Original article

Total Phenolic Content and Antioxidant Activity of Germinated Black Rice Variety *Krisna* Extract, Indonesia

Sudana Fatahillah Pasaribu[1], Budiyanti Wiboworini[2], Lilik Retna Kartikasari[3]

Abstract:

Background: Indonesia is an agricultural country and the third-largest rice producer in the world. Pigmented rice tends to contain a lot of nutrients and bioactive compounds, namely phenols, anthocyanins, fiber, amino acids, tocopherols, and *γ-oryzanol,* and has antioxidant properties. Phenols are bioactive compounds and antioxidants which are beneficial for health. Since the synthetic antioxidants have carcinogenic properties, natural antioxidants used in this study. One of the plants that have the potential to have antioxidant activity and phenol content is black rice variety *krisna* from Sleman district, DI Yogyakarta, Indonesia. The germination process in rice can theoretically increase its bioactive compounds, thereby increasing the potential for antioxidant activity. ***Objective:*** To analyze the antioxidant activity and total phenol content of the germinated black rice extract variety *krisna* in Sleman district, D.I. Yogyakarta, Indonesia. ***Materials and Methods:*** This type of research was quantitative with laboratory experimental methods. The research sample was germinated black rice of a variety *krisna* from Indonesia. The extraction method used maceration, the total phenol analysis method used the Folin-Ciocalteu method, and the antioxidant activity analysis used the DPPH-method Free Radical Scavenging Assay. ***Results and Discussion:*** The germinated black rice extract of varieties *krisna* Indonesian has a total phenol content of 2269 mg/g and antioxidant activity 71.05%. ***Conclusion:*** Germinated black rice extract of variety *krisna* Indonesian contains total phenol and antioxidant activity so that it has the potential to have health benefits.

Keywords: Germinated black rice, antioxidant, phenol, Indonesia.

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Introduction

Indonesia is an agricultural country where significant part of the population plays a role in the crop and food sector[1]. This is in line with the BPS Research Report 2018, in which Indonesia is the third-largest rice producer in the world[2]. According to *World Agricultural Production* 2020, rice is a staple food ingredient for some people in the world and including Indonesia, with a total rice consumption of 90%[1,3,4]. Rice consists of various types, namely white, brown, red, purple, and black rice[5]. Pigmented rice tends to contain a lot of nutrients and bioactive compounds, namely phenols, anthocyanins, fiber, amino acids, tocopherols, and γ-oryzanols, therefore consuming they will have health benefits because they are antioxidants[6].

Antioxidant compounds in food are generally called exogenous antioxidants[7]. Exogenous antioxidants are needed by the body to prevent and reduce

1. Dept. of Nutrition, Postgraduate Program, Universitas Sebelas Maret, Surakarta, Indonesia.
2. Dept. of Nutrition, Faculty of Medicine,Universitas Sebelas Maret, Surakarta, Indonesia.
3. Dept. of Animal Science, Faculty of Agriculture, Universitas Sebelas Maret, Surakarta, Indonesia.

Correspondence to: Sudana Fatahillah Pasaribu, Dept. of Nutrition Sciences, Postgraduate Program, Universitas Sebelas Maret, Surakarta, Indonesia. E-mail: sudanafatahillah@gmail.com

oxidative stress in the body[8]. The antioxidant mechanism works, namely by giving one electron to a compound that has oxidant properties. Antioxidant activity is the ability of components of a nutrient or bioactive compound to counteract oxidation reactions from free radicals which can be determined from the amount of antioxidants[9]. Free radicals are compounds that have unpaired electrons which are reactive, unstable, and cause damage to important molecules and cells in the body. The body can also produce free radicals in the form of secondary oxygen from metabolism. The body can also balance the production of endogenous antioxidants, but if there is oxidative stress, its activity can decrease[7].

Phenolic compounds are secondary metabolite bioactive compounds produced in shikimic acid and pentose phosphate through phenylpropanoid metabolism. Phenolic compounds are antioxidants and have one or more hydroxyl groups attached to the benzene ring and have variations from simple molecules to complex polymers[10]. Phenol compounds are divided into several subgroups, namely phenolic acids, flavonoids, tannins, lignans, lignins, coumarins, and stilbenes[11]. These phytochemical compounds have many functions such as giving color and taste to food and are beneficial for health[12]. Several studies reported phenol compounds and antioxidant activity to have many health benefits such as antidiabetic, anti-inflammatory, anti-cancer, and anti-hypertensive[13–17]. Therefore, humans need antioxidants to prevent and treat disease.

The human body does not have large amounts of antioxidant reserves, so it is in dire need of exogenous antioxidants. Antioxidants based on the source consist of two, namely natural and synthetic antioxidants. Natural antioxidants are antioxidant compounds that are sourced from fruits, vegetables, spices, and other plants[18]. Meanwhile, synthetic antioxidants are artificial antioxidants such as Butylated Hydroxytoluene (BHT), Propyl Galat (PG), and Butylated Hydroxyanisole (BHA). These synthetic antioxidants are carcinogenic, so it is feared that they can have side effects that are harmful to health[19]. There are concerns about the side effects of synthetic antioxidants so that natural antioxidants are an alternative to be developed in this study. Plants that have antioxidant activity and phenol compounds are mostly found in grain plants[20]

One of the grains that have the potential to have antioxidant activity and high phenol content is the black rice *krisna* variety from Sleman district, DI Yogyakarta, Indonesia.

According to research Wanti and Parnanto,[21] black rice (*Oryza sativa L)* contains components of phytochemical compounds that can act as high antioxidants compared to brown and white rice[21]. Black rice can also be enhanced the content of bioactive compounds with process germination[3]. Germination is a process of embryo growth that causes physiological changes in black rice after absorbing water. The germination process will change the value of phytochemical compounds and the nutritional content to be better[22]. This is because in germination an active enzymatic process occurs, thus affecting changes in bioactive compounds[23]. Therefore, germinated black rice extract variety of *krisna* has the potential to contain antioxidant activity and total phenol. However, currently, the research that examines the analysis on the germinated black rice extract of *krisna* variety has never been carried out. Based on this study, the researchers aimed to analyze the antioxidant activity and total phenol content of the germinated black rice extract *krisna* variety in Sleman district, DI Yogyakarta, Indonesia.

Materials and Methods

Research Design

The type of research was quantitative with laboratory experimental methods. Preparation of germinated black rice, extraction of germinated black rice, phenol analysis, and antioxidant activity were carried out at the Laboratory of the Center for Food and Nutrition Studies, Gadjah Mada University, Yogyakarta, from March to May 2021. The rice samples used were local black rice varieties krisna obtained from Sleman Yogyakarta, Indonesia.

Materials

Perforated trays, flannelette, filters, digital scales, containers, cabinet dryer, disk mill machines, 60 mesh filters, rotary evaporators, filter paper, spatulas, measuring cups, micropipettes, orbital shakers, black rice spectrophotometers, water, germinated black rice flour, 80% ethanol, 3% citric acid and distilled water.

Germination of Black Rice

Black rice was sorted and cleaned, then soaked black rice with water (1: 1 = water: rice) for 6

hours at room temperature then drain. In the next stage, the black rice was sprinkled on a hollow container that has been placed in a wet flannel and covered with a wet flannel cloth. Every 12 hours, flush with water for 48 hours. If the black rice has germinated with a shoot length of 0.5-1 mm, then the germinated black rice can be dried in a cabinet dryer with a temperature of 50°C for 5 hours. Then the germinated black rice was powdered with a disk mill and filtered using a 60 mesh filter[3,24].

Germinated Black Rice Extract

The germinated black rice flour weighed 100 g and add 80% ethanol at a ratio of 1: 5. The solvent was acidified with 3% citric acid (ratio 85:15 (v / v) then let stand for 1 x 24 hours, and the solution is stirred with an orbital shaker for 4 hours at a speed of 150 rpm. Furthermore, the solution was centrifuged at a speed of 4000 rpm for 30 minutes. The supernatant was filtered with a paper filter and rotary evaporator temperature of 50°C to obtain germinated black rice extract[3,25,26].

Determination of Antioxidant Activity

Determination activity antioxidant extracts of germinated black rice used DPPH-Free Radical Scavenging Assay[27].

1. Determination of the maximum wavelength of DPPH by measuring 4.0 ml of 50 ppm DPPH solution with a Uv-Vis spectrophotometer in the wavelength range (400-600 nm), so that the maximum wavelength was found.
2. The extracted sample was diluted to a concentration of 10 mg / mL using methanol which was put into a test tube.
3. Reagent solution was added 1 mL of 2,2-Diphenyl-1- Picrylhydrazyl (DPPH) 200 µM.
4. The mixed solution was then incubated in a dark room for 30 minutes.
5. The solution was further diluted to 5 ml used methanol. Make a blank solution (1 ml of DPPH solution + 4 ml of ethanol).
6. Then take the absorbance measurement with a wavelength of 515 nm using a spectrophotometer Uv-Vis.
7. The measurement of the percentage of free radical scavenger (% total antioxidant activity) was calculated used the equation:

$$\%\ \text{Total Antioxidant Activity} = \frac{(A0 - A1)}{A0} x\ 100\%$$

A0: Absorbance Standard

A1: Absorbance Sample

Determination of Total Phenol Levels

Phenol levels were determined using the Folin-Ciocalteu method, and the research of Chatatikun et al. was referred[28]. It was observed weighed a sample of 50 µl of germinated black rice extract, dissolved in 50 µl of distilled water, and made with a concentration of 10,000 ppm. The solution was added with 50 µl of 10% folin and 50 µl of bicarbonate (60 g / L). The solutions were incubated for 60 minutes at room temperature. The absorbance was seen using a spectrophotometer at a wavelength of 730 nm. The absorbance obtained was recorded and then calculated using the phenol standard curve. The standard solution for the total phenol test used was phenolic acid 114 mg diluted with 1000 ml distilled water at a concentration so that the phenol solution used was 0.114 mg/ml.

Results

Data on percentage yield of germinated black rice extract

Dry powder of germinated black rice was macerated using 80% ethanol and 3% citric acid as solvents. Maceration is done by soaking germinated black rice powder with solvent and allowed to stand for 24 hours. The obtained filtrate was concentrated to produce a thick extract of germinated black rice with a dark purple color. The results of the thick germinated black rice extract were listed in Table 1. Table 1 shows that the percentage yield of germinated black rice extract was 9.94%, from the initial weight of 100g of Simplicia. After becoming a thick extract, it was 9.94 g.

Table 1. Percentage results of yield germinated black rice extract

Sample	Flour Weight	Thick Extract	Yield
Germinated black rice	100 g	9.94 g	9.94%

Data Antioxidant Activity Analysis

Antioxidant activity of the germinated black rice extract was analyzed using the DPPH-Free Radical Scavenging Assay parameters % method. Table 2 shows that analysis of the highest germinated black rice extract was found in the second analysis, which was 71.11%, while the second analysis of

antioxidant activity was 71%. The results of the analysis antioxidant activity of the two germinated black rice extracts when averaged, results were 71.05%. Then, the standard deviation value from the first and second analysis of antioxidant activity was calculated, which was ± 0.07.

Table 2. Antioxidant activity of germinated black rice extract

Result	Antioxidant activity	Mean	SD
Analysis 1	71.00%	71.05%	± 0.07
Analysis 2	71.11%		

Data Total Phenol Analysis

Total phenolic extract germinated black rice was analyzed by using the Folin-Ciocalteu method by making a standard phenol curve to produce a linear regression equation. Total phenolic content was calculate using a regression linear presented in Figure 1. Then the results of these equations are used to calculate the total phenolic content in the germinated black rice extract, by entering the absorbance of the extracted sample obtained on the spectrophotometer as the Y value. The results of analysis total phenol are given in Table 3. Table 3 shows that the analysis of the highest germinated black rice extract was found in the first analysis, which was 227.4 mg/g, while the second analysis of total phenol was 226.5 mg/g. The results of the analysis of the total phenol of the two germinated black rice extracts when averaged, the results were 226.9 mg/g. Next, the standard deviation value from the first and second analysis of antioxidant activity was calculated, which was ± 0.63.

Table 3. Total phenol germinated black rice extract

Result	Total phenol	Mean	SD
Analysis 1	227.4 mg/g	2269 mg/g	± 0.63
Analysis 2	226.5 mg/g		

Discussion and Conclusion

The germinated black rice was used Krisna from local varieties, Sleman, D.I.Yogyakarta, Indonesia. The germination process is carried out to increase the bioactive content of black rice. Cho et al.,[29] found pigmented rice after germination process would changed in bioactive chemical compound so that it will affect the addition of new bioactive chemical compounds, increased of bioactive chemical compounds, and nutrients in germinating rice. After the black rice was germinated, the drying process was carried out to reduce the moisture content in the germinated black rice to prevent damage and rot which is influenced by enzymatic reactions, fungi, and bacteria[30,31].

The germinated black rice flour used was 100g, then extracted using the maceration method. Maceration method was an extraction method that used room temperature and recommended for this study. In addition, hot extraction methods will have an impact on decreasing the total phenol content and antioxidant activity in germinated black rice. In general, maceration extraction uses a solvent. The function of solvent fluids was to push out bioactive compounds from the cell through the entry of solvents into the cell walls and into the cell cavities in plants that have bioactive substances[30]. In another study Dhianawaty et al.,[32] observed that that maceration methods was applied in isolating total phenolic and antioxidant activity. In addition, maceration extraction yields high yield amounts[33–36].

The germinated black rice flour was then macerated with 80% ethanol and 3% citric acid as a solvent (85:15). In a similar study by Kristiana et al.,[37]found ethanol and citric acid solvents more effective than others to binding phenol bioactive compounds, in addition mold cannot live in ethanol. Ethanol solvent compared to aquadesh has a high effect on binding phenolic compounds[25]. The thick purple extract results were listed in Table 1 showing the amount of thick extract was 9.94 g and the yield of germinated black rice extract was 9.94%.

The thick extract obtained in Table 1 has a dark purple color with a yield of 9.94%. A recent study stated the greater yield produced would more efficient treatment applied without compromising other properties[38]. The yield of this study was higher than that of Maulida and Guntari[39]. it was found that the average yield value of black rice extract was 6.7%. The difference in results is presumably because the filter size used was 20/40 mesh so that it affects the size of the simplicia particles filtered. Ardyanti et al.,[40] reported that different of yield influenced by particle size of simplicia and maceration time so that our research findings are in line with Antari's study stated the particle of simplicia with 60 mesh filtering had the highest average yield value compared to the simplicia particles filtering 40 mesh.

Total Phenolic

The compounds are the largest group of distributed secondary metabolites that have an aromatic ring[20]. Phenolic compounds can be found in both edible and non-edible plants. Phenolic bioactive components are widely found in cell walls and plant vacuoles which have a distinctive aroma and function to prevent decay[20,42].

The analysis of the total phenolic content of germinated black rice extract was shown in Table 2. These results were obtained using the Folin-Ciocalteu method. This method was often used as a standard in assessing total phenolic content because it was a fast and simple method. The absorbance of that suspension was Spectrophotometrically measured at 730nm wavelength and then total phenolic was calculated using a regression linear Y = 8.137X + 0042, with a correlation value of R2= 0.997. The linear calibration curve showed in Figure 1.

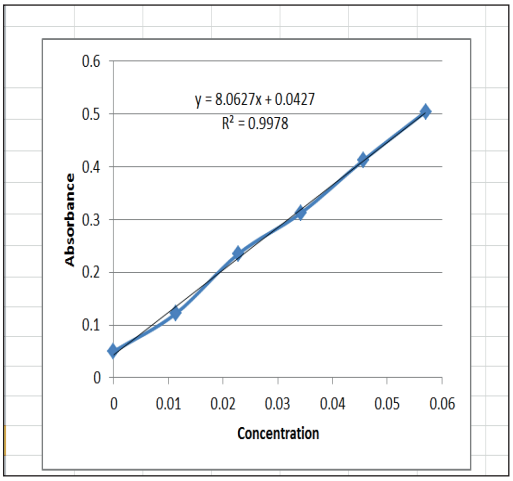

Figure 1: The linear calibration curve for phenol standards

Based on the results of the calculation of the total phenolic content in the germinated black rice extract, it was 226.9 mg/g. Phenolic compounds are bioactive substances that have antioxidant properties, especially through their redox properties, which allow phenolic compounds to act as reducing agents, hydrogen donors, and have biological activity that can help maintain the body's metabolic system[43].

Total phenolic of germinated black rice extract in our study has potential to beneficial effects as antioxidant.

Antioxidant Activity

The activity of phenolic compounds has different structures such as OH bonds, dissociation energy, delocalization of phenol radical resonances, and steric inhibitions derived from hydrogen substitution in an aromatic ring[44]. The Phenolic compounds as antioxidants through their ability to scavenge free radicals. The higher the percentage of synthetic free radical inhibition, the greater the antioxidant potential[45].

DPPH is a method of analyzing antioxidant activity which is widely used in plants. DPPH oxidant activity analysis method is to use stable free radicals on DPPH which have unpaired electrons on the nitrogen atomic bridge[46]. The solvent used in the DPPH analysis is methanol or methanol buffer, each of which is appropriate as an analysis of the antioxidant activity of extracts that are less polar or nonpolar. DPPH assay is considered a simple, easy, fast and sensitive method to applied therefore it requires a small sample size. It is easy to apply because the DPPH radical compound used is relatively stable compared to other methods[47].

The working principle of the DPPH method is through a donation of hydrogen atoms (H +) from the substances tested on the DPPH radicals to become non-radical compounds of diphenyl picryl hydrazine which will be shown by color changes. DPPH will react to the reducing agent according to the electrons so that it becomes paired, and the solution loses color stoichiometry depending on the number of electrons taken[47,48]. The extract contains antioxidant activity which can be seen through its ability to reduce the purple color of the DPPH radical so that it becomes a yellow color of the DPPH-H compound which can be detected at 515-517 nm[49].

The results of our study reported that germinated black rice extract has ability to scavenging free radicals showed in Table 3. The data show that the inhibitory ability of phytochemical compounds in germinated black rice extract was 71.05% + 0.07 and it is caused germinated blck rice extract has phenolic compounds. Phenolic compounds have been shown to provide hydrogen atoms to purple DPPH free radicals to form yellow DPPH compounds. The presence of antioxidant activity in germinated black rice extract shows potential as a source of antioxidants.

The balance of oxidants and antioxidants is very important because it is related to body health. Antioxidants will protect the body's cells against oxidative damage and inhibit the formation of

oxidative products. The imbalance resulting from high oxidants compared to antioxidants has an impact on the production of Reactive Oxygen Species (ROS) and excess oxidative stress cause several degenerative diseases[50,51]. Several studies have also stated that antioxidants have many health benefits such as anti-diabetes, anti-obesity, anti-aging anti-inflammatory, anti-hypertension, prevention of cardiovascular disease, and Alzheimer's[52–56].

Conclusion

Based on the results and discussion of this study, it can be concluded that the germinated black rice extract of the Indonesian *krisna* variety has a total phenol content of 226.9 mg/g and an antioxidant activity value of 71.05%. Germinated black rice extract also has the potential to have health benefits such as anti-diabetes, anti-dyslipidemia, anti-obesity, and other health benefits due to its phenolic content and antioxidant activity.

Recommendations

It is necessary to carry out further research related to the effect of giving germinated black rice extract on various degenerative diseases.

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Data gathering and idea owner of this study: Sudana Fatahillah Pasaribu

Study Design: Sudana Fatahillah Pasaribu

Writing and submission of manuscript: Sudana Fatahillah Pasaribu, Budiyanti Wiboworini, Lilik Retna Kartikasari.

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