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Short Communication
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Total Phenolic Content in Black Rice (*Oryza sativa* L. *indica*) Bran Ethanolic Extract from Two Different Regions in Java, Indonesia

Perbandingan Kadar Fenolik Total pada Ekstrak Etanol Beras Hitam (*Oryza sativa* L. *indica*) dari Dua Provinsi di Pulau Jawa, Indonesia

Afifah Kusuma Vardhani[1], Nadia Bunga Anggraini[1], Mahdi Jufri[2], Erni Purwaningsih[3], Hidayah Sunar Perdanastuti[4]

[1]Department of Pharmacy, Faculty of Health Sciences, Esa Unggul University,
Jl. Arjuna Utara No.9, Duri Kepa, Kec. Kb. Jeruk, Kota Jakarta Barat, Daerah Khusus Ibukota Jakarta 11510, Indonesia
[2]Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, University of Indonesia,
Margonda Street, Pondok Cina, Beji, Depok, West Java 16424, Indonesia
[3]Department of Pharmacy, Faculty of Medicine, University of Indonesia,
Salemba Street, Kenari, Senen, Central Jakarta 10430, Indonesia
[4]School of Pharmaceutical Sciences, Universiti Sains Malaysia, Chancellory, Georgetown, Penang 11800, Malaysia

**Corresponding author: afifah.kusuma@esaunggul.ac.id; (+62)85229280880*

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ABSTRACT

Rice bran, a rice by-product yielded by the milling process, has recently been recognized as a potential source of natural active constituents. Black rice bran has the highest antioxidant capacity compared to white and brown rice bran. It is correlated with the contribution of phenolic compounds in rice bran. The purpose of this study was to determine the total phenolic content in black rice (*Oryza sativa* L. indica) bran ethanolic extract from two different regions in Java, Indonesia, using Folin-Ciocalteu Reagent (FCR). Black rice bran samples used in this study were planted in Ciletuh Geopark Sukabumi, West Java (BRBE1), and Karanganyar Regency, Central Java (BRBE2). The parameters observed were total phenolic content, and the result was statistically tested with an unpaired t-test by GraphPad Prism 8.3.0 application. The samples contained 175.48 mg GAE/g extract in BRBE1 and 174.39 mg GAE/g extract in BRBE2. There was no significant difference based on these results. It can be concluded that both samples contained phenolic compounds and statistically, there was no significant difference.

Keywords: black rice bran, ethanolic extract, total phenolic content, Folin-Ciocalteu Reagent

ABSTRAK

Bekatul merupakan produk sampingan yang dihasilkan dari proses penggilingan padi. Dewasa ini, bekatul telah popular dengan adanya potensi komposisi aktif alami yang dimilikinya. Dibandingkan dengan bekatul beras putih dan merah, bekatul beras merah memiliki kapasitas antioksidan yang paling tinggi. Hal ini sebanding dengan kontribusi komponen fenol pada bekatul beras hitam. Tujuan dari penelitian ini adalah untuk menetapkan kadar fenolik total pada ekstrak etanol bekatul beras hitam (*Oryza sativa* L. *indica*) yang diperoleh dari hasil pertanian di Pulau Jawa menggunakan *Folin-Ciocalteu Reagent*. Ekstrak bekatul beras yang digunakan berasal dari Ciletuh Geopark, Sukabumi, Jawa Barat (BRBE1) dan Kabupaten Karanganyar, Jawa Tengah (BRBE2). Parameter yang ditetapkan yaitu kadar fenolik total dan hasilnya secara statistik diuji menggunakan uji-t 2 sampel independen pada aplikasi GraphPad Prism 8.3.0. Kadar fenolik total pada BRBE1 dan BRBE2 menunjukkan hasil sebesar 175.48 mg GAE/g ekstrak dan 174.39 mg GAE/g ekstrak. Pada BRBE1 dan BRBE2 tidak terdapat perbedaan kadar fenolik total yang bermakna. Ekstrak etanol beras hitam dari Ciletuh Geopark, Sukabumi, Jawa Barat dan Kabupaten

Karanganyar, Jawa Barat mengandung komponen fenolik dan tidak terdapat perbedaan yang signifikan secara statistik pada keduanya.

Kata Kunci: bekatul beras hitam, ekstrak etanol, kadar fenolik total, *Folin-Ciocalteu Reagent*

INTRODUCTION

Rice bran is a by-product of rice processing obtained through the milling process (Zaky et al., 2022). Rice bran contains fiber and minerals (Nam et al., 2006), fat, protein, amino acids, phenolic components (Parrado et al., 2006; Zaky et al., 2022), gamma oryzanol (Chen & Bergman, 2005), some bioactive compounds such as vitamin E (tocopherols and tocotrienols) and anthocyanins, which are mainly found in pigmented rice (Nam et al., 2006).

Data on world black rice production in 2003 stated that China was the country with the highest black rice production (62%). The next black rice-producing country is Sri Lanka (8.6%), followed by Indonesia (7.2%), India (5.1%), the Philippines (4.3%), Bangladesh (4.1%), Myanmar, Malaysia, and Thailand (Chaudhary, 2003).

Depending on the region, several different names exist for this type of rice in Indonesia. In Solo, black rice is known as "beras wulung", while the people of the Cibeusi area, Subang, West Java, are called "beras gadog". "Cempo ireng", "beras jlitheng", and "beras melik" are names for black rice in the Bantul and Sleman areas, Yogyakarta (Raharjo et al., 2019).

The taxonomic position of black rice according to Tjitrosoepomo (2005), is:

Kingdom: Plantae
Division: Spermatophyta
Subdivision: Angiosperms
Class: Monocotyledoneae
Nation: Poales (Glumiflorae)
Tribe: Poaceae (Graminea)
Clan: Oryza
Species: *Oryza sativa* L. *indica*

Black rice is one of the most potential local commodities for further development in Ciletuh Geopark, Sukabumi, West Java (Rini & Sofiani, 2018). While Karanganyar is one of Central Java areas with black rice as one of its main agricultural products (Putra et al., 2017).

Rice bran is obtained through the rice milling process (Zaky et al., 2022). The ethanolic extract of black rice bran contains gamma oryzanol of 118.572 mg/g and inhibits the work of the tyrosinase enzyme with an IC50 value of 74.8 g/mL (Vardhani et al., 2020). Gamma oryzanol in rice bran is a collection of ferulic acid, which is a phenolic compound (Ruen-Ngam et al., 2014; Trinovita et al., 2018). Ferulic acid is the dominant phenolic acid in the bran (Laokuldilok et al., 2011), which is 56-77% of the total phenol (Goufo & Trindade, 2014).

In this study, the phenolic content was determined in the extract of black rice bran from Ciletuh Geopark, Sukabumi, West Java (hereinafter referred to as BRBE1) and Karanganyar Regency, Central Java (hereinafter referred to as BRBE2).

METHODS

1. Black Rice Bran Extract

Black rice bran (Oryza sativa L. indica) BRBE1 and BRBE2 were extracted using the maceration method with 96% ethanol as the solvent, with a solvent:sample ratio of 4:1 (Ruen-Ngam et al., 2014).

2. Standardization: Specific and Non-specific Parameters of Black Rice Bran Extract

Parameters measured in this study were water content using Moisture Analyzer, ash content and insoluble ash content using the gravimetric method (Singh et al., 2013), heavy metal detection using the Atomic Absorption Spectrophotometry method (Akman et al., 2007), and molds/yeasts detection using the pour cup method (Sanders, 2012).

3. Total Phenolic Content Count

The gallic acid standard was utilized because of its stability. A standard curve was prepared with concentrations of 4 ppm, 8 ppm, 12 ppm, 16 ppm, and 20 ppm. A total of 20 µl of standard solution and 100 µl of *Folin-Ciocalteu Reagent* (FCR) were mixed homogeneously in a 96-well microtiter plate and then incubated at room temperature for 5 minutes. 80 µl of sodium carbonate solution was added to the mixture. Incubated for 120 minutes at room temperature again. The absorbance was measured at 720 nm using a microplate reader. Each standard solution of gallic acid was analyzed for absorption by linear regression equation:

$$Y = a + bx$$

A total of 20 µl of extract in absolute methanol and 100 µl of FCR were mixed homogeneously in a 96-well microtiter plate. Then, incubation was carried out at room temperature for 5 minutes. An 80 µl of sodium carbonate solution was added to the mixture. Incubated for 120 minutes at room temperature again. The absorbance was measured at 720 nm using a microplate reader. Calculating the total phenolic content yielded gallic acid equivalents per g (GAE/g) of extract extrapolated from the gallic acid calibration curve (Farasat et al., 2013; Shannon et al., 2018).

The result was statistically analyzed with an unpaired t-test by GraphPad Prism 8.3.0 application.

RESULTS & DISCUSSION

Ethanol extract of black rice bran was obtained with a solvent:sample ratio of 4:1 (Ruen-Ngam et al., 2014). Galanakis et al. (2013) stated that the hydroxyl group in phenol can form hydrogen bonds with oxygen in ethanol. The alcohol groups in the phenol group, such as ferulic acid and vanillic acid, also have hydrogen bonds with oxygen in ethanol. In rice bran, the ferulic acid content in total phenol is 56-77% (Goufo & Trindade, 2014). 96% ethanol has more water content than pure ethanol, resulting in its ability to attract more hydrophilic compounds to phenol. Organoleptically, the extract obtained was in the form of a dark purple viscous extract, odorless.

The results of plant determination showed the identity of the Latin name *Oryza sativa* L black rice with the part used, namely bran. In the measurement of the water content of the extract, the results shown were 7.29% in BRBE1 and 7.16% in BRBE2. These results have met the standard parameters of the water content in the extract, which is below 10%. Measuring water content using a Moisture Analyzer has the advantage of obtaining water content results quickly. Other non-specific standard parameters on BRBE1 and BRBE2 showed ash content of 3.34% and 3.05% and acid-insoluble ash content of 0.03% and 0.26%, respectively. In each extract, no heavy metals and molds/yeasts were detected. These results have met the standard extract parameters (Materia Medika Indonesia vol. IV, Farmakope Indonesia ed. V).

The black rice bran extracts (BRBE1 and BRBE2) obtained met the extract standardization guidelines (Materia Medika Indonesia vol. IV, Farmakope Indonesia ed. V). Furthermore, the standardized extract was determined to count the total phenolic content. The gallic acid standard was used to measure total phenol content using the *Folin-Ciocalteu* Reagent. This reagent reacts with the OH group on phenol to form a chromogen. Thus, it could be detected using visible light. The color formed is blue, detected using wavelengths above 580 nm (Hudz et al., 2019). The gallic acid was utilized as a standard because it is a stable phenol group, easy to obtain, and able to represent the total phenol content (Singleton et al., 1999).

The study conducted by Jung et al. (2017) showed a strong correlation between phenol and ORAC/oxygen radical absorbance capacity values in 21 types of Korean rice bran, including black rice bran. The ORAC value obtained reached 1101.31 M, which is equivalent to trolox/g. ORAC indicates the number of antioxidants that the body can absorb.

Total Phenolic Content Count

The gallic acid calibration curve is shown in **Figure 1**. The total phenol content in BRBE1 was known to be 175.48 mg gallic acid equivalent in 1 g extract (17.5%), while in BRBE2, it was known to be 174.39 mg gallic acid equivalent in 1 g extract (17.4%).

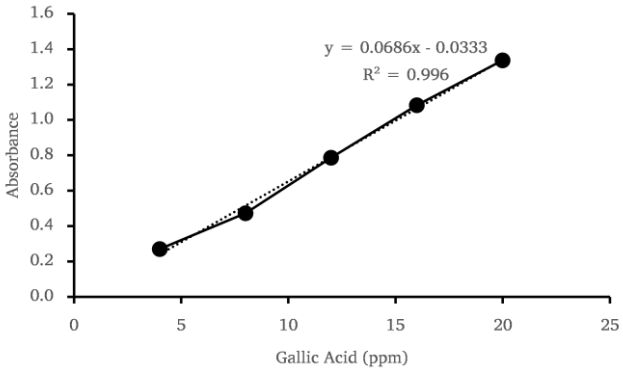

Figure 1. The gallic acid calibration curve (Sukrasno, 2017)

This value is higher than the total phenol content in the pure ethanol extract of black rice bran grown in Semarang, Central Java, which is 122.4 mg gallic acid equivalent / g extract (Sukrasno et al., 2017). An unpaired t-test was performed using the GraphPad Prism 8.3.0 application. There was no significant difference between the phenol content of BRBE1 and BRBE2 with p-value = 0.9306.

In this study, the total phenol content in black rice bran grown in Ciletuh Geopark Pelabuhan Ratu, Banten, and Karanganyar Regency, Central Java (BRBE1 and BRBE2) was higher than the total phenol content in black rice grown in Semarang, Central Java. Planting location, soil texture, water pressure,

and agricultural techniques cannot be ignored to produce high-quality rice products (Dou et al., 2016; Sanusan et al., 2010). Temperature, humidity, water availability, and altitude in the Karanganyar area are suitable for planting organic black rice for most of the population (Putra et al., 2017). The high soil fertility in Ciletuh Geopark is caused by tectonic and volcanic eruptions in the past (UNESCO, 2017), as well as geodiversity such as rivers, waterfalls, and geysers (Ardiansyah et al., 2019).

CONCLUSION

Ethanol extract of black rice bran (*Oryza sativa* L. *indica*) grown in Ciletuh Geopark, Sukabumi, West Java and Karanganyar Regency, Central Java contains phenolic compound. Black rice bran from those areas can be utilized to obtain the health benefit of gamma oryzanol.

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