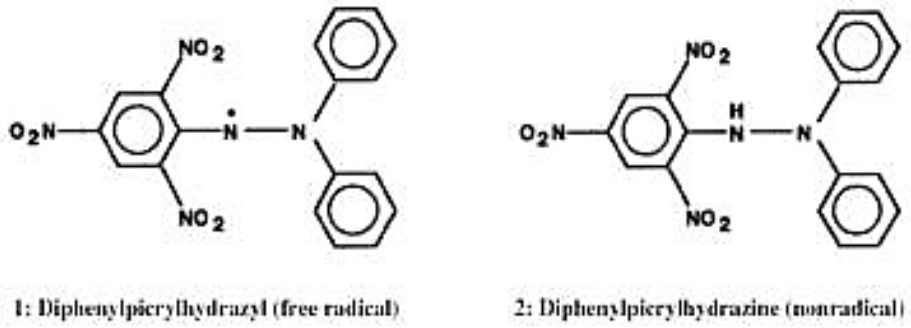

Figure 1: Reaction of DPPH with Antioxidant Compounds.

Antioxidant activity was further analyzed using a specimen tool. Antioxidant activity is then analyzed using UV-vis spectrophotometry based on the change in absorbance of DPPH at a certain wavelength. One of the parameters used to interpret the results of antioxidant activity testing with the DPPH method is the Efficient Concentration 50% (EC50) value, better known as the Inhibitory Concentration 50% (IC50). The results of DPPH test compounds are usually compared with the IC50 value of vitamin C, vitamin E, or quercetin, which are natural antioxidant compounds [7].

2. Method

The research type in this study is pure experimental research. The research location was in Fenafafan and Namrole sub-districts of South Buru Regency, Maluku Province,

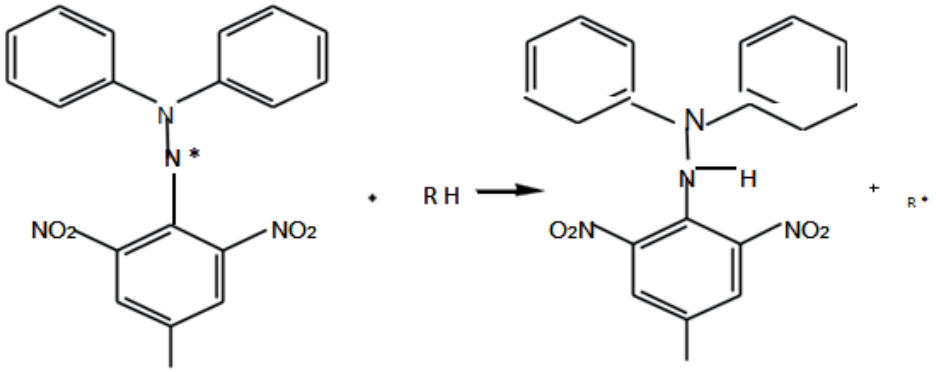

Figure 2: DPPH Reduction of Antioxidant Compounds.

Indonesia (Figure 3). This research has been taking place on February 2023 to June 2023.

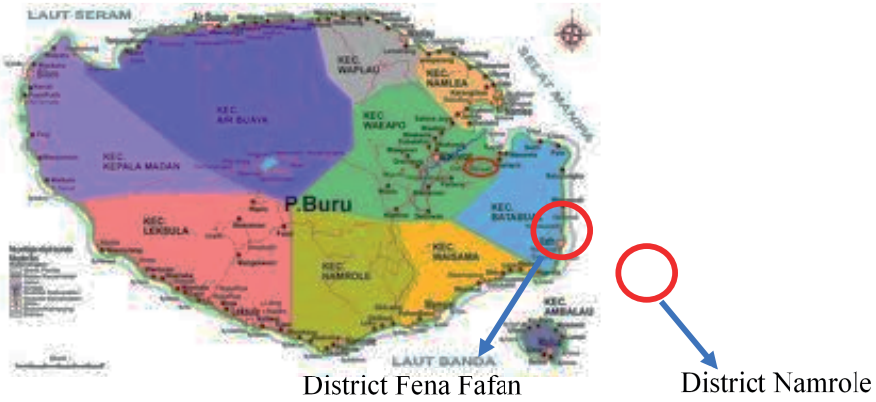

Figure 3: Research Location.

The population in this study is hotong (*Setaria italica*) seeds food ingredients located in Namrole District (Emori Hamlet, Km.7 and Km.9) and Fenafafan District (Waekatin and Mangaswaen). The sample in this research is Hotong Buru (*S. italica*) from Namrole District and Fenafafan District, South Buru Regency. Determination of variables in this study, namely independent variables: Type of Hotong Buru (*S. italica*). Active variable: antioxidant activity.

2.1. Materials

The tools used in this study are: Petri dish, oven, analytical scales, desiccator, 100 ml kjeldahl flask, 1000 ml kjeldahl flask, burette, hot plate, furnace, soxhlate, porcelain cup, erlenmeyer tube, funnel, filter paper, blender, centrifuge, rotary evaporator, vortex,

glassware, stirrer, water heater, shimadzu 1240 mini UV spectrophotometer. The materials used in this study are: Hotong Buru. NaOH, ethanol, methyl red, BCG, boric acid, H_2SO_4, hexanes, HCl alcohol, ascorbic acid, acetone, and 1,1- diphenyl-2-picrylhydrazyl (DPPH).

2.2. Analysis

Antioxidant test was conducted using 1,1-dephenyl-2-picryl-hydrazyl (DPPH) as a free radical. Samples were extracted using methanol. Wet samples such as Fena Fafan Namrole Sub-district 6, as much as 5 g, were cut into small pieces (+1 cm) then mashed, then put into an erlenmeyer and soaked with 100 ml of methanol for 1 hour on a shaker. The soaking solution was filtered and the filtrate was evaporated with a shaker. The immersion solution was observed and the filtrate was evaporated with a rotary evaporator at 400 C. The dried extract was then put into vials for testing. Testing began with the preparation of concentration series, namely 10, 25, 50, 75, 100, ppm. Each concentration series added 0.1 nM DPPH solution as much as 3 ml (1:3 v/v). Then, the sample and DPPH were vortexed for 1 minute then incubated for 30 minutes at room temperature. The absorbance was measured using a U-1240 Shimadzu mini-UV Spectrophometer at a wavelength of 517 nm. Inhibitory Concetration Value (IC50) was recorded as the amount of sample concentration to reduce DPPH concentration by 50%. Percentage of inhibition was calculated using the following formula:

$$Inhibition\ (\%) = \frac{Control\ OD - Sample\ OD}{Control\ OD} \times 100$$

Description :

Control OD = absorbance of control

Sample OD = absorbance of test sample.

The IC value50 is a number that indicates the concentration of the test sample that provides 50% silencing (able to inhibit or reduce the oxidation process by 50%). The IC value50 is determined by creating a linear curve between thse concentration of the test solution (x-axis) and the presntation of immersion (y-axis) from the equation y = a + bx and can be calculated with the IC 50 value using the formula [8,9]:

Description: y = % inhibition (50)

a = intercept (the intersection of the line on the y-axis)

b = slope

x = concentration

2.3. Data analysis technique

The data obtained in this study was analyzed using descriptive and inferential analysis.

Descriptive analysis was used to describe the chemical composition and quality of hotong buru in tables and graphs.

Inferential analysis using one way anova technique to analyze the effect of food quality of hotong buru-buru on panelists. Before analyzing using the anova technique, the data were first tested by Shapiro Wilk to determine the normality of the data and Levene'stest to determine the homogeneity of the data. The confidence level used is 0.05. One way is anova analysis, normality and homogeneity using SPSS for Windows 1 software.

3. Results and Discussion

3.1. Results of Proximate Analysis of Hotong Buru Seeds Ingredients (Table 1.)

TABLE 1: Results of Proximate Analysis of Hotong Buru Seeds Ingredients.

No.	Sample	Average				
		Fat	Ash	Water	Protein	Carbohydrate
1	Hotong Balls	19.76	1.27	9.31	6.23	64.43
2	Hotong Chips	24.59	0,98	6.39	4.89	63.57
3	Hotong Green	18.76	0,91	6.95	5.77	65.02
4	Hotong Raisins	13.40	1.07	6.34	7.09	66.19
5	Hotong Snowy	14.16	1.05	7.68	6.65	64.28
6	Hotong Croquettes	19.08	0,92	6.14	6.43	66.23

Based on Table 1. It can be explained about the results of proximate analysis of hotong seed-based food ingredients are the highest in carbohydrates.

3.2. Results of Antioxidant Activity

The results of antioxidant activity analysis of Hotong Buru (*S. italica*) seeds ingredients can be seen in Table 2.

Based on Table 2. above, it can be explained that the results of the phytochemical test of Hotong Buru (*S. italica*) seeds ingredients contain antioxidant ingredients in Hotong

Table 2: Phytochemical Test Results of Hotong Buru (*S. italica*) Seeds Ingredients.

Compounds	Phytochemical Test Results of Hotong Seeds from South Buru	Color
Alkaloid	Positif (+)	Yellow
Flavonoid	Positif (+)	Yellow
Terpenoid	Positif (+)	Red
Steroid	Positif (+)	Blue
Tanin	Positif (+)	Foam
Saponin	Positif (+)	Foam

Source: Primary Research Data, 2023

Buru (*S. italica*) products, namely Alkaloid, Flavonoid, Terpenoid, Steroid, Tannin and Saponin.

Table 3: DPPH (1,1-dephenyl-2-picrylhydrazyl) Assays.

No.	Sample	Concentration (%)	Absorbants	Inhibition Value	IC50 ppm
1.	Raw Material	40	0,507	27.36	481.5642
		80	0,504	27.79	
		120	0,498	28.65	
		160	0,459	34.24	
2.	Hotong based food	40	0,541	22.49	806.3867
		80	0,535	23.35	
		120	0,521	25.36	
		160	0,512	36.16	

Source: Primary Data, 2023

Table 3 shows the antioxidant activity test results and IC50 of raw materials and processed foods from hotong seeds. The average inhibition value of raw materials is 29.51 and the average inhibition value of hotong seed-based food is 26.84. this indicates that the inhibition value of raw materials is higher when compared to the inhibition value of hotong seed-based food. However, the IC50 test results for raw materials and hotong seed-based food showed that the IC50 value of hotong seed-based food was greater than the IC50 value of raw materials.

4. Discussion

The results of the research that has been carried out, namely antioxidant-analysis of Hotong Buru (S. italica) materials, it can be explained that from the Results of

the phytochemical test of culinary materials assessed diphenyl-1-piorhydrocyll (DPPH), shows that Hotong Buru has many antioxidants recorded. Antioxidants are proton-giving compounds that can counteract or reduce the negative effects of oxidants. Antioxidants work by donating one electron to the oxidant compound so that the activity of the oxidant compound can be inhibited [10].

Based on the results of observation, each sample has antioxidant activity as seen from the color change from purple to yellow. According to [11], that the change of purple to yellow color complex on antioxidant activity test indicates the presence of antioxidant compounds in a sample (Figure 1). The purple color complex on DPPH is due to antioxidant compounds that undergo a reduction process/give hydrogen ions and form DPPH-H will cause the purple color complex to fade.When the purple DPPH solution merges with the electron donor material, DPPH will be reduced, causing the purple color to fade and mixed with yellow [12].

Flavonoids which are polyphenolic compounds have the ability to protect hydrogen atoms in free radical compounds, so the antioxidant activity of polyphenolic compounds can be obtained in the neutralization reaction of free radicals or stopping the chain reaction that occurs [13]. Saponins are able to reduce superoxide through the formation of hydroperoxide intermediates so as to prevent biomolecular damage from free radicals [14]. Antioxidant activity in steroid and triterpenoid compounds is a class of phenolic compounds, which are compounds with OH groups directly bound to aromatic carbon carbon groups.

This phenolic compound has the ability to protect hydrogen atoms, so that free radical DPPH can change into a more stable form. The more hydroxyl groups the phenolic compound has, the higher the antioxidant activity obtained [15]. Phenol and flavonoid compounds have a linear contribution to antioxidant activity, so the higher the phenol and flavonoid levels, the better the antioxidants [16]. The high total phenolic content in the stem bark extract is also thought to have an important role as an antioxidant.

The implications of the results of this study on biology learning in the form of a practicum guide for students. This research is related to the course on biology learning, namely biochemistry. Biochemistry is the chemistry of materials and processes that occur in the bodies of living things, and attempts to understand the process of life from the chemical side. In biochemistry learning is inseparable from practicum activities, practicum activities themselves are carried out with the aim of providing more understanding through a series of processes that are experienced themselves so that a learning material can be understood.

5. Conclusion

Phytochemical test results show that there is antioxidant activity in Hotong Buru seeds. This antioxidant activity is characterized by the presence of alkaloids, flavonoids, steroids, tannins, terpenoids and saponins. The IC50 test results for raw materials and hotong seeds based food showed that the IC50 value of hotong seeds based food was greater than the IC50 value of raw materials.

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