



The Effect of Giving Forest Guava Fruit Yogurt (*Bellucia pentamera Naudin*) on the Growth of *Esterichia coli* Bacteria in Mice (*Mus musculus*)

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Abstract: This research investigates the effect of forest guava fruit yogurt (*Bellucia pentamera Naudin*) on the growth of *Escherichia coli* bacteria in mice (*Mus musculus*). The study utilized an experimental design with treatment and control groups. Mice were divided into four groups: three treatment groups received forest guava fruit yogurt at concentrations of 5, 10, and 15%, respectively, administered at 0.5 ml per dose daily for 14 days, while the control group was given plain yogurt. *E. coli* growth was assessed by analyzing fecal samples from each group. The findings revealed that the growth of *E. coli* bacteria in the mice decreased significantly after administration of forest guava fruit yogurt, with the most pronounced reduction observed in the group receiving the 15% concentration. In contrast, the control group, which received plain yogurt, exhibited a less marked decrease in *E. coli* growth. These results suggest that forest guava fruit yogurt has a stronger inhibitory effect on *E. coli* bacteria compared to plain yogurt. In conclusion, forest guava fruit yogurt appears to be an effective natural agent for reducing *E. coli* growth in mice, potentially offering added health benefits beyond those of conventional yogurt.

Keywords: *E. coli*; Jambu hutan (*Bellucia pentamera Naudin*); Mencit (*Mus musculus*); Yoghurt

Introduction

Stunting is a significant nutritional problem in Indonesia, characterized by impaired physical growth due to poor nutrition, infections, and insufficient care. Children under five years old are particularly vulnerable, with stunting potentially causing long-term developmental issues in physical, mental, and cognitive health (de Onis & Branca, 2016; De Sanctis et al., 2021; Marissa et al., 2024; Wirottama et al., 2023). One of the factors contributing to stunting is Environmental Enteric Dysfunction (EED), a disorder of the small intestine associated with an imbalance of gut microorganisms. EED in stunted children often leads to chronic

inflammation, reduced nutrient absorption, and an overgrowth of harmful pathogens such as *Escherichia coli* (*E. coli*), which further inhibits growth (Crane et al., 2015; Gabain et al., 2023; Harper et al., 2018; Hutasoit, 2020; Rinanda et al., 2023; Zhang et al., 2022).

Escherichia coli is one of the main species of gram-negative bacteria in the form of short rods that have a length of about 2 µm, a diameter of 0.7 µm, a width of 0.4-0.7 µm and are facultative anaerobes. *E. coli* becomes pathogenic if the number of these bacteria in the digestive tract increases or is located outside the intestines. Excessive growth of pathogenic bacteria in the digestive tract caused by infection and low immunity

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will cause the probiotics in the digestive tract to decrease (Basavaraju & Gunashree, 2022; Hutasoit, 2020).

Probiotics, particularly lactic acid bacteria (LAB), have been shown to have beneficial effects in balancing gut microbiota and inhibiting pathogenic bacteria like *E. coli*. LAB produce antimicrobial compounds that help prevent the growth of enterotoxigenic bacteria (Anumudu et al., 2024; Dempsey & Corr, 2022; Riadi et al., 2017; Shokryazdan et al., 2014). Research by Windiyanti et al. (2023) has demonstrated that forest guava fruit extract can inhibit the growth of *E. coli*, suggesting its potential as a natural remedy for gut-related issues.

Forest guava fruit (*Bellucia pentamera Naudin*) is a local fruit that, when processed into yogurt, offers several benefits, including extended shelf life and enhanced practicality for consumption. The addition of probiotics through forest guava fruit yogurt could help restore microbial balance in the gut, potentially reducing harmful bacterial growth and improving nutrient absorption, which is critical for addressing stunting in children (Marisa & Salni, 2023; Ayu Rochmiyah et al., 2022). This research will test the effect of forest guava fruit yogurt on the growth of *Escherichia coli* bacteria using mice. Mice are widely used as laboratory animals because they have advantages such as a relatively short life cycle, easy to handle, and reproductive characteristics similar to other mammals (Dewi et al., 2023).

This study aims to evaluate the effect of forest guava fruit yogurt on the growth of *E. coli* in mice (*Mus musculus*), using them as a model for testing its potential probiotic benefits. By reducing *E. coli* in the digestive tract, the yogurt could contribute to improving gut health and nutrient absorption, with the hope of extending these findings to stunted children. This research could provide a new dietary approach to help reduce stunting by promoting better digestion and growth.

Method

This research uses the experimental method with a treatment/experimental group design and a control group. The samples in this study used white mice (*Mus musculus*) with 3 treatment groups by giving 0.5 ml of forest guava fruit yogurt in concentrations of 5, 10, and 15% every day for 14 days. The control group used pure yogurt. The mice used were 7 mice for each group. *E. coli* bacteria examination was carried out before and after the intervention. The bacteria measured came from mice feces taken on the day the bacterial examination was to be carried out.

Procedure of Forest Guava Fruit Yogurt

Prepare 100 grams of forest guava fruit. Wash and peel then cut the unused parts of the guava. Crush the forest guava fruit that has been cut. Squeeze the juice from the forest guava fruit with a cloth. Prepare a frying pan, add 500 ml of mineral water and 60 grams of powdered milk then stir until smooth. Stir and heat over low heat to 40 °C, don't let it boil. Add 50 grams of granulated sugar and forest guava juice (according to the concentrate) then heat while stirring until the temperature is 80 °C, then turn off the heat and wait for the temperature to drop to 40 °C. Add 25 ml of yoghurt starter, stir well then transfer to a bottle. Incubate the yoghurt at room temperature 30 °C for 24 hours. After 24 hours, store the yoghurt in the chiller section of the refrigerator and the yoghurt is ready to use.

Isolation and Identification of *E. coli* Bacteria

The mouse feces samples were crushed and mixed with NaCl at a dilution of up to 10-10. The dilution results were then planted on eosin methylene blue agar (EMBA) media using the spread method and incubated for 24 hours at 37 °C. After 24 hours, the growing colonies can be counted using a colony counter. *E. coli* colonies that have been confirmed by Gram staining are then confirmed using the IMVIC test. The indole test is carried out by growing *E. coli* in a tube containing sulfide indole motility (SIM) medium, the methy red and Voges proskauer test with MR-VP medium, and for the citrate test with Simon citrate. The tubes were incubated for 24 hours at 37 °C.

Data analysis

Data analysis used the Dependent T test to determine differences before and after treatment and the ANOVA test to determine differences between groups.

Result and Discussion

Guava fruit yoghurt has a characteristic white, slightly brownish color with a thick texture like yoghurt, a dominant sour and slightly sweet taste.

Table 1. Validation Test

Group (%)	Number of Colony	
	Before (cfu/ml)	After (cfu/ml)
5	4	3
10	5	4
15	4	5
Control	5	4

Before checking the number of *E. coli* and Lactobacillus bacteria, a validation test is first carried out. This validation test functions as a comparison to determine whether there is contamination, both from the

equipment and media used during the measurement. In this validation test, the number of colonies that grow must be < 10, if the number of colonies that grow in the petridish is > 10, then the entire examination must be repeated because the examination is contaminated and cannot be used. In the validation measurements, only EMB media was used, dissolved in distilled water, then poured into a petridish without containing the mouse feces samples, then incubated in an incubator at a temperature of 37 °C for 24 hours. The results of the validation test showed that the number of colonies was < 10, which indicates that the media and materials were not exposed to contamination from external factors.

Table 2. The Average Growth of *Escherichia coli*

Group (%)	Before	After
5	5.8x10 ⁷	31x10 ⁴
10	5.5x10 ⁷	2.3x10 ⁴
15	5.2x10 ⁷	1.6x10 ⁴
Control	7.9x10 ⁷	2.3x10 ⁵

The average number of *Escherichia coli* growth in mice before being treated with 5% guava fruit yoghurt was 5.8 x 10⁷, while the average number of *Escherichia coli* growth in mice after being treated with 5% guava fruit yoghurt was 3.1 x 10⁴. The average growth of *Escherichia coli* in mice before being treated with 10% guava fruit yoghurt obtained an average number of 5.2 x 10⁷ while

Table 4. Paired Sample T-Test before and after Intervention

Group (%)	Min (cfu/ml)	Max (cfu/ml)	Mean (Before)	Mean (After)	Delta	Standard Deviation	Sig
5	1.1 x 10 ⁴	9.1 x 10 ⁷	5.8 x 10 ⁷	3.1 x 10 ⁴	-5.7 x 10 ⁷	2 x 10 ⁷	0.001
10	1.1 x 10 ⁴	6.9 x 10 ⁷	5.5 x 10 ⁷	2.3 x 10 ⁴	-5.4 x 10 ⁷	1 x 10 ⁷	0.018
15	1.4 x 10 ⁴	8.7 x 10 ⁷	5.2 x 10 ⁷	1.6 x 10 ⁴	-5.1 x 10 ⁷	2 x 10 ⁷	0.000
Control	1.1 x 10 ⁵	9.4 x 10 ⁷	7.9 x 10 ⁷	23 x 10 ⁵	-7.8 x 10 ⁷	1 x 10 ⁷	0.018

The difference results obtained were that the reduction in *Escherichia coli* bacteria was greatest in the 15% group. In all treatment groups, the significance value was < 0.05. The results of the Independent T Test in the intervention and control groups are as follows

Table 5. Independent T Test in the Intervention and Control Groups

Group	Sig.(2-tailed)
Control - 5%	0.002
Control - 10%	0.002
Control - 15%	0.002

It was concluded that there was a significant difference between the average growth of *Escherichia coli* bacteria in mice in the control group and the intervention group.

the average growth number of *Escherichia coli* in mice after being treated with 10% guava fruit yoghurt was 2.3 x 10⁴. The average growth of *Escherichia coli* in mice before being treated with 15% guava fruit yoghurt obtained an average number of 5.5 x 10⁷ while the average growth number *Escherichia coli* in mice after being treated with 15% guava fruit yoghurt was 1.6 x 10⁴.

In this research, a normality test was first carried out to see whether the resulting data was normally distributed or not so that we could continue with the statistical test of mean differences.

Table 3. Normality Test

Group (%)	Sig.	
	Before	After
5	0.383	0.051
10	0.725	0.000
15	0.991	0.420
Control	0.244	0.000

The results of Shapiro-Wilk normality test which shows that the data for each data has a sig value > 0.05, which means that the group data of 5, 10, 15% and control group are normally distributed, except control group and 10% are not normally distributed.

The results for differences in the average growth of *Escherichia coli* for each group before and after giving forest guava fruit yoghurt is shown in Table 4.

The difference in the amount of *Esherichia coli* bacteria growth between groups can be seen in the One Way Anova test results.

Table 6. One Way Anova Test on All Treatment Groups

Group (%)	Min	Max	Delta	Mean	Std. Deviation	Sig
5%	1x10 ⁷	9x10 ⁷	-2x10 ⁷	5x10 ⁷	2x10 ⁷	0.51
10%	2x10 ⁷	6x10 ⁷	-4x10 ⁷	5x10 ⁷	1x10 ⁷	
15%	2x10 ⁷	8x10 ⁷	-7x10 ⁷	5x10 ⁷	2x10 ⁷	
Control	5x10 ⁷	9x10 ⁷	-4x10 ⁷	7x10 ⁷	1x10 ⁷	

It was concluded that there was no significant difference in the growth of *Esherichia coli* bacteria between groups. Yoghurt is made through a fermentation process using a mixture of *Lactobacillus bulgaricus*, *Lactobacilus acidophilus*, and *Streptococcus thermophilus* bacteria which can break down milk sugar (lactose) into lactic acid. Lactic acid bacteria (LAB) have basic properties as probiotics and produce antimicrobial compounds. The antimicrobial compounds produced by

LAB are able to inhibit the growth of enterotoxigenic bacteria such as *Escherichia coli* (Li et al., 2017; Popović et al., 2020; Riadi et al., 2017; Sorensen et al., 2025).

Decrease in bacteria *Escherichia coli* that were greater in mice given 15% yoghurt was related to the results of research of Windiyanti et al. (2023) which found that forest guava fruit contains secondary metabolite compounds such as alkaloids, flavonoids, saponins, tannins and terpenoids. The greater the guava fruit concentrate used in yoghurt, the greater the reduction in bacteria *e. coli* due to the content of secondary metabolite compounds in forest guava which can inhibit or even kill the growth of bacteria *Escherichia coli*.

From the results of this research, the growth of bacteria *Escherichia coli* in mice after being given forest guava fruit yoghurt decreased. Decrease in bacteria were most *Escherichia coli* abundant in the group given forest guava fruit yoghurt with a concentration of 15%. In the plain yoghurt group, bacterial growth *Escherichia coli* decreased less than the group given forest guava yoghurt. This is in line with research conducted by Agusanty et al. (2023) which found that yoghurt with the addition of forest guava fruit had an inhibitory value for bacteria *Escherichia coli* which is better than pure yoghurt.

Alkaloids and tannins can disrupt the formation of bacterial cell walls and even cell death. According to Wulandari et al. (2019) alkaloids interfere with the components of peptidoglycan formation, while tannins interfere with peptidoglycan synthesis. Inhibition also occurs due to flavonoid and saponin compounds. Flavonoid compounds are thought to cause damage to cell membranes which can interfere with bacterial growth. Saponin compounds are able to reduce the surface tension of cell walls which causes the cell walls to lyse and antibacterial substances to enter the cells. Apart from that, terpenoid compounds are also found in cashew fruit extract. Terpenoid compounds can reduce the permeability of bacterial cell walls which causes bacterial cells to lack nutrition so that their growth will be hampered (Arabski et al., 2012; Mawan et al., 2018; Cankaya & Somuncuoglu, 2021).

Conclusion

In conclusion, forest guava fruit yogurt (*Bellucia pentamera Naudin*) has been shown to effectively inhibit the growth of *Escherichia coli* bacteria in mice (*Mus musculus*), with higher concentrations, particularly 15%, demonstrating the greatest efficacy. This suggests that forest guava fruit yogurt offers greater health benefits compared to plain yogurt, particularly in promoting gut health. The findings of this study have broader

implications, especially in the context of public health. As a natural probiotic source, forest guava fruit yogurt holds potential for inclusion in nutritional intervention strategies aimed at improving gut health in children, particularly in areas prone to digestive issues caused by pathogenic bacteria like *E. coli*. Furthermore, this yogurt could play a complementary role in efforts to reduce stunting, given the critical role of gut health in optimal nutrient absorption. Future studies are necessary to confirm these benefits through clinical trials on humans and to explore the commercial development of this product for wider community access.

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Author Contributions

Conceptualization, methodology, software, preparation of original writing draft, formal analysis, investigation, resource, S.F.A.; project administration, data curation, supervision, writing-review and editing, visualization, M.S. and F.C.

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Conflicts of Interest

The authors declare that there is no conflict of interest.

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