Effect of Fermentation Time on Antibacterial Activity of Fermented Red Rice Bran by *Rhizopus oryzae* in Inhibiting *Staphylococcus aureus* and *Escherichia coli*

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Abstract. Rice bran is a by-product of the rice milling process which is rich in nutrients and bioactive compounds such as flavonoids, alkaloids, triterpenoids, and so on. Pigmented rice bran such as red rice bran is richer in bioactive compounds because it contains anthocyanin compounds. The number of bioactive components found in red rice bran can be used as an antibacterial. Fermentation with the fungus *Rhizopus oryzae* can increase the content of active compounds in rice bran. The purpose of this study was to determine the effect of variations in fermentation time on the antibacterial activity of red rice bran fermented with *Rhizopus oryzae*. Red rice bran of Inpari 24 variety was prepared and fermented with variations of 3, 4, 5, and 6 days of fermentation at 37 °C. The fermented bran was extracted by maceration method using ethanol solvent. The bran extract obtained was then analyzed for its antibacterial activity using the agar diffusion method. The test bacteria used were *Stapylococcus aureus* and *Escherichia coli* bacteria. The average results of the largest antibacterial activity test were 2.53 mm for *S. aureus* and 3.90 mm for *E. coli*. Based on the One way statistical test (ANOVA) it was found that the variation in fermentation time had an effect on the inhibition zone produced in the antibacterial activity test of *S. aureus* and *E. coli*.

Keywords: Rice Bran · fermentation · Rhizopus oryzae · antibacterial

1 Introduction

Bacterial infection is one of the most common health problems in Indonesia. The types of bacteria that often cause infection are *Staphylococcus aureus* and *Escherichia coli*. *Staphylococcus aureus* is a bacterium that causes dermatitis and respiratory tract infections, while *Escherichia coli* is a bacterium that causes diarrhea. One way to prevent infection from bacteria is by giving antibacterial substances that can kill or inhibit bacterial activity [1].

Prevention of bacterial infections is usually done with antibiotics. However, antibiotics have negative effects such as making bacteria resistant. Another way of prevention

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is to use antibacterial substances from plants to inhibit the activity of pathogenic bacteria [2]. Antibacterial substances in plants can be in the form of bioactive compounds such as flavonoid compounds, phenolics, tannins, steroids, triterpenoids, or plant pigment substances. One part of the plant that is rich in antibacterial compounds is red rice bran [3]. A pigmented natural ingredient will contain more bioactive compounds such as red rice, as well as red rice bran [4]. Thushara *et.al* [5] reported that a traditional Sri Lankan rice variety with red pericarp had high antibacterial activity against bacteria, especially gram-positive bacteria.

There are many bioactive compounds such as phenolics in plants bound to the plant cell wall [6]. Phenolic compounds are bound to the plant matrix so they cannot be extracted with water or a mixture of water and organic solvents [7]. Rice bran fermentation with *R. oryzae* was able to increase the levels of phenolic compounds in the extract by releasing bound phenolic compounds using certain enzyme complexes [8].

The fermentation of red rice bran with microbes can help enrich the antibacterial compounds in the bran extract. Faizah *et.al* [9], stated that bran fermentation for 72 h can increase the total phenolic compounds up to 19.76%. Jannah *et.al* [10] said that the inhibition zone produced by fermented brown rice bran extract for 5 days against *Salmonella typhi* bacteria could reach 13.03 mm.

2 Research Methods

2.1 Ingredients

The materials needed for this research are red rice bran Inpari 24, *Rhizopus oryzae*, *Staphylococcus aureus*, *Escherichia coli*, potato dextrose agar, nutrient agar, nutrient broth, dimethyl sulfoxide, blank disk, and antibiotic disc.

2.2 Methods

2.2.1 Fermentation and Extraction of Bran Active Compound

20 g sterile bran sample was mixed with sterile distilled water in a ratio (bran: aquadest) 1:1 [11]. *Rhizopus oryzae* inoculum was added to the rice bran as much as 10% (v/w) aseptically and shaken with a sterile spatula. Samples were incubated at 37 °C with variations in incubation time of 3, 4, 5, and 6 days. The results obtained were dried in an oven at a temperature of 50 °C for 24 h. The fermented bran was dissolved in 60 ml of ethanol as a solvent [12]. The sample and solvent were shaken with a shaker incubator at 150 rpm for 5 h at room temperature. As control used red rice bran without fermentation.

2.2.2 Antibacterial Activity Test for Rice Bran

Aseptically poured 100 microlites of each test bacteria into a petri dish. Next, the liquid Nutrient Agar media was poured into a petri dish that already contained the inoculum of the test bacteria. The mixture was homogenized and allowed to solidify. Prepared disc paper to be soaked in fermented bran extract which had been added with DMSO. The positive control used was chloramphenicol antibiotic, while the negative control used

Table 1. The Yield of Red Rice Bran Extract

Ethanolic red rice bran extract	Yield (%)
Control (nonfermentation)	8.22
Fermentation 3 days	10.48
Fermentation 4 days	14.19
Fermentation 5 days	14.57
Fermentation 6 days	14.96

was DMSO. The paper disc is placed on the surface of the Nutrient Agar media using sterile tweezers with a little pressure. Incubate on media with disc paper at 37 °C for 24 h until inhibition areas appear.

3 Result

3.1 % Yield Fermented Red Rice Bran

Based on Table 1, it can be seen that the results obtained from the 6-days fermentation treatment with *R. oryzae* had the highest yield of 14.96%. In the fermentation treatment, there was an increase in the number of bioactive compounds extracted due to the activity of *R. oryzae* which hydrolyzes the bran cell wall fibers so that nutritional compounds and secondary metabolites such as bound phenolics can be available in free form and are also extracted by solvents [13]. The resulting yield increases with increasing fermentation time, the longer the fermentation time, the greater the yield obtained. While in the control treatment, the yield obtained was smaller than that of fermented bran extract because there was no fermentation activity of *R. oryzae* in the control treatment.

3.2 Phytochemical Test

The phytochemical test on fermented red rice bran extract aims to determine the presence of secondary metabolite compounds qualitatively. The types of phytochemical tests carried out were phenolic, saponin, flavonoid, steroid, terpenoid, and alkaloid tests.

Based on Table 2, the results of the phytochemical test showed that the rice bran without fermentation was positive for phenolics, terpenoids, and flavonoids. While the fermented rice bran extract was positive for phenolics, flavonoids, steroids, and terpenoids. 5 days of fermented red rice bran extract contained more types of phytochemical compounds with high intensity than other variations, namely phenolic compounds, flavonoids, and terpenoids with high concentrations and steroid compounds with medium concentrations.

The diversity of types and concentrations of different bioactive compounds is thought to be due to the activity of the cellulase enzyme from *R. oryzae* in degrading bran cellulose fibers, so that the levels of bioactive compounds in the extract can increase. The longer fermentation process, until the optimum time making bioactive compounds contained

Table 2. The Pytochemical Test

Compound	Changes in color / precipitate formed	Extract Red Rice Bran				
		Tf	F3	F4	F5	F6
Phenolic	Dark Green	+ +	+ +	+ + +	+ + +	+ + +
Saponin	Formed foam	-	-	-	-	-
Flavonoid	Orange Yellow	+	+	+	+ + +	+ +
Steroid	Green	-	+ +	+ + +	+ +	-
Triterpenoid	Brown Ring	+ +	+ + +	+ + +	+ + +	+ + +
Alkaloid (Meyer)	Yellow	-	-	-	-	-
Alkaloid (Dragendorff)	Orange Precipitate	-	-	-	-	-

Information
+ + +: high intencity.
+ + : medium intencity.
+: low intencity.
-: no content.
Tf: non fermentation.
F3 : fermentation 3 days.
F4: fermentation 4 days
F5: fermentation 5 days.
F6: fermentation 6 days.

in the extract increase. When fermentation exceeds the optimum time, there will be a decrease in active compounds.

In the 6-day fermented bran extract, the bioactive compounds contained were less, although the yield was large. This may be due to the decreased production of cellulose-degrading enzymes produced by *R. oryzae* as it begins to enter the death phase and the degradation of active compounds such as gallic acid into aliphatic compounds [14]. The high yield of fermented bran extract for 6 days was thought to be caused by other compounds contained in the bran extract such as protein.

3.3 Antibacterial Activity Test

Based on Table 3, the largest inhibition zones for *Staphylococcus aureus* and *Escherichia coli* were obtained in the 5-day fermentation time treatment. The average value of the highest inhibition zone in the 5-day fermentation treatment for *Staphylococcus aureus* was 2.53 mm and *Escherichia coli* was 3.90 mm. The magnitude of the inhibition zone of fermented bran extract for 5 days when compared to other variations was probably due to the large number of bioactive compounds contained in the extract which were more and more concentrated than other variations. The 5-day fermented rice bran extract contains phenolic compounds, flavonoids, and terpenoids with high concentrations and steroid

Table 3. Antibacterial Activity of Red Rice Bran Extract

Treatment Variations	Average of Inhibition Zone (mm)	
	S. Aureus	*E. coli*
Non-Fermentation	0	0
Fermentation 3 days	1.33	1.73
Fermentation 4 days	2.23	2.80
Fermentation 5 days	2.53	3.90
Fermentation 6 days	0.80	1.23
K + (Chloramphenicol)	19.83	25.23
K- (DMSO)	0	0

compounds with moderate concentrations. The more bioactive compounds contained in the extract, the greater the inhibition zone for the test bacteria [15].

According to Winastri *et.al* [16] an inhibition zone of less than or equal to 5 mm is in the weak category, 6–10 mm is in the moderate category, 11–20 mm is in a strong category, and more than equal to 21 is the strong category. The results of the acquisition of inhibition zones for the treatment without fermentation, fermentation for 3 days, 4 days, 5 days, and 6 days were categorized as weak. The results of the inhibition zone obtained by the positive control of chloramphenicol were in the strong category, while the negative control of DMSO did not produce an inhibition zone which indicated the absence of antibacterial activity.

4 Conclusion

The conclusion is that there is an effect of variations in fermentation time on the antibacterial activity of fermented brown rice bran *R. oryzae*. The variation of 5 days of fermentation showed the average results of the greatest antibacterial activity on *Staphylococcus aureus and Escherichia coli*, respectively 2.53 mm and 3.90 mm, where the antibacterial activity was categorized as a weak inhibition zone.

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