Indonesian Journal of Pharmaceutical Science and Technology
Journal Homepage : http://jurnal.unpad.ac.id/ijpst/
Research Article

Determination of Gamma Oryzanol from Ethanolic Extract of Indonesian Rice Bran (*Oryza sativa*)

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Submitted 04 April 2023; Revised 08 June 2023; Accepted 24 June 2023; Published 01 February 2024

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Abstract

Rice bran contains gamma oryzanol, a chemical compound mostly consisted of complex ester trans-ferulate with phytosterols. Gamma oryzanol has a strong antioxidant activity, explained by the free radical scavenging activity. This study was aimed to determine gamma oryzanol from ethanolic extract of Indonesian rice bran. Quantitative analysis was conducted using HPLC analysis with mixture of met hanol:acetonitrile:isopropanol (50%:40%:10%) as mobile phase and Inertsil ODS-3 5 µm as stationary phase. The UV detector wavelength was set to 327 nm, and the flow rate was set to 1 mL/min. Gamma oryzanol standard and rice bran ethanolic extract 96% was injected into HPLC system. Ethanolic extract of rice bran (*Oryza sativa*) sample showed four major peaks as those of gamma oryzanol standard and contained 105,981 ppm of gamma oryzanol. As the conclusion, ethanolic extract of Indonesian rice bran (*Oryza sativa*) contains 10.59% gamma oryzanol.

Keywords: Gamma oryzanol, HPLC, Rice Bran

Penetapan Kadar Gamma Oryzanol dari Ekstrak Etanol Bekatul Beras (*Oryza sativa*) di Indonesia

Abstrak

Bekatul mengandung gamma oryzanol, suatu senyawa kimia yang sebagian besar terdiri dari kompleks ester transferulat dengan fitosterol. Gamma oryzanol memiliki aktivitas antioksidan yang kuat, yang ditunjukkan oleh aktivitas penangkal radikal bebas. Penelitian ini bertujuan untuk menentukan kadar gamma oryzanol dari ekstrak etanol bekatul beras. Analisis kuantitatif dilakukan menggunakan analisis HPLC dengan campuran metanol: asetonitril: isopropanol (50%:40%:10%) sebagai fase gerak dan Inertsil ODS-3 5 µm sebagai fase diam. Panjang gelombang detektor UV diatur pada 327 nm, dengan laju aliran 1 mL/min2. Standar gamma oryzanol dan ekstrak etanol 96% bekatul beras diinjeksikan ke dalam sistem HPLC. Sampel ekstrak etanolik bekatul beras (*Oryza sativa*) menunjukkan empat puncak utama yang sesuai dengan standar gamma oryzanol serta mengandung 105.981 ppm gamma oryzanol. Sebagai kesimpulan, ekstrak etanol bekatul beras (*Oryza sativa*) mengandung 10,59% gamma oryzanol.

Kata Kunci: Gamma oryzanol, HPLC, Bekatul beras

1. Introduction

Rice is of special importance for the nutrition of large reaches of the population in Asia parts of Latin America and the Caribbean and, increasingly so, in Africa. As a result, it plays a pivotal role for the food security of over half the world population. The rice varieties grown across the world belong overwhelmingly to the Oryza sativa species.[1]

Rice bran contains fibers and minerals, fat, protein, gamma oryzanol, as well as several bioactive compounds such as vitamin E quercatin, and anthocyanins, which commonly found in pigmented rice bran.[2,3,4,5] Gamma oryzanol is a chemical compound, mostly consisted campesteryl ferulate, campestanyl ferulate, stigmastanyl ferulate, and steryl ferulate.[6]

Gamma oryzanol has a strong antioxidant activity, explained by the free radical scavenging activity. Other potential range of therapeutic properties exhibited by gamma oryzanol include anti-carcinogenic, anti-inflammatory, antidiabetic, anti-ageing, neuroprotective and hepatoprotective effects, most of which can be attributed to its potent antioxidant capacity.[7] In preclinical study, gamma-oryzanol offers significant protection against changes in body weight, elevated triglyceride levels, kidney damage, and both structural and functional abnormalities in the heart.[8] With its wide range of biological activities, it is beneficial to determine gamma oryzanol content in natural products.

Gamma oryzanol of various Japanese pigmented rice extracted with hexane/ ethanol mixture (4:3, v/v) yielded 43.3 - 54.2 mg/100g dried weight.[9] The ethanolic extract of Indonesian black rice bran (*Oryza sativa* L. *indica*) contained 118.572 mg/g of gamma oryzanol.[10]

This study aimed to determine gamma oryzanol content from the ethanolic extract of Indonesian rice bran. Ethanol has several benefits as a solvent, including low toxicity, good operational security, as well as being obtained from a bio-renewable resource.[11]

2. Method

2.1. Tools

The tools used include High-performance liquid chromatography (HPLC).

2.2. Materials

The materials used include Rice bran sample was obtained from CV Vigur Organik, Malang, East Java. Ethanol for maceration was purchased from CV Satya Darmawan, Depok, West Java. Standard gamma oryzanol was purchased from Sigma-Aldrich Chemical Co., St. Louis, Missouri, United States of America, and grade solvents (methanol, acetonitrile, and isopropanol) were obtained from Merck KGaA, Darmstadt, Germany.

2.3. Methods

2.3.1. Extraction of Rice bran

Rice bran was extracted using 96% ethanol by maceration 1:4 (w:v) during 3 days. After extraction, extract was evaporated by water bath at 80°C for 6 hours to obtain the slimy mixture.[12]

2.3.2. High-Performance Liquid Chromatography (HPLC) Analysis

20 µL of both standard (Sigma-Aldrich Chemical Co., St. Louis, Missouri, United States of America) and rice bran ethanolic extract were injected into the HPLC system (Shimadzu Corp., Kyoto, Japan), equipped with Inertsil ODS-3 5 µm in a 250x4.6 mm HPLC-column (GL Sciences, California, United States of America). A mixture of metha nol:acetonitrile:isopropanol (50%:40%:10%) was used as the mobile phase under isocratic conditions. The ultraviolet (UV) detector wavelength was set to 327 nm and the flow rate was set to 1 mL/min.[13]

3. Result

Quantitative determination of gamma oryzanol was conducted using HPLC Analysis. The calibration curve of gamma oryzanol standard in the range of concentration of 20 – 120 at wavelength 327 nm was shown in Figure 1. Gamma oryzanol standard solution with a concentration of 70 ppm was analyzed to produce the chromatogram as demonstrated in Figure 2.

Figure 2 showed four peaks of gamma

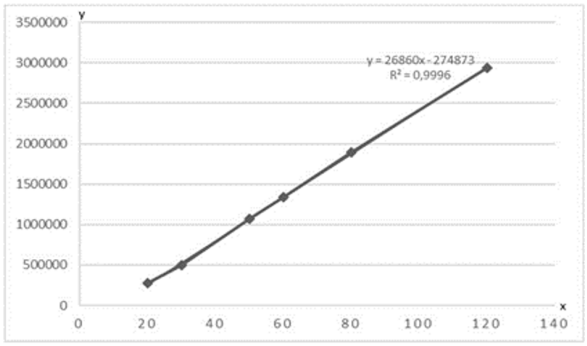

Figure 1. Calibration curve of gamma oryzanol standard

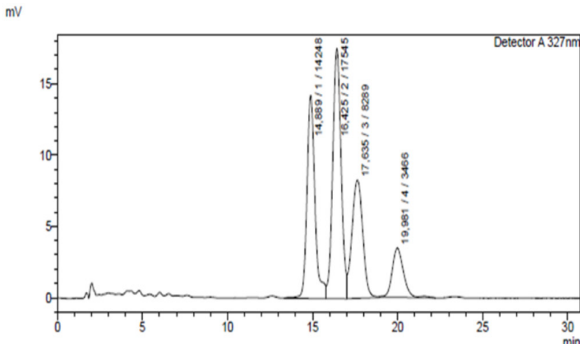

Figure 2. Chromatogram of gamma oryzanol standard solution (70 ppm)

oryzanol standard solution (70 ppm), with a retention time of 14.889; 16.425; 17.635 and 19.981 (in minutes). The total width area of the standard solution injected calculated by HPLC system was 1,591,206. 70 ppm of gamma oryzanol standard solution was chosen because there was only a slight difference in width area between the standard and the ethanolic extract. Ethanolic extract of rice bran sample with the concentration of 600 ppm was analyzed and provided chromatogram shown in Figure 3 as the result. The retention time were 15.360; 16.712; 17.814 and 20.351 (in minutes). The total width area of the extract injected calculated by HPLC system was 1,433,116.

Gamma oryzanol quantification from ethanolic extract of rice bran was calculated using the calibration curve. Regression equation y= ax + b with y as the total width area of extract injected.

1433116 = 26860x – 274873

x = 63.589

After (x) was determined, gamma oryzanol content was calculated using the following formula:

Content = (x (mcg/mL) x sample volume (mL)) /weight volume (g)

= (63.589 mcg/mL x 10mL) / 0.006 g

= 105,981 mcg/gram

= 105,981 ppm

According to the result of the calculation, gamma oryzanol content was revealed to be 105.981 ppm.

4. Discussion

The area of the 600 ppm bran extract chromatogram is 1,433,116. Based on the area of the calibration curve on Figure 1, rice bran extract contains 105.981 mg/g gamma oryzanol. A study by Kumar et al (2019)[14] reported rice bran from India, containing 62,010 ± 0,12 ppm gamma oryzanol, using n-hexane as solvent.

However, the stability of gamma oryzanol extracted using hexane is lower than the one extracted using polar solvent, which is more resistant to temperature change such as isopropanol and ethanol.[15] This is due to the more volatile nature of hexane compared to ethanol, so that the hexane extract has a lower

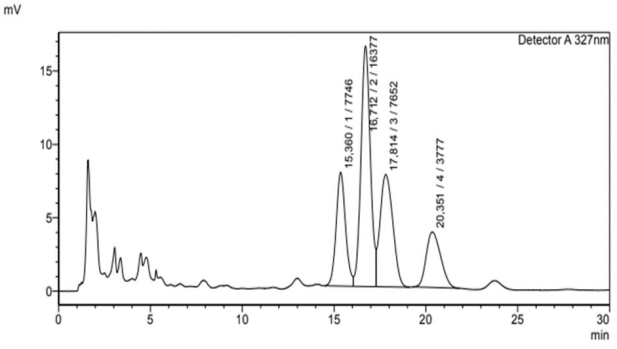

Figure 3. Chromatogram of ethanolic extract of rice bran (600 ppm)

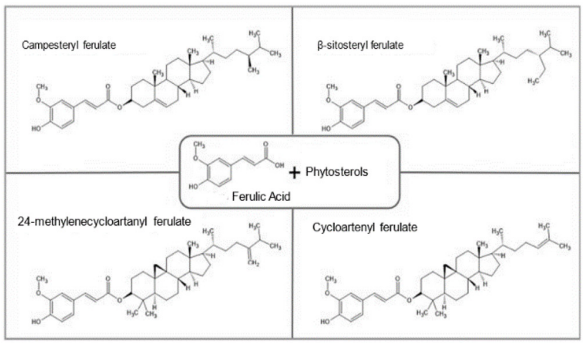

Figure 4. The Structure of the Gamma Oryzanol Components Detectable by HPLC[15]

acid value, an indicator of its oil quality.[16] Imsanguan et al. (2008)[17] reported that ethanol is a better solvent for the extraction of gamma oryzanol than hexane. Study by Kumar et al (2018)[14] revealed lower amount of gamma oryzanol and is in accordance with Imsanguan (2008)[17] who reported that ethanol was a better solvent for gamma oryzanol extraction compared to hexane.

Gamma oryzanol is a group of ferulic acid consisting of 10 compounds: Δ7-stigmastenyl ferulate, stigmasteryl ferulate, cycloartenyl ferulate, 24-methylene cycloartanyl ferulate, Δ7-campestenyl ferulate, campesteryl ferulate, Δ7-sitostenyl ferulate, sitosteryl ferulate, campestanyl ferulate, and sitostanyl ferulate.[9] Referred to Lerma-Garcia et al (2009)[18] study, the four major compounds of gamma oryzanol are 24-Methylene cycloartanyl ferulate, cycloartenyl ferulate, campesteryl ferulate, and β-sitosteryl ferulate. The component of gamma oryzanol with the highest antioxidant activity is 24-methylene cycloartenyl ferulate.[19]

Ethanolic extract of rice bran sample in this study produced a chromatogram with four peaks in a pattern similar to those of gamma oryzanol standard solution. It indicated that the sample contained gamma oryzanol. HPLC chromatogram of rice bran extract indicated the identification of four main constituents of gamma oryzanol.9 Based on Figure 3, the peaks identified were: [1] cycloartenyl ferulate (21.78%), [2] 24-methylene cycloartanyl ferulate (46.06%), [3] campesteryl ferulate (21.52%), and [4] sitosteryl ferulate (10.62%). The structure of the gamma oryzanol components detectable by HPLC was shown in figure 4.[15]

Gamma oryzanol in rice bran is a collection of ferulic acid which is a phenolic compound.[12,13] Gamma oryzanol extracted with ethanol as solvent demonstrated a higher amount of gamma oryzanol as a result, 105,981 ppm, compared to the previous studies. Solvent may significantly affect the extractability of gamma oryzanol from rice bran.[9] According to Huang and Lai (2016), gamma oryzanol content in each variety of rice will be different.[20] It opens the probability that different varieties give different levels of biological activities. With a higher amount of gamma oryzanol on its rice bran, hypothetically it also has a higher strength of biological activities.

5. Conclusion

Ethanolic extract of rice bran from Malang, East Java, Indonesia, contains 10.59 % of gamma oryzanol. It needs further studies to measure the biological activities of gamma oryzanol inside each variety of rice bran.

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