Acta Medical and Health Sciences, 2(2): 73-80 (2023)

Advance Access Publication Date: October 2023
DOI : 10.35990/amhs.v2n2.p73-80

ORIGINAL ARTICLE

Studying the influence of guava fruitghurt on *Escherichia coli* coloniesin rats' digestive tracts

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ABSTRACT

A balanced microbiota can play a role in the host's defense mechanism against pathogenic infections. *E. coli* is a harmless and beneficial commensal. An increase in bacteria will change their nature to become pathogens that can cause gastrointestinal disease, such as diarrhea. The management of such condition can be managed in several ways, including the consumption of probiotics. Fruitghurt is a variant of yogurt that is made using fruit juice. *L. acidophilus* is resistant to stomach acid; it can inhibit the growth of pathogenic bacteria and can maintain the number of live bacteria up to 10^7/ml. The research design used was experimental. The research subjects were white rats (Rattus norvegicus), with the Wistar strain as many as 27 rats. They were divided into 3 groups: 1. K (-),a negative control group that was not induced by *E. coli* and was not given fruitghurt; 2. K (+)a positive control group that was given *E.coli*, but not treated by fruitghurt; and 3. P1, the treatment group that was induced with *E.coli* and treated by fruitghurt. The results showed that guava fruitghurt could reduce *E. coli* colonies. The number of *E. coli* colonies after being given fruitghurt was 161, while in the rats that were not given fruitghurt, there were 258 colonies. Guava fruitghurt can reduce *E. coli* because of the flavonoid, tannin, and polyphenol contents found in red guava, which act as protein coagulators, antibacterials, and toxins, as well as *L. acidophilus* bacteria that can inhibit the growth and attachment of pathogenic bacteria in the digestive tract and inhibit the spread of pathogenic bacteria.

Keyword: *E. coli*, Fruitghurt, *L. acidophillus*, Probiotic, Microbiota

Received: 2023-06-21, Revised: 2023-09-11 Accepted: 2023-10-20, Published: 2023-10-31.

How to cite :
Dewi, A.K.P., Mahendra, G.D. and Ambarukmi, F. (2023) "Studying the influence of guava fruitghurt on Escherichia coli coloniesin rats' digestive tracts", Acta Medical and Health Sciences, 2(2).p73–80. doi: 10.35990/amhs.v2n2.p73-80

INTRODUCTION

Red guava fruitghurt is a variant of yogurt made of red guava fruit and *L. acidophilus* bacteria. One of the advantages of this fruitghurt is that it can be consumed by individuals who are sensitive to milk or who have lactose intolerance.[1,2] Red guava is abundant and produce fruit throughout the year in various regions of Indonesia. It is a fat-free and lactose free fruit, which contains vitamin C, flavonoids, and tannins that are highly beneficial for the human body.[3,4] Furthermore, red guava has the advantage of being a source of prebiotics for the digestive tract. These prebiotics serve as food for probiotic bacteria.[2] *Lactobacillus acidophilus* is a lactic acid bacteria (LAB) that can be used because it is resistant to gastric acid, can inhibit the growth of pathogenic bacteria, and can maintain a viable bacterial count of up to 10^7 colony-forming units per milliliter (CFU/mL).[5] LABs are a group of bacteria that can convert carbohydrates into lactic acid. Carbohydrates fermentation may result in the lactic acid production, which can lower the pH. The decrease in pH can inhibit the growth of other microorganisms, especially pathogenic bacteria.[6] Probiotics become beneficial microorganisms for human health when given in the right amount. Probiotics may present in the form of food supplements containing non-pathogenic live bacteria that are resistant to stomach acid and can establish colonies in the colon.[7]

The microbiota refers to a collection of microorganisms that live within the host's body. In humans, the microbiota primarily consists of various types of bacteria but also includes viruses, fungi, and other eukaryotic organisms. The human body provides a suitable environment for these microorganisms, particularly within the digestive tract, skin, and respiratory system. The human microbiota plays a crucial role in maintaining the health and balance of the body.[8,9] They assist in the processes of food digestion, synthesis of vitamins, protection against pathogens, and regulation of the immune system.[10,8,11] A balanced microbiota (eubiosis) can play a role in maintaining the immune response by interacting with intestinal epithelial cells, thereby promoting a tolerant condition in the digestive tract.[8,12] A changing composition of microbiota can disrupt the homeostatic mechanisms of the microbiota, a condition known as dysbiosis. Dysbiosis is a state in which there are qualitative and quantitative alterations in the composition, distribution, and metabolic activities of microbes, which can lead to detrimental effects on the host.[12] The disruption of the balance of the microbiota in the digestive tract is associated with various diseases that can occur, such as inflammatory bowel diseases, irritable bowel syndrome, metabolic disorders, and allergies.[12,8,13] *Escherichia coli* is one of the important microbes in the human gastrointestinal tract.[8] In healthy individuals, this bacterium is harmless commensal and beneficial to humans. However, under conditions where there is an increase in the number of these bacteria, its nature can change to become a pathogen that can cause gastrointestinal diseases in animals and humans.

Based on the background above, this study aims to determine the influence of administering guava fruitghurt on the number of *E. coli* colonies in the digestive tract of experimental animals.

METHODS AND SUBJECTS

Research Subjects

The research subjects used in this study were white rats (Rattus norvegicus) of the Wistar strain. The rats selected for this research met the following criteria: healthy and active, aged 2-3 months, weighing 200-300 grams, and of the male gender. Any rats that showed signs of sickness during the acclimatization period and had any anatomical abnormalities were excluded from the subjects of this research.

Research Procedure

To reidentify of *Escherichia coli* and *Lactobacillus acidophilus* bacteria to be used, several macroscopic observations were employed including microscopic examination with Gram staining, cultivation on differential media, and biochemical tests. The process of bacterial reidentification on a macroscopic level was carried out by observing the bacterial colonies formed on the surface of EMBA after incubation for 24 hours in a CO2 incubator at a temperature of 37°C with a CO2 concentration of 7.5%. The *Escherichia coli* colonies in Figure 1 appear metallic green in color.

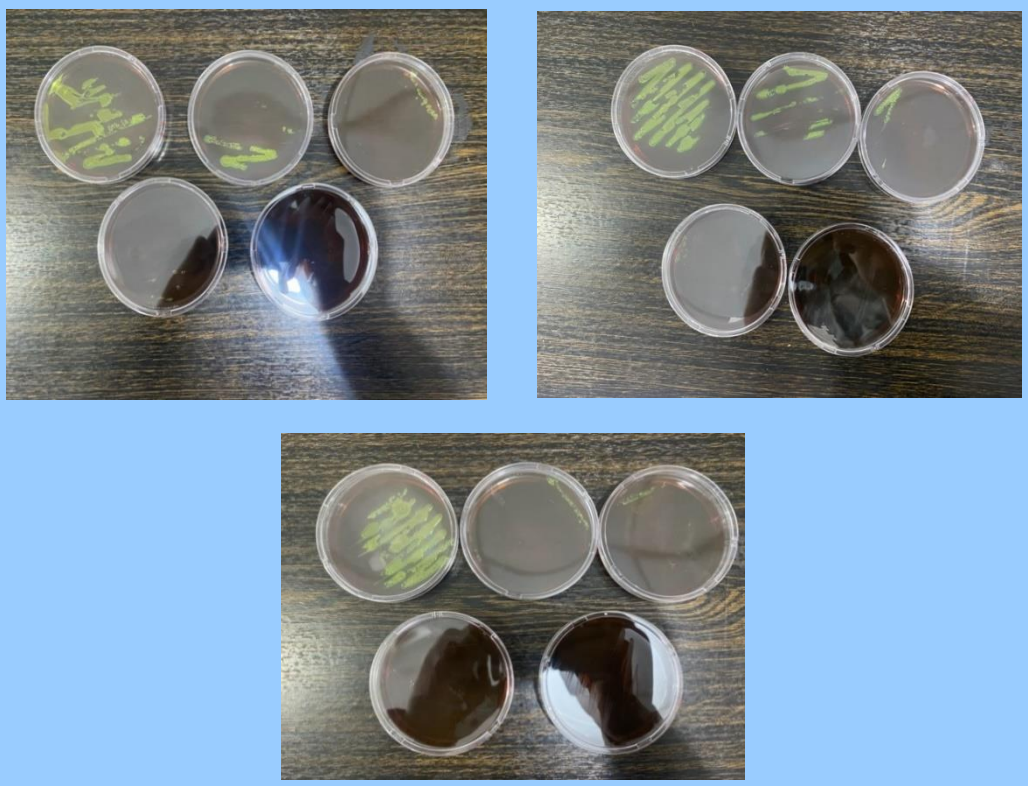

Figure 1. The growth of *E. coli* on EMB

The process of bacterial reidentification on a microscopic level was performed by observing the shape and color of the bacteria under a microscope at a magnification of 1000x after Gram staining. The characteristics of *Escherichia coli* bacteria include being Gram-negative, rod-shaped, and facultatively anaerobic.

The reidentified bacteria were then rejuvenated by culturing them on MRSB medium with 7.5% CO^2 at 37°C for 48 hours. The red guava that was used in this study was selected at its ripeness and freshness, showing a yellowish skin color and pink to red flesh with a sweet taste. The guavas that were included in the subjects' criteria were thoroughly washed and placed into a juicer at a speed of 47 rpm to obtain the fruit juice. The juice was then mixed with water in a 1:1 ratio. The mixture is then filtered multiple times until a clear, free-fiber guava juice is obtained. A total of 225 ml of red guava juice was mixed with 25 grams of sucrose. The mixture is then sterilized through autoclaving for 15 minutes at 121°C.[1] Prior to being used in the study, the experimental animals were acclimatized to standard environmental conditions (temperature of 22-28°C, 12-hour light-dark cycle) and provided with standard feed and ad libitum access to water for 7 days. After the acclimatization process, the rats were fasted for 60 minutes before the

subsequent treatment and divided into three groups, with each group consisting of nine rats. The positive control group and treatment group were induced by orally administering *Escherichia coli* suspension at a dosage 1 ml x 10^5 CFU/ml per kg body weight of the rats for 1 day. Fruitghurt was administered orally using a syringe and given vertically at a dosage of 1 ml x 10^8 CFU/ml to the treatment group (P1) for 14 days.

Table 1. Research Groups

No.	Groups	Standard Feed	*Escherichia Coli*	Fruitghurt	Duration of Treatment
1.	Control group (KN)	+	-	-	23 days
2.	Positive control group (KP)	+	1 ml	-	23 days
3.	Treatment group (P1)	+	1 ml	1 ml	23 days

Note:
KN: Control group, rats not induced with *Escherichia coli* and not given fruitghurt.
KP: Positive control group, rats induced with *Escherichia coli* and not given fruitghurt.
P1: Treatment group 1, rats induced with *Escherichia coli* and given fruitghurt.

The samples used to count *E. coli* were feces from the experimental animals. The feces were aseptically collected directly from the rectum and transferred to sterile plastic containers. The feces were then weighed and homogenized in a physiological saline solution (0.9% NaCl), followed by serial dilutions. On day 23, the fecal samples were collected after the administration of fruitghurt. The media used was EMB agar as a differential media for *E. coli*. The colonies that were grown on EMB agar were counted using the *total plate counting* (TPC) method. TPC method is used to estimate the number of bacterial colonies that grow on the medium. In this research, all colonies that appear as *metallic green colonies* on the EMB (*Eosin Methylene Blue*) medium were counted, indicating that these colonies are *E. coli*.

Data normality was tested using the Shapiro-Wilk test before conducting statistical analysis, as the data size was less than 50. The data were then analyzed using the Levine test to assess homogeneity. If the data were found to be homogeneous, an Analysis of Variance (ANOVA) test was performed to determine the differences between groups.

Ethical Aspects of the Research

This study was conducted after obtaining ethical approval from the Research Ethics Committee of the Faculty of Medicine, Universitas Jenderal Achmad Yani, with the approval number 002/UH1.08/2022. The committee reviewed the research proposal involving animal subjects and ensured that the study adhered to ethical principles and guidelines. The welfare and well-being of the animals were prioritized throughout the research process. Informed consent was not applicable in this study as it involved animal subjects. The research procedures followed the ethical standards and regulations set by the committee to minimize any potential harm or discomfort to the animals.

In research that involves the use of animal models, there are ethical aspects specific to animal experimentation. This is done as an effort to avoid inhumane treatment when using animal subjects. The ethical standards employed encompass three principles known as the 3R concept: replacement, reduction, and refinement. The concept aims to eliminate inhumane practices towards test animals in accordance with the ARRIVE Guidelines

(*Animal Research: Reporting of In Vivo Experiments*) in Animal Research. The purpose of conducting research with animal models is to gain new knowledge for the benefit of humans without needlessly sacrificing the welfare of the animals involved. In this study, mice were chosen as the animal model due to their anatomical and bio-physiological similarities to humans.

Refinement refers to treating animals in a humane manner. Test animals should be free from thirst and hunger, and they should be provided with suitable cages. In this study, mice were placed in cages measuring 30 x 50 x 15 cm, with each cage housing 9 mice to allow them to engage in their normal activities. The cages were lined with 3 cm bedding that was changed every 2 days. The mice were housed in the animal facility of the Faculty of Medicine at Universitas Jenderal Achmad Yani. The facility provides a well-ventilated room with proper airflow, lighting from lamps, and sunlight entering through closed windows, maintaining a room temperature ranging from 26-28°C.

RESULTS AND DISCUSSION

Table 2. Bacterial Colony Counts

	Dilution						
Treatment	**10^1**	**10^2**	**10^3**	**10^4 A**	**10^4 B**	**10^5 A**	**10^5 B**
Control group (KN)	TMTC	TMTC	TMTC	320	306	144	138
Positive control group (KP)	TMTC	TMTC	TMTC	426	406	270	246
Treatment group (P1)	TMTC	TMTC	TMTC	346	332	180	142

Note:
KN: Negative control group, rats not induced with *Escherichia coli* and not given fruitghurt
KP: Positive control group, rats induced with *Escherichia coli* and not given fruitghurt
P1: Treatment group 1, rats induced with *Escherichia coli* and given fruitghurt
TMTC – Too many to count

In Table 2, it can be observed that there is a decrease in the number of *E. coli* colonies in the gastrointestinal tract of the experimental animals. These results are consistent with previous studies.[14] Fruitghurt, the fermented juice of red guava with *Lactobacillus acidophilus*, demonstrats inhibitory effects on the growth of *Escherichia coli*, as indicated by a reduction in colony count. This can be attributed to the chemical constituents of red guava, such as flavonoids, which can damage the bacterial cell wall and impair its permeability. Additionally, tannins present in red guava exhibit antibacterial properties by binding to proteins, inhibiting the formation of bacterial cell walls. Furthermore, polyphenols play a role as toxins in the bacterial protoplasm, causing damage and penetration of the cell wall.[14,15] Similar findings were obtained in a study conducted by Gary Efraim Girsang, indicating that fruitghurt, the fermented juice of red guava with *Lactobacillus acidophilus*, has the ability to inhibit the growth of *Escherichia coli* bacteria. This can be attributed to the properties of *Lactobacillus acidophilus*, which can inhibit the growth and adhesion of pathogenic bacteria and impede their spread.[16]

In the P1 treatment group, there is a decrease in the number of *Escherichia coli* bacteria after the administration of red guava fruitghurt. One of the important components in red guava is tannin, which

has antibacterial properties. Tannins can inhibit the growth of *Escherichia coli* bacteria by breaking down the bacterial cell walls.[17,18]

The administration of red guava fruitghurt is able to inhibit the colony count of microbiota in the gastrointestinal tract, especially *Escherichia coli*. This is due to the presence of *lactic acid bacteria* in fruitghurt, which can inhibit the growth of microbiota by increasing the production of metabolites such as biosurfactants, bacteriocins, organic acids, and H2O2, which can inhibit the growth of pathogenic bacteria.[19, 20]

Table 3. The effect of Fruitghurt on *Escherichia coli* colonies

Groups		p-value
KN	KP	0.000
	P1	0,000
KP	P1	0,000

Note:
KN : Negative control group, rats not induced with *Escherichia coli* and not given fruitghurt
KP : Positive control group, rats induced with *Escherichia coli* and not given fruitghurt
P1 : Treatment group 1, rats induced with *Escherichia coli* and given fruitghurt

In Table 3, the p-value is 0.000, which is smaller than the significance level (sig = 0.05). Thus, there is sufficient evidence to state that there is a significant difference in the number of bacterial colonies after induction with *Escherichia coli* and induction with *Escherichia coli* + Fruitghurt in the gastrointestinal tract of Wistar rats.

It can be observed that the ANOVA test shows a very low *p-value* (0.000) for the comparison between the treatment group and the positive control group, as well as the comparison between. The very low *p-value* indicates a significant difference between the groups.

The decrease in the number of *Escherichia coli* colonies is likely due to the action of fruitghurt fermented by *Lactobacillus acidophilus*. This bacterium is a lactic acid bacteria that can inhibit the growth of microbiota by increasing the production of metabolites such as biosurfactants and bacteriocins, which can inhibit the growth of pathogenic bacteria.[21] In addition, *Lactobacillus acidophilus* produces lactic acid as part of its metabolism, which exhibits antimicrobial properties and can inhibit the growth of *Escherichia coli* bacteria.[19,21]

The decrease in the number of *Escherichia coli* colonies may also be attributed to the presence of flavonoids, tannins, and polyphenols found in red guava. Flavonoids act by damaging the bacterial cell wall, leading to loss of cell permeability. Tannins, on the other hand, exhibit antibacterial properties by binding to proteins and inhibiting the formation of bacterial cell walls. Polyphenols, acting as toxins within the bacterial protoplasm, disrupt and penetrate the cell wall.[17,18]

The findings of this study align with the previous research, which suggests that red guava can inhibit the growth of *Escherichia coli* due to its content of flavonoids. Flavonoids are known to have protein coagulating properties, which can interfere with the formation of bacterial cell walls.[22,23] In addition, the role of fermented fruitghurt containing *Lactobacillus acidophilus* can inhibit the growth of *Escherichia coli* colonies due to the production of lactic acid, which exhibits antimicrobial properties and hampers the growth of *Escherichia coli* bacteria.[14]

CONCLUTION

Guava fruitghurt could reduce *E. coli* colonies in the gastrointestinal tract of Wistar rats. The number of *E. coli* colonies after the administration of fruitghurt were 161 colonies, while in rats with no administration of fruitghurt were 258 colonies.

DECLARATION OF INTERESTS

The author hereby declares that there is no conflict of interest in the scientific articles that we have written.

ACKNOWLEDGEMENTS

Our gratitude goes to the UNJANI Research and Community Service Institute, which has provided funds and opportunities to conduct this research as well as to the Dean and his staff who have given the author the opportunity to conduct and complete this research.

The authors also thank the professionals who have helped in the preparation and research such as colleagues, students, assistants to the Microbiology Laboratory and Animal Laboratory, Faculty of Medicine, Universitas Jenderal Achmad Yani. And all parties who have helped so that this research can be carried out.

FUNDING

This research received funding from the Institute Research and Community Service UNJANI Bandung.

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