

Enhancement of Antioxidant and Colon Anticancer Activity from Fermented Rice Bran Extract

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ABSTRACT

Colon cancer is a serious disease that can lead to death. This disease is caused by unhealthy eating habits such as low fiber intake, high consumption of animal products, and processed foods. Every year, the number of colon cancer patients increases. Therefore, further research is needed to find effective and potential alternative treatments. One of the substances that has antioxidant content as a cancer preventive compound is rice bran. The increase in bioactive compounds in rice bran can be done through fermentation. This study aims to investigate how fermentation affects the antioxidant activity and colon anticancer potential of rice bran extract. The research results show fermentation can increase the total phenol content and antioxidant activity. The highest total phenol content is found in fermented black rice bran using *R. oligosporus*, which is 7.96 µgGAE/g. The highest antioxidant activity is exhibited by fermented red rice bran using *R. oryzae*, which is 78.92%. There is a positive correlation between phenolic content and antioxidant activity, indicating that fermented rice bran extract exhibits higher antioxidant properties compared to non-fermented ones. The phenolic compound content and the role of microorganisms in fermented rice bran contribute to the increased antioxidant activity and colon anticancer effects.

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1. INTRODUCTION

Colon cancer is one of the types of cancer that affects the surface of the large intestine and is a serious and potentially fatal disease. Colon cancer is one of the leading causes of death in several countries, including Indonesia. Unhealthy dietary habits such as low fiber intake, high consumption of animal products, and processed foods significantly contribute to the occurrence of colon cancer [1]. The number of colon cancer cases increases each year. Globally, there were 1.1 million diagnosed cases of colon cancer in 2020, and it is estimated to rise to 1.9 million cases by 2040 [2][2]. Therefore, further research is needed to find effective and potential alternative treatments for colon cancer.

Black rice bran is a byproduct of the process of milling black rice to remove the husk. Previous studies have shown that rice bran contains various bioactive compounds, including antioxidants. Antioxidants play a role in protecting the body's cells from damage caused by free radicals, which can lead to various degenerative diseases, including cancer. Rice bran contains bioactive compounds such as oryzanol, ferulic acid, caffeic acid, tricin, coumaric acid, phytic acid, vitamin E, phytosterol, and carotenoids [3]. These bioactive compounds have been found to have anticancer benefits [4]. Based on the research by [5], the antiproliferative activity with MTT test showed IC₅₀ values of bran extract on vero cells (728.55-1718.90 µg/mL) higher than WiDr cells (163.52-481.73 µg/mL). This indicates that the induction of bran extract actively targets WiDr cancer cells compared to vero cells.

One way to enhance bioactive compounds in food products, including rice bran, is through the fermentation process. Fermentation is a traditional processing method used to improve the nutritional value, biological activity, and acceptability of various food products. The increase in bioactive compounds depends on the type of inoculum and fermentation time. For example, fermenting rice bran for 72 hours results in the highest increase in antioxidant activity in the fermented rice bran [6]. The research by [7] reported that the cytotoxic activity on WiDr colon cancer cells was higher for fermented

rice bran with an IC50 value of 650.7 µg/mL compared to non-fermented rice bran with an IC50 value of 831.8 µg/mL.

Based on these findings, exploring the potential of fermented rice bran extract in preventing colon cancer is of great interest for further investigation. This review article aims to provide a comprehensive overview of the enhanced antioxidant and antiproliferative activities of fermented black rice bran extract on WiDr colon cancer cells. By compiling and analyzing data from recent studies, it is hoped that a deeper understanding of the potential application of fermented black rice bran extract in colon cancer prevention can be obtained. Furthermore, this research is expected to contribute valuable information to the added value of food processing by-products.

2. METHOD

This study utilizes systematic review to gather evidence related to the potential of fermented rice bran as a functional food for colon cancer prevention. The initial phase of the research involves identifying questions based on PICO (Population, Intervention, Comparator, and Outcome). Fermented rice bran is the population, antioxidants and bioactive compounds are the intervention, there is no comparator, and colon anticancer is the outcome. Next, a protocol is developed using Meta analysis (PRISMA/Preferred Reporting Hans for Systematic Reviews and Meta-analysis) as a guideline for literature selection. To obtain relevant data, searches are conducted in various databases using keywords "or" and "and". The obtained data is then screened based on the abstracts of journals, and a quality assessment is performed. The data quality is measured by considering the research article's methodology.

After conducting searches in the databases, 67 articles are found. Then, the articles are screened based on their titles and assessed for data quality, resulting in 5 articles that are suitable and included in the data extraction phase. The data extraction phase involves collecting core information (quality assessment) from scientific articles that have passed the screening process. This process includes full-text articles and summarizing information systematically. The use of PRISMA in this article can be observed in Figure 1.

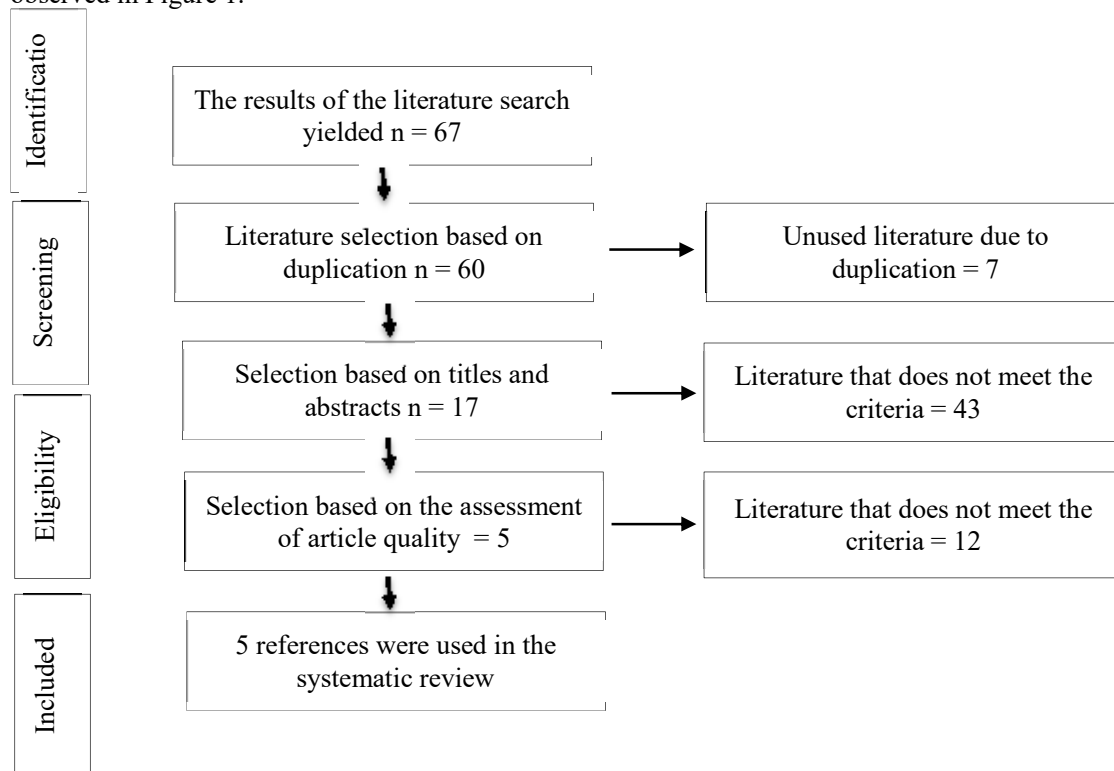


Figure 1. The use of PRISMA

3. RESULTS AND DISCUSSION

Fermented Rice Bran.

Rice bran is the outer layer of rice, produced as a by-product during the rice milling process. Rice bran contains various nutrients, such as starch, dietary fiber, fats, proteins, vitamins, and minerals [8], [9]. The chemical composition of rice bran is highly diverse and influenced by various factors such as rice variety, milling process, rice cultivation environment, distribution of chemical components within rice grains, outer layer thickness, grain size and shape, grain resistance to damage, and the analytical method used [10]

The bioactive compounds in rice bran, such as γ -tocotrienol, γ -oryzanol [11], and ferulic acid [12], have been reported to possess the ability to inhibit the growth of colon cancer cells. Through the fermentation process, rice bran can experience improved digestibility and an increase in saturated fatty acid content. Fermentation of rice bran can result in enhanced nutritional value, total phenol, γ -oryzanol, and antioxidant activity compared to non-fermented rice bran [6].

Based on the results of the article search, 6 articles were found that were synthesized in a narrative manner. The description of the article is explained in Table 1 as follows:

Table 1. Data Extraction Results in Systematic Review

No	Ingredient	Total phenolic content ($\mu\text{gGAE/g}$)	Antioxidant activity (%)	In vitro (IC_{50})/in vivo colon cancer	Reference
1	Black rice bran [5]	3.70	-	Vero cell 1564.86, WiDr cell 461.50	Zulfafamy <i>et al.</i> 2018
2	Fermented black rice bran (<i>R. oryzae</i>) [5]	5.10	-	Vero cell 1694.27, WiDr cell 163.52	
3	Fermented black rice bran (<i>R. oligosporus</i>) [5]	5.30	-	Vero cell 1509.81, WiDr cell 343.41	
4	Red rice bran [6]	3.29	75.07	-	Faizah <i>et al.</i> 2020
5	Fermented red rice bran (<i>R. oryzae</i>) [6]	3.94	78.92	-	
6	Rice bran [6], [7]	3.13	76.50	-	
7	Fermented rice bran (<i>R. oryzae</i>) [6]	3.62	73.92	-	
8	Fermented black rice bran (<i>R. oryzae</i>) [7]	7.48	65.80	Vero cell 1001.9, WiDr cell 873.9	Safrida <i>et al.</i> 2022
9	Fermented black rice bran (<i>R. oligosporus</i>) [7]	7.96	72.01	Vero cell 811.1, WiDr cell 650.7	
10	Black rice bran [13]	-	-	LAB colony number 8.99 log CFU/g, MDA level 1.88 μmol Acetate acid 11.9 mM, Butyrate acid 3.4 mM, Propionate acid 2.3 mM	[13]Putri <i>et al.</i> 2023
11	Fermented black rice bran (<i>R. oligosporus</i>) [13]	-	-	LAB colony number 9.04 log CFU/g, MDA level 1.41 μmol Acetate acid 14.4 mM, Butyrate acid 2.8 mM, Propionate acid 2.1 mM	
12	Defatted rice bran [14]	-	-		Tajasuwan, <i>et al.</i> 2023

Acetate acid 18.7
mM
Butyrate acid 5.7 mM
Propionate acid 6.2
mM
Production of
beneficial bacteria
(*Alloprevotella*,
Prevotellaceae UCG-
001, *Ruminococcus*,
Roseburia,
Butyricoccus) and
lessened the
production of
harmful bacteria
(*Turicibacter*,
Clostridium sensu
stricto,
Escherichia-
Shigella,
Citrobacter)

Total Phenolic Content of Fermented Rice Bran

There was a significant difference in the total phenolic content of fermented rice bran (FBRB) and non-fermented rice bran (NFBRB). The total phenolic content (TPC) in the FBRB extract was higher compared to the NFBRB extract. Based on the research data in Table 1, fermented rice bran has a higher total phenol content compared to non-fermented rice bran. Fermented black rice bran with *R. oligosporus* has the highest total phenol content, which is 7.96 $\mu\text{gGAE/g}$. Fermentation process in rice bran can increase the total phenolic content. After a 72-hour fermentation process with *R. oligosporus* and *R. oryzae* inoculums, the phenolic content in rice bran increased by 22.5% and 14.7% [7]. The fermentation process increased the availability of free phenolic acids, resulting in a higher TPC in the FBRB extract compared to the NFBRB extract [5]. This increase was due to enzymes produced by fungi that can release phenolic compounds' bonds in the substrate during the fermentation process. Some fungal enzymes known to release phenolic compounds in the substrate include β -glucosidase, cellulase, and α -amylase [15].

Antioxidant Activity

Antioxidants are reported to play a role in protecting cells from damage caused by oxidative stress resulting from the formation of free radicals, and oxidative stress is a major cause of cancer cases. Several groups of compounds act as antioxidants, including phenolic acids, anthocyanins, flavonoids, tocotrienols, tocopherols, γ -oryzanol, and phytic acid [16]. These compounds can be found in rice bran. The antioxidant function of these compounds is influenced by the presence of phenolic hydroxyl groups in the ferulic acid part of their structure. The phenolic content and antioxidant activity have a positive correlation. The phenolic content in rice bran actively contributes to the enhancement of its antioxidant activity.

There was an increase in antioxidant activity in fermented rice bran compared to non-fermented rice bran. Based on the research data in Table 1, fermented rice bran experiences an increase in antioxidant activity. Fermented red rice bran with *R. oryzae* has the highest antioxidant activity, which is 78.92%. The percentage of increase in antioxidant activity for rice bran fermented by *R. oligosporus* was approximately 60.2-72%, while rice bran fermented by *R. oryzae* showed an increase of about 59.5-65.8%.

Both types of fungi demonstrated similar potential in enhancing antioxidant activity; however, rice bran fermented by *R. oligosporus* exhibited higher antioxidant activity. This increase in antioxidant

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activity was attributed to the presence of phenolic acid and flavonoid compounds, as these compounds act as antioxidants by donating hydrogen atoms or transferring electrons. This finding explains why fermented rice bran has higher antioxidant activity compared to non-fermented rice bran based on the content of phenolic and flavonoid compounds [7]. In the methanol extract of rice bran fermented for 72 hours using *R. oryzae* and *R. oligosporus*, there is an increase in antioxidant activity compared to non-fermented rice bran [5]. The rice bran sample fermented for 72 hours using *R. oryzae* exhibited the highest antioxidant activity compared to other fermentation times. The antioxidant activity of the fermented rice bran extract reached 78.92% [6].

Colon Anticancer Activity

The mechanism of colon cancer cell prevention by bioactive compounds in rice bran has various effects, including acting as antioxidants that protect against free radicals, altering the cell cycle, inhibiting cell growth, regulating the immune system, inducing apoptosis through cascade pathways, and safeguarding the mucosal layer by influencing microbial transformation through the high fiber content in rice bran [3].

These mechanisms also differ among the same or different groups of bioactive compounds, such as ferulic acid and p-coumaric acid, both of which belong to the phenolic compound group and can inhibit the development of Caco-2 colon cancer cell cycle but through different inhibition pathways. Ferulic acid delays the S phase pathway, affecting centrosome-regulating genes and DNA damage checkpoint genes like CEP2, CETN3, and RABGAP1. On the other hand, p-coumaric acid induces the G2/M phase pathway and influences other cell cycle-regulating genes such as MYC, CDKN1A, PCNA, CDC25A, ODC1, CCNA2, and CCNB1 [12].

Black rice bran extract has a lower cytotoxic effect on Vero cells compared to WiDr cells. This indicates that black rice bran extract is more effective in inhibiting the growth of WiDr cancer cells compared to normal Vero cells, making non-fermented black rice bran extract or fermented black rice bran extract considered safe for normal human cells. Based on IC50 values, categorized natural extracts as active ($\leq 20 \mu\text{g.mL}^{-1}$), moderate ($>20-100 \mu\text{g.mL}^{-1}$), weak ($>100-1000 \mu\text{g.mL}^{-1}$), or inactive ($>1000 \mu\text{g.mL}^{-1}$) [17]. Thus, black rice bran extract from both ethanol and methanol fractions has the potential to prevent colon carcinogenesis, despite having weak cytotoxic activity against WiDr colon cancer cells. Additionally, research findings show a significant difference in cytotoxic activity between fermented black rice bran and non-fermented black rice bran samples, where fermentation increases cytotoxic activity higher than the control group [5].

Fermented and non-fermented black rice bran have a positive effect in increasing the number of lactic acid bacteria colonies, short-chain fatty acid concentration, and reducing malondialdehyde levels in azoxymethane-induced mouse colons. At the end of the intervention, the number of lactic acid bacteria colonies in the mouse feces from the fermented black rice bran group (9.04 log CFU/g) and the non-fermented black rice bran group (8.99 log CFU/g) was higher than the positive control group (8.33 log CFU/g). The concentration of short-chain fatty acids in the mouse group receiving non-fermented and fermented black rice bran was higher than in the control group. Moreover, the malondialdehyde levels in the mouse group receiving non-fermented black rice bran (1.88 μmol) and fermented black rice bran (1.41 μmol) were lower than in the positive control group (2.02 μmol) [13].

The group of mice given black rice bran experienced an increase in the total number of lactic acid bacteria and short-chain fatty acids. As a result, the pH value and β -glucuronidase activity decreased. Histopathological evaluation of the mucosal tissue showed inhibition of tumor growth rate in the group of mice fed with black rice bran compared to the group fed with a standard diet. Additionally, the expression of proliferating cell nuclear antigen decreased significantly, while the expression of caspase-8 and caspase-3 increased notably in the group fed with black rice bran diet. These findings indicate that black rice bran is effective in preventing colon carcinogenesis [18].

Defatted rice bran (DRB) can enhance the growth of beneficial bacteria (*Alloprevotella*, *Prevotellaceae UCG-001*, *Ruminococcus*, *Roseburia*, *Butyricoccus*) and reduce the growth of harmful bacteria (*Turicibacter*, *Clostridium sensu stricto 1*, *Escherichia-Shigella*, *Citrobacter*) present in the feces, mucosa, and tumors of the colon. Additionally, DRB also aids in the production of SCFA (acetate, propionate, butyrate) in the cecum. Moreover, DRB can restore Goblet cell loss and improve the

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thickness of the mucus layer in the colon tissue. These findings suggest that DRB has the potential as a prebiotic supplement to regulate gut microbiota imbalance, reduce the risk of CRC, and thus warrant further research on the utilization of DRB in various nutritional health products to enhance beneficial bacteria in the colon [14].

4. CONCLUSION

Fermentation enhances the ability of rice bran extract as an antioxidant and anticancer agent. During the fermentation process, the total phenol content and antioxidant activity of rice bran extract increases. The highest total phenol content is found in fermented black rice bran using *R. oligosporus*, which is 7.96 µgGAE/g. The highest antioxidant activity is exhibited by fermented red rice bran using *R. oryzae*, which is 78.92%. The phenolic content and the role of microorganisms in fermented rice bran contribute to the increased antioxidant activity and colon anticancer effects. Additionally, fermented rice bran extract shows potential in inhibiting the growth and spread of colon cancer cells through apoptosis mechanisms.

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