



Probiotic Contents and Antioxidant Activity in Probiotic Drinks Patikala Fruit Juice (*Etilingera elatior* (Jack) R.M.Smith) from Kolaka

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Abstract

Background: Patikala fruit (*Etilingera elatior*) can be used as food and medicine to boost the immune system. Patikala is a fruit with a high nutritional value that can be used to make a probiotic drink. This study aimed to see if patikala fruit juice could be used as a probiotic and antioxidant drink as an immune booster after the COVID-19 pandemic. **Methods:** The patikala fruit used is typical of the Kolaka area. This research used a randomized block design using different fermentation time factors. Each treatment was carried out three times repetitions. Making a probiotic drink from patikala (*Etilingera elatior*) is done by taking patikala fruit juice with a mixture of powdered skim milk and *Lactobacillus casei* bacteria, then carrying out fermentation treatment for 20 hours, 24 hours, and 28 hours. A dilution process is carried out to test probiotic levels, and antioxidant activity tests are carried out. **Results:** The results obtained from the analysis of probiotic contents and antioxidant activity show that the potential of patikala fruit juice to be the best probiotic and antioxidant drink is at a time variation of 28 hours. During this time, the probiotic contents reached a maximum of 9.6×10^{14} cfu/ml, which had the best ability to ward off free radicals and had 9.900% inhibition. **Conclusions:** Patikala fruit juice drink (*Etilingera elatior*) contains probiotics and potential antioxidant activity to improve the immune system after the COVID-19 pandemic.

Keywords: antioxidant; immune; patikala, probiotic

Introduction

Indonesia is a country with high biodiversity. If biodiversity can be processed and utilized optimally, it will provide high economic value for life. Biodiversity can be used as a source of raw materials for industries such as medicines (ethnomedicine) (Hadanu et al., 2022), beauty (ethnocosmetics) (Putri & Kaliu, 2022) and food ingredients (ethnofood) (Sari et al., 2021).

Among the many flora that grow in Indonesia, thousands of plants have medicinal properties and are used by the community to treat various diseases (Hadanu et al., 2022). One of the plants in Indonesia is Patikala. Patikala with the Latin name *Etilingera elatior* (Jack) R.M. Smith can be used as food (Sari, et al., 2021) and also used in medicine (Fahrudin, et al., 2016). Patikala can grow in yards and gardens or grow wild in Kolaka. Various tribes, such as the Tolaki, Bugis, Toraja, and Makassar tribes, inhabit Kolaka. Even though the tribes in this area are pretty complex, they still maintain local wisdom, such as using local plants to help the family economy.

Patikala fruit can play a role in improving the immune system. The immune system has a vital role in maintaining health. Moreover, currently, Indonesia is in the post-pandemic period of COVID-19, or what is known as the New Normal. The COVID-19 pandemic has significantly impacted the health and economic sectors. The public is advised to implement



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health protocols, including wearing masks, maintaining distance, washing hands, and maintaining body immunity. One way to keep the immune system is by consuming probiotic drinks because the digestive tract also influences the human immune system (Cabinian et al., 2018). Thus, medical experts agree that a healthy digestive tract reflects general health.

The components in patikala fruit that can improve the immune system are flavonoid compounds (Wahyuni et al., 2017). Apart from that, according to Ahmad et al. (2015), patikala fruit also contains phenolic compounds, which function as antioxidants. Patikala is one of the fruits that can be used as a probiotic drink because it contains nutrients that are good for the body, namely 4.4g carbohydrates, 1.2 g dietary fiber, 1.0 g fat, 1.3 g protein, 91 g water, 32 mg calcium, 4 mg iron, 27mg magnesium, 30mg phosphorus, 54 mg potassium and 0.1 mg zinc. Microorganisms can use these nutrients as an energy source for their growth (Simatupang et al., 2018).

Probiotics are live microorganisms that benefit the host's health, both animals and humans. The working principle of probiotics is to utilize the ability of these organisms to break down long chains of carbohydrates, proteins, and fats (A'yuni et al., 2020). Making probiotic drinks from starch juice uses lactic acid bacteria as a probiotic agent, which must remain alive from the time they are consumed until they reach the human intestine. Apart from that, the required probiotic bacteria must be able to produce antimicrobial substances to suppress pathogenic bacteria's growth, grow well in vitro, and have high stability so they are safe for human consumption. One of the bacteria that has the above criteria is *Lactobacillus casei*.

Milk concentration factors also influence the quality of probiotic drinks. The milk must be sterile to increase the nutritional value of the probiotic drink from patikala essence. Meanwhile, the length of fermentation will determine the ability of the bacteria to break down nutrients in the medium optimally (Retnowati, 2013). Hence, the research method we use to analyze the probiotic levels of this patikala juice is by using variations in fermentation time.

Several studies have been conducted on probiotic drinks containing fruit juice. For example, research on probiotic drinks containing strawberry juice and *Lactobacillus casei* isolates (A'yuni et al., 2020) shows that the longer the fermentation time, the higher the antioxidant and probiotic content. Meanwhile, research using snake fruit juice (Utami, 2018) demonstrates the effect of adding milk and varying fermentation times on the properties of probiotic drinks.

Based on this explanation, this research will examine the potential of patikala fruit juice (*Etlingera elatior*) (Jack) R.M.S.m), which is abundant in Kolaka and contains many nutrients, to become a probiotic and antioxidant drink.

Methods

This research was carried out experimentally, namely deliberately giving treatment to the research object and then looking at the consequences of the treatment provided. So the levels of probiotics and antioxidant activity contained in patikala (*Etlingera elatior*) (Jack) R.M.Smith) fruit juice can be reached out. This research used a randomized block design using different fermentation time factors: 20 hours, 24 hours, and 28 hours. Each treatment was carried out three times repetitions. The population of this study included 5 kg of patikala fruit typical of Kolaka, powdered skim milk, and *Lactobacillus casei* bacteria.

The samples used in this research were 400 ml of patikala fruit juice, 500 ml of powdered skim milk, and 100 ml of *Lactobacillus casei* bacteria. The sample size was 450 ml of patikala fruit juice probiotic drink (Patikala fruits are mixed with plenty of water and then filtered) with a proportion of 40% patikala fruit juice, 50% skim milk, and 10% *Lactobacillus casei* bacteria. Data collection for this research used a one-shot case study. This research was conducted in July and August 2023. Data collection regarding probiotic levels used the pour plate method. The pour plate method is by taking 1 ml of each sample then putting each into a test tube containing 9 ml of sterile NaCl solution (10^{-1} dilution),

then taking 1 ml of the 10⁻¹ dilution and putting it back into a test tube containing 9 ml NaCl (dilution 10⁻²), this process is carried out continuously until dilution 10⁻¹³. Then, 1 ml of the last three dilutions was taken and poured into a cup containing sterile MRSA in warm conditions. The following process is incubation at 37°C for 48 hours. The colonies that grew on each plate were recorded, and the TPC number in 1 ml was calculated by multiplying the average number of colonies by the dilution factor used in Colony Forming Units/ml. Calculation of the number of bacterial colonies is by using the Standard Plate Count (SPC) formula (Jackson et al., 2000):

$$\text{Number of colonies} = \frac{1}{\text{dilution factors}}$$

Testing the antioxidant activity of this drink uses a DPPH solution. DPPH solution was prepared by dissolving 50 mg DPPH with 70% methanol. This study also tested antioxidant activity by measuring the absorption value. The sample was left at room temperature in the dark for 30 minutes, and then the absorbance value was estimated at a wavelength (λ) of 517 nm. The blank used was made based on the procedure above and replaced with aquademineralization-deionization then the inhibitory activity was calculated using the equation (Santoso, 2017):

$$RSA = \frac{Abs.BI - Abs.S}{Abs.BI} \times 100\%$$

Result

Probiotic Contents

Samples fermented for 20 hours, 24 hours, and 28 hours produced the same mean of total LAB (lactic acid bacteria), which was 9.6 × 10¹⁴ CFU/ml.

Table 1. Results of probiotic contents analysis

Fermentation Time	Total colonies			Mean of total colonies (CFU/ml)
	Repetition 1	Repetition 2	Repetition 3	
20 hours	16	6	7	9,6 × 10 ¹⁴
24 hours	8	16	5	9,6 × 10 ¹⁴
28 hours	9	16	4	9,6 × 10 ¹⁴

This indicates that the maximum bacterial growth rate is reached between 20 and 28 hours of fermentation so that the bacterial cells do not multiply further. This is because the bacteria are stationary, meaning that the number of living cells equals the number of dead cells (Risna et al., 2022).

Antioxidant activity

The qualitative antioxidant activity test in Figure 1. (1) shows a picture of the DPPH solution before being mixed into a test tube containing patikala fruit juice. Then, in Figure 1. (2), the DPPH solution is added to the patikala fruit juice drink. DPPH was used as a free radical to prove whether patikala fruit juice extract has free radical scavenging activity. The picture below shows positive results regarding the antioxidant activity of patikala fruit juice. The activity of the sample resulted in a color change in the DPPH solution in methanol, which was initially purple after being mixed with the sample solution by homogenizing it using a vortex and then changed to a pale yellow color.

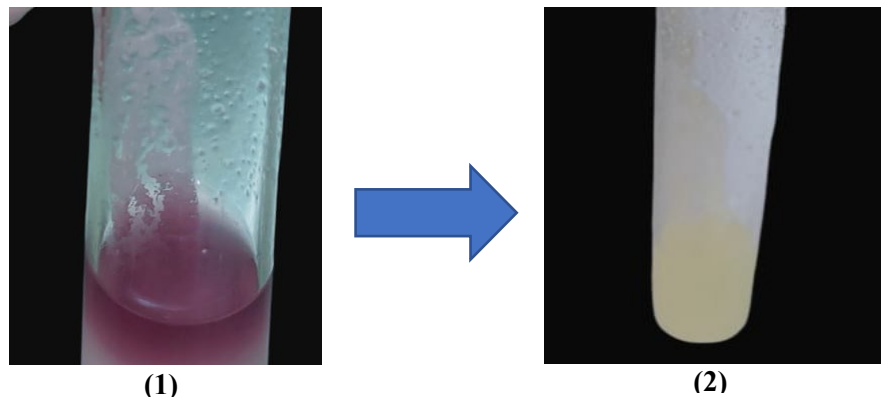


Figure 1. DPPH solution in methanol (1), Patikala probiotic drink + DPPH solution (2).

Table 2. Analysis data in the form of inhibition percentage

Fermentation Time	% Inhibition			Mean Inhibition %
	Repetition 1	Repetition 2	Repetition 3	
20 hours	7,054%	9,959%	5,904%	7,369%
24 hours	7,081%	10,324%	8,243%	8,549%
28 hours	8,432%	11,675%	9,594%	9,900%

Discussion

In Table 1. Samples fermented for 20 hours, 24 hours, and 28 hours produced the same mean of total LAB, namely 9.6×10^{14} CFU/mL. This shows no difference in probiotic levels in the patikala fruit juice (*Etilingera elatior*) probiotic drink. Different fermentation time treatments for the patikala fruit juice probiotic drink produced the same probiotic levels. This is due to several factors, including substrate concentration and number of starters.

In addition to the time factor, substrate concentration also influences bacterial growth. The lower the dilution rate and the longer the fermentation time, the more lactic acid bacteria tend to increase. This may be due to glycolysis during the fermentation process by lactic acid bacteria for metabolism (Primordia et al., 2014). A thinner surface increases the concentration of nutrients in the substrate, growing glycolysis for lactic acid bacteria and leading to higher growth. The longer the fermentation time, the more sugar is broken down, and the easier lactic acid bacteria can grow.

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Table 2. shows that with increasing fermentation time, the percentage of antioxidant/inhibition of the patikala fruit juice drink increases. These results show that the longer the variation in fermentation time, the greater the amount of antioxidants.

Changes in phytochemical compounds cause this; namely, during the fermentation process, there is an increase in phenolic compounds because, during fermentation, enzyme activity occurs, which increases the release of phenolic compounds from bound form to free form. Lactic acid bacteria can degrade ferulic acid and cinnamic acid in polysaccharides in fruit (A'yuni et al., 2020). During fermentation, the amount of phenolic compounds increases due to the hydrolysis of sugar by lactic acid. Microbial enzymes hydrolyze phenolic glycosides and release aglycones that have antioxidant activity. An increase in total phenolic compounds can increase antioxidant activity because these phenolic compounds have the potential to act as antioxidants.

Conclusions

The research results show that patikala fruit juice extract has the potential to be a probiotic drink and has antioxidant activity. The results obtained from the analysis of probiotic contents and antioxidant activity show that the potential of patikala fruit juice to be the best probiotic and antioxidant drink is at a time variation of 28 hours. During this time, the probiotic contents reached a maximum of 9.6×10^{14} CFU/ml, which had the best ability to ward off free radicals and had 9.83% inhibition.

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Declaration statement

The authors reported no potential conflict of interest.

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