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Potential of extract rice bran fermented by *Rhizopus oryzae* as antibacterial against *Salmonella typhi*

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Abstract. The availability of bioactive compounds in rice bran can be increased through fermentation. This study aim to determine the effect of rice bran fermentation time by *Rhizopus Oryzae* and rice bran extract concentration on antibacterial activity against *Salmonella thypi*. The time of rice bran fermentation were 5 and 7 days while as a control was rice bran without fermentation. Antibacterial test used the disk diffusion method with various concentrations of fermented and unfermented rice bran ethanol extracts which were 25%, 12.5% and 6.25% (w/v) as positive control using chloramphenicol and DMSO as negative control. The highest zone inhibitory analysis showed that the antibacterial activity of fermented rice bran extract for 5 days was 13.03 ± 3.08 mm, while for 7 days was 7.9 ± 3.44 mm and unfermented rice bran was 9.73 ± 1.1 mm. The results of this study showed that the fermented rice bran ethanol extract for 5 days had the highest antibacterial activity for inhibiting the growth of *Salmonella thypi*.

1. Introduction

Typhoid fever is a disease that arises due to *Salmonella typhi* bacterial infection [1], which attacks the small intestine and causes endemic diseases in Indonesia [2]. *Salmonella typhi* infection in humans, caused by the body's immune system which has decreased activity so it is unable to kill *Salmonella typhi* bacteria. This results in *Salmonella typhi* which is in the bloodstream can survive, develop, invade and damage body cells [3].

Infection due to *Salmonella typhi* is usually treated with antibiotics. Antibiotics are also known to have many side effects [4]. The use of oral and parenteral antibiotics in patients is increasingly causing the emergence of pathogenic microbes that are resistant to antibiotics [5]. One of the pathogenic bacteria that is often resistant to antibiotics is *Salmonella typhi*. To overcome this problem, one of them is by using natural plant-based antibacterial which contains bioactive compounds. Natural antibacterial compounds are usually phenolic or polyphenolic derivatives such as the flavonoid group [6] alkaloids, saponins, steroids, and tannins [7], some of these compounds are found in rice bran.

Rice bran is the outermost skin of rice and the innermost skin of the husk which is peeled off through the process of grinding and polishing. Rice bran is a waste of rice milling which is abundant in Indonesia and contains bioactive compounds. Microbial fermentation methods can increase the availability of bioactive compounds in rice bran, some studies have used mushrooms for rice bran fermentation. Rice bran fermented by *Rhizopus oryzae* has increased the phenolic compound up to two times to 765 mg/g [8]. Razak et al. [9] stated that rice bran fermented by the fungus *Aspergillus*



oryzae and *Rizhopus oryzae* can increase total phenolic levels, especially ferulic acid up to 43.19 mg/mL. Ribeiro et al. [10] reported that bran fermentation by *Rhizopus oryzae* was able to increase phenolic compounds and digestibility of rice bran proximate nutrients namely protein, fiber, lipids and minerals. Fermentation can increase the active compound which has the potential as an antibacterial, this study aims to determine the effect of bran fermentation duration and concentration of rice bran extract on antibacterial activity against *Salmonella typhi*.

2. Materials and methods

2.1. Materials

IR 50 type rice bran (rice milling in Indonesia), *Rizhopus oryzae* and *Salmonella typhi* (collection of Lab. Faculty of Medicine, Universitas Brawijaya Malang Indonesia), potato dextrose agar (Merck, Germany), nutrient agar (Merck, Germany), nutrient broth (Merck, Germany), muller Hilton agar (Himedia, india), dimethyl sulfoxide (Merck, Germany), and Blank and antibiotic disc (Oxoid, UK).

2.2. Methods

2.2.1. *Fermentation and extraction of bran active compounds.* 50 gr of sterile rice bran was added with 50 mL of sterile distilled then added 5 mL suspension of *Rizhopus oryzae* that equivalent with 10^5 spores/mL aseptically. Rice bran were incubated at 37 °C for 5 and 7 days then dried at 50 °C for 24 h. As control used rice bran without fermentation. All of rice bran were extracted with ethanol 95% (1: 5, w/v) at 120 rpm for 5 h. Rice bran ethanolic extracts were used for further analysis.

2.2.2. *Antibacterial activity test for rice bran extract.* *Salmonella typhi* was grown in nutrient broth at 30 °C for 18 h. *Salmonella typhi* suspension (0.3 mL) was used with turbidity equivalent to 0.5 Mc farland (10^8 cfu/mL). The blank disks were attached to muller hilton agar plates medium containing *Salmonella typhi* and then added 25 μ L rice bran extracts, as a positive control using antibiotic disc and DMSO as a negative control. The plates were incubated at 37 °C for 24 h then measured the diameter of the inhibition zone. The concentrations of rice bran extracts for antibacterial test were 25%, 12.5% and 6.25%.

2.2.3. *Data analysis.* Quantitative determination of rice bran extract was the average of *triplicate* independent experiments and presented as average \pm standard error

3. Results and discussion

Tabel 1. Yield of Rice bran Extract.

Rice bran extract	Yield (%)
Non fermentation	7.3603
Fermentation (5 day)	9.4330
Fermentation (7 day)	9.6910

Based on Table 1, the yield of fermented rice bran is greater than the treatment without fermentation. The highest yield in 7 days fermented rice bran was 9.6910%. During the fermentation process with the fungus *Rhizopus oryzae* there is a breaking of the bonds in the bran cellulose so that many phenolic compounds that are bound in the bran are extracted. These compounds include antibacterial compounds found in rice bran.

Table 2 shows the differences in nutrient levels (fat, protein, ash and fiber) in non-fermented rice bran extract and fermented rice bran. The fermentation will reduce the levels of crude fiber in rice bran because during fermentation phenolic compounds and others will be released due to hydrolysis by cellulase enzymes that produced by *Rhizopus oryzae*. The use of the genus *Rizhopus oryzae* is a promising thing in the fermentation process, because the fungus of this genus does not produce toxic

substances [11]. So that bran bioactive compounds are not wasted much during the extraction process. *Rizhopus oryzae* fungus is able to produce cellulase enzymes [12], namely β -glucosidase exo-1,4- β -D glucanase, and endo-1,4- β -D-glucanase [13].

Table 2. Proximate Analysis of Rice Bran Extract.

Parameter	Rice Bran Extract		
	Non fermentation	Fermentation (5 day)	Fermentation (7 day)
Fat	8.70±0.02	9.77±0.02	10.03±0.14
Protein	12.89±0.08	14.79±0.14	13.33±0.11
Ash	12.80±0.02	13.76±0.22	13.64±0.04
Fiber	10.28±0.24	15.82±0.05	15.36±0.09

3.1. Phytochemical test

Phytochemical test on rice bran extract aims to determine the presence of secondary metabolite compounds contained in rice bran extract qualitatively. Phytochemical tests conducted are phenolic, flavonoid, saponin, steroid and terpenoid tests, as well as alkaloids. In phytochemical testing, the three extracts gave positive results with different colour intensities, except that the saponin test showed negative results. Fermented rice bran extract 5 days gives a more intense colour intensity compared to unfermented and fermented 7 days. The result of phytochemical test are listed in Table 3.

Table 3. Phytochemical Test.

Active Compound Group	Extract		
	Rice bran without fermentation	Fermented rice bran 5 days	Fermented rice bran 7 days
Phenolic	+	+++	++
Flavonoid	+	++	++
Saponin	-	-	-
Steroid	++	+++	+
Terpenoid	+	++	+
Alkaloid	Dragendorff	+++	+++
	Mayer	+	++

Abbreviations: +++ : high content ++ : medium content
+ : little content - : no content

3.2. Antibacterial activity of fermented rice bran by agar diffusion method

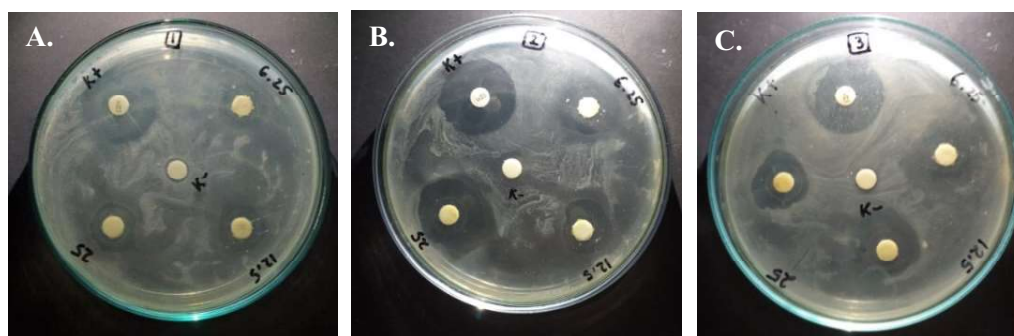


Figure 1. Fermented rice bran extraction: non fermentation (A), 5 days fermentation (B) and 7 day fermentation (C) against *Salmonella typhi*.

According to Susanto et al. [14] if the diameter of the inhibition zone is less than 5 mm, the antibacterial activity is categorized as weak, the diameter of the inhibition zone of 6-10 mm is

moderate, the diameter of the inhibition zone of 11-20 mm is categorized as strong and if more than 21 mm then the antibacterial activity is categorized stronger. Based on Table 2, the highest antibacterial activity is 13.03 mm, there are 5 days fermentation treatment and extract concentration is 25%, while the lowest antibacterial activity is 7.13 mm on 7 days fermentation treatment and extract concentration is 6.25% and is categorized as a weak inhibitory zone. Inhibition zones produced by bran extracts in various treatments are presented in Figure 1.

Table 4. Antibacterial activity of rice bran extract

Extract of bekatul	Extract concentration (%)	Inhibition zones (mm)
Non fermentation	6.25	8.13±0.90
	12.50	8.17±0.45
	25.00	9.73±1.10
Fermentation (5 days)	6.25	8.9±2.7
	12.50	9.7±1.22
	25.00	13.03±3.08
Fermentation (7 days)	6.25	7.13±0.81
	12.50	7.63±1.29
	25.00	7.9±3.44
Positive control (Chloramphenikol)		19.23±1.4

Table 4 shows the highest antibacterial activity in the 5-day fermentation treatment with 25% extract concentration was 13 mm and categorized as strong inhibitory zone. The lowest antibacterial activity at 7 days fermentation treatment with a concentration of 6.25% was 7.13 mm and categorized as medium inhibitory zone.

4. Conclusion

The highest zone inhibitory analysis showed that the antibacterial activity of fermented rice bran extract for 5 days was 13.03 ± 3.08 mm, while for 7 days was 7.9 ± 3.44 mm and unfermented rice bran was 9.73 ± 1.1 mm. The results of this study showed that the fermented rice bran ethanol extract for 5 days had the highest antibacterial activity for inhibiting the growth of *Salmonella thypi*.

References

- [1] Sidabutar S and Satari H I 2010 *Sari Pediatri* **11** (6) 434
- [2] Rakhman A, Humardewayanti R and Pramono D 2009 *Berita Kedokteran Masyarakat* **25** (4) 167-75
- [3] Ugrinovic S, Menager N, Goh N and Mastroeni P 2003 *Infect Immune*. **71** 6808-19
- [4] Rangan C and Barceloux D 2009 *Dis Mon.* **2** (55) 293-311
- [5] Mardiasuti H, Kurniawati A and Kadarsi R 2007 *Majalah Kedokteran Indonesia* **57** (3) 75-79
- [6] Ambujakshi H R, Surendra V, Haribabu T and Goli D 2009 *J. Pharm. Res.* **2** (6) 1028
- [7] Ningsih D R and Zufahair D K 2016 *Molekul* **11** (1) 101-111
- [8] Schmidt C G, Leticia M G, Luciana P, Helen S H and Eliana B F 2013 *Food Chem. J.* **146** (12) 371-77
- [9] Razak D L A, Rashid N Y A, Jamaluddin A, Sharifudin S A, Kahar A A and Long K 2016 *Jurnal Teknologi* **78** (11-12) 29-34
- [10] Ribeiro A C, Graca C D S, Chiattoni L M, Massarolo K C, Duarte F A, Mellado M D L S and Soares L A D S 2017 *Journal of Microbiology and Biotechnology* **6** (2) 45-52
- [11] Oliveira M S, Cipolatti E P, Furlong E B and Soares L de S, 2012. *Food Sci. Technol. Camp.* **32** (3) 531-37
- [12] Yosmar R, Suharti N, and Rasyid R 2013 *Jurnal Farmasi Andalas.* **1** (1) 5-12
- [13] Ikram U H, Javed M M, Khan T S and Shiddiq Z 2005 *J. Agric & Biol. Sci.* **1** 329
- [14] Susanto D, Sudrajat and Ruga R 2012 *Mulawarman Scientifie* **11** (2) 181-90