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Antibacterial Activity of Rosella Tea Kombucha Against The Growth of *Escherichia coli* Based on Fermentation Time Variations

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ABSTRACT

Background: Public awareness has begun to experience changes in maintaining consumption patterns, such as consuming functional drinks. Rosella Tea Kombucha is able to produce important compounds for the body such as polyphenols, organic acids, vitamin B complex, vitamin C, enzymes and antibiotics. The use of antibiotics as a treatment for *Escherichia coli* can cause side effects and resistance so this Rosella Tea Kombucha can be an alternative. The aim of this research was to determine the activity of Rosella Tea Kombucha as an antibacterial against *Escherichia coli* bacteria. **Methods**: This type of research is a true experimental with a posttest-only group design. Antibacterial test uses the disc diffusion method with the Ciprofloxacin antibiotic as positive control and sterile distilled water as negative control. **Results**: The results showed that Rosella Tea Kombucha was able to inhibit the growth of *Escherichia coli* bacteria based on variations in fermentation time on 6, 8, 10, 12 and 14 days with the diameter of the inhibition zone respectively being 7.27 mm; 7.97mm; 8.43mm; 9.57mm; and 12.73 mm. Statistical tests using the One Way ANOVA test obtained a sig of (0.000) < 0.05, which means there are significant differences in the growth inhibition zone of *Escherichia coli* at various fermentation times of Rosella Tea Kombucha. The largest inhibitory zone diameter was shown in the fermentation time variation of 14 days with strong inhibitory category.

Keywords: Antimicrobial Activity, Rosella Tea Kombucha, Fermentation Time Variations.

INTRODUCTION

Kombucha tea is a traditional drink that has unique characteristics, because the process to make this tea by fermenting natural ingredients or various types of tea assisted by symbiotic culture. The symbiotic culture used has various names, one of which is Symbiotic Culture of Bacteria and Yeast (SCOBY). The kombucha culture or SCOBY is round and pale white and has a rubbery, gel-like texture. This follicle culture is made from cellulose resulting from the metabolism of acetic acid bacteria which is able to float and sometimes sink in the kombucha liquid. In general, kombucha is known as tea mushroom which is fermented for 1-14 days or according to the desired acidity level. In the fermentation of kombucha tea, the growth of yeast and bacteria occurs, the sugar content increases, ethanol production increases, and organic acid production increases (Restuati, 2011). The results of fermentation and oxidation of microorganisms in kombucha are useful for preventing cancer, improving liver function, helping control high blood pressure, preventing stroke, relieving throat pain, reducing fat and cholesterol, maintaining body stamina and maintaining skin cleanliness. The antimicrobial content in kombucha can inhibit the growth of Shigella sonnei, Escherichia coli, and Salmonella typhimurium. The combination of glucuronic acid and lactic acid in kombucha is very effective at destroying microorganisms (bacteria, viruses and fungi). Apart from that, the acetic acid contained in kombucha is known to be able to inhibit and kill a number of gram-positive and gram-negative bacteria (Naland, 2008). Kombucha tea contains various compounds that are very important for the body, such as polyphenols, organic acids (acetic acid, gluconic acid, lactic acid), vitamin B complex, vitamin C, enzymes and antibiotics (Firdaus et al., 2020). With the various ingredients contained in kombucha, it is believed that kombucha can overcome health problems such as high blood pressure, rheumatism, obesity, arthritis, migraines, diabetes and others (Naland, 2008).

Kombucha tea can be mixed and matched with various kinds of tea, for example rosella. Rosella is an annual plant with single leaves, oval-shaped and has serrations on the edge of the leaves. In general, the length of Rosella leaves ranges from 6-15 cm and width 5-8 cm. Rosella has a single flower where on each flower stalk there is one flower bud which has 8-11 hairy petals (Haidar, 2016). Rosella as a health drink is believed to cure various diseases such as hypertension, diabetes, diuretic. Rosella flower petals contain active substances such as hibisci glucose, gossypetin, and anthocyanin. Anthocyanins are compounds that can produce a red color and are classified as flavonoids. Anthocyanins are believed to be able to cure degenerative diseases (Djaeni et al., 2017). Apart from that, rosella contains catechins, vitamins C, B1, B2, carotenoids, organic acids, saponins and alkaloids which can damage bacterial cell proteins and cause cell death. With this antibacterial content, it is able to kill several types of bacteria such as Pseudomonas aeruginosa, Klebsiella pneumoniae, Staphylococcus aureus and Escherichia coli (Arifianti, 2015).

Escherichia coli is a bacteria belonging to the Enterobacteriaceae. Escherichia coli bacteria are normal flora in the human body which can be pathogenic, causing various diseases such as

diarrhea. Escherichia coli bacteria is the cause of diarrhea with a percentage of 21.5% apart from Shigella dysenteriae, Salmonella spp, Vibrio Cholerae and Campylobacter jejuni (Estri, 2015). In 2020, the coverage of diarrhea sufferers throughout Indonesia reached 44.4% at all ages and 28, 9% in toddlers (Kementerian Kesehatan RI , 2021). The use of synthetic antibiotics as a treatment for diseases caused by Escherichia coli bacteria can cause side effects and resistance. Alternative treatment is needed as an antibacterial using traditional medicine made from natural ingredients that are safer for daily consumption, namely Rosella Tea Kombucha. Therefore, in this study, the antibacterial activity of Rosella Tea Kombucha was tested against Escherichia coli bacteria using the disc disk test method based on varying fermentation times of 6, 8, 10, 12 and 14 days.

METHOD

This research is true experimental research with the design used is Posttest-Only Group Design, namely by measuring the inhibition zone of five types of Rosella Tea Kombucha samples with varying fermentation times. The research was conducted at the Bacteriology Laboratory, Department of Medical Laboratory Technology, Health Polytechnic of Denpasar from February to June 2022. The sample used in this research was Rosella which was made into kombucha. The Rosella used is 250 grams of dried Rosella flowers obtained from Denpasar area where the parts taken are the flower petals to be made into tea. For kombucha, we use kombucha seeds from Inidhya Kombucha in the Sesetan area, South Denpasar. This research is an experimental research in which the treatment used is the fermentation of Rosella Tea Kombucha with the same concentration but different fermentation times, namely samples on the 6th, 8th, 10th, 12th, 14th day, positive control (antibiotic ciprofloxacin) and negative control (sterile distilled water). This test was 3 replication at each time variation with the same concentration, 15 treatment sample data were obtained. Positive and negative controls were repeated 2 times to obtain 4 control data. The total data obtained was 19 data.

The instruments used in this research were a digital balance, analytical balance (RADWAG AS220.R2), cover glass, stir bar, gas stove, beaker (IWAKI TE-32), disposable Petri disk (ONEMED), vernier caliper, hot plate (JISICO), magnetic stirrer, measuring pipette (IWAKI), ball pipette, test tube (IWAKI), tube rack, tube, autoclave (TOMY SX-500), Erlenmeyer (IWAKI), dropper pipette, incubator (ESCO Isotherm), spirit lamp , Mc Farland Densitometer (biosan DEN-1B), Biosafety Cabinet (BSC-1800 II B2-X). The materials used in this research were dried rosella, SCOBY, sugar, mineral water, blank disk (OXOID), Escherichia coli bacteria, kombucha starter solution, distilled water, MHA media (OXOID), Ciprofloxacin disk (OXOID), physiological NaCl solution 0, 85%, 70% alcohol, sterile cotton cap, aluminum foil, Mc Farland standard 0.5%.

A. Procedure for making Rosella Tea Kombucha

Boil 250 grams of dried Rosella in 2500 ml of water until it turns red. 10% sugar from the total volume is added to the roselle tea solution then stirred until homogeneous then waited until the solution cools (temperature below 50˚C). The cooled Rosella tea is divided into 5 different containers, amounting to 500ml per

container, then added SCOBY and 10% starter of the total volume per container, then covered with gauze. Each container is labeled according to the fermentation day, namely days 6, 8, 10, 12 and 14. Kombucha tea that has been labeled is fermented according to the specified time and taken according to the time period stated on the label.

B. Antimicriobial test against Escherichia coli bacteria

Empty discs were saturated with kombucha tea according to the day of fermentation, then placed on an empty plate. The sterile cotton swab that has been prepared is then dipped into the prepared bacterial suspension, then lifted and squeezed by attaching the cotton swab to the wall of the tube. Bacteria are inoculated on the surface of the MHA media until they are evenly distributed over the entire surface of the media and the media is closed again. The media that has been inoculated with the suspension is left for 15 minutes so that the bacterial suspension can seep into the media. Discs that had been saturated from each kombucha tea according to the day of fermentation were attached to the inoculated MHA medium using tweezers. Discs that have been attached to the media surface must not be moved or shifted. Positive controls and negative controls were attached to MHA media that had been inoculated which was different from the kombucha tea media. The media to which the disc was attached was incubated at 37ºC for 24 hours in an inverted position.

Data was collected through laboratory examination by measuring the diameter of the inhibition zone using a caliper. Measurements were carried out on the growth of Escherichia coli bacteria formed from various samples, namely fermentation results on the 6th, 8th, 10th, 12th and 14th days expressed in millimeters (mm). The data analysis used in this research is quantitative analysis, carried out using the One Way ANOVA statistical test to determine the differences between various variations in the fermentation time of Rosella Tea Kombucha in the growth inhibition zone of Escherichia coli. To determine the significant differences in the inhibition zone between each of the variations in the fermentation time of Rosella Tea Kombucha which can inhibit the growth of Escherichia coli, the Least Significant Deference (LSD) test was used.

RESULTS

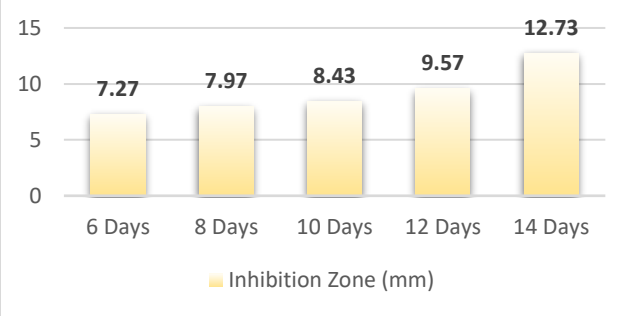
Results of the Rosella Tea Kombucha inhibition zone test on the growth of Escherichia coli bacteria with five treatments, namely fermentation days 6, 8, 10, 12 and 14 with 3 replication using the disc diffusion method. The results showed inhibition of the growth of Escherichia coli bacteria which was characterized by the formation of a clear zone around the disc that had been given the test substance, namely Rosella Tea Kombucha on MHA media.

Table 1. Results of Antibacterial Activity of Rosella Tea Kombucha

Fermentation Time (Days)	Inhibition zone(mm)			Mean of Inhibition Zone (mm)	Inhibition Zone Category
	I	II	III		
6	7,60	7,70	6,50	7,27	Medium
8	7,90	8,40	7,60	7,97	Medium
10	9,00	8,50	7,80	8,43	Medium
12	9,50	9,90	9,30	9,57	Medium
14	11,60	13,10	13,50	12,73	Strong

Based on table 1, there are differences in diameter results from the first to the third repetition. The diameter category of Rosella Tea Kombucha's inhibitory zone on the growth of Escherichia coli bacteria was the largest produced on the 14th day of fermentation (13.50 mm) with strong category, while the smallest diameter was produced on the 6th day of fermentation (6.50 mm) with medium category. Meanwhile, the results obtained from control group (40.95 mm) in the sensitive category and in the negative group there was no inhibition zone produced.

Picture 1. Rosella Tea Kombucha



Picture 2. Antibacterial Activity Graph of Rosella Tea Kombucha

Table 2. One Way ANOVA and LSD Statistical Test Results

LSD			ANOVA
Fermentation Time (Days)	Significant Time (Days)	Sig.	
6	12	.001	
	14	.000	
8	12	.012	
	14	.000	
10	14	.000	
12	6	.001	.000
	8	.012	
	14	.000	
14	6	.000	
	8	.000	
	10	.000	
	12	.000	

In this study, a normality test was carried out using the Shapiro-Wilk test. In this test the data is said to be normally distributed (symmetrical) because all the data gets a Sig value. > 0.05. Based on the results of the One Way ANOVA test, a sig of (0.000) < 0.05 was obtained, which means that there were differences in the growth inhibition zones of Escherichia coli at various times of rosella tea kombucha fermentation. Furthermore, the LSD results from the five fermentation time variations showed that there were significant differences in the inhibition zone on the 14th day of fermentation compared to the other time variations.

DISCUSSION

Based on the inhibition zone diameter category for natural ingredients, fermentation times on days 6, 8, 10 and 12 are included in the medium category (the range of inhibition zones produced has a diameter of 6 – 10 mm). Meanwhile, on the 14th day of fermentation, it was included in the strong category, because the range of inhibition zones produced had a diameter of 11-20 mm. The clear zone or

inhibition zone is formed due to the activity of secondary metabolites and acetic acid contained in the test sample. In this study, the test sample used was Rosella Tea Kombucha, which contains components such as flavonoids, tannins, catechins, alkaloids and saponins. Flavonoids are able to inhibit bacterial growth by inhibiting nuclear acid synthesis so that the cytoplasmic membrane and bacterial metabolism are disrupted and cell death can occur. The mechanism of action of saponin as an antibacterial is by reducing surface tension, resulting in increased cell permeability or leakage and resulting in intracellular compounds coming out. Furthermore, the tannin content contained in rosella is able to inhibit the enzyme reverse transcriptase and DNA topoisomerase so that bacterial cells cannot form. Catechins as antibacterials are able to damage the lipid bilayer membrane which will result in loss of cell structure and function so that cells can experience death (Arifianti, 2015).

Another compound produced in the formation of kombucha is acetic acid, which acts as an antibacterial by denaturing proteins which can cause the metabolic activity of bacterial cells to stop and can inhibit the growth of gram-positive and gram-negative bacteria (Cholidah, et al, 2020). The cell wall structure of gram-negative bacteria is more complex than the cell wall structure of gram-positive bacteria. Gram-negative bacteria consist of three inner layers, while gram-positive bacteria only have a single layer of their cells. The relatively complex cell structure of gram-negative bacteria will also make it more difficult for antibacterial compounds to enter the bacterial cells (Hamidah, et al, 2019). Based on the results of the inhibition zone diameter, it can be seen that the inhibition zone increases in proportion to the increase in fermentation time, however in certain situations the antibacterial inhibition zone formed will not always be directly proportional to the increase in the fermentation time of the antibacterial. This occurs due to differences in the speed of diffusion of antibacterial compounds on the agar media as well as the types and concentrations of different antibacterial compounds (Hamzah, 2019). The results of the diameter of the inhibition zone at various fermentation times for Rosella Tea Kombucha on the growth of Escherichia coli bacteria in this study showed an increase in results from various fermentation times. This increase was due to the longer the fermentation time, the more active substances produced increased. The longer the fermentation time, the larger the inhibition zone formed.

The results of measuring the inhibition zone diameter obtained from research on Rosella Tea Kombucha in inhibiting the growth of Escherichia coli bacteria showed that there were differences at each fermentation time. The data obtained was then processed using statistical tests. The first test carried out is the normality test or Shapiro Wilk test, then proceed with the One Way ANOVA test and LSD test. The Shapiro Wilk test showed the data was normally distributed. The One Way ANOVA test to determine inhibition zones differences in the growth of Escherichia coli using Rosella Tea Kombucha with different fermentation times. After analyzing the data obtained, Sig. (0.000) < (0.05), which means that there are differences in the inhibition zone for the growth of Escherichia coli at various times of Rosella Tea Kombucha fermentation. The LSD test was carried out to determine the difference in the zone of

inhibition between each concentration which could inhibit the growth of Escherichia coli. The results obtained on the 14th day of fermentation time were significant for all variations in fermentation time, namely p (0.000) < α (0.05), the 14th day of fermentation was able to form an inhibition zone better than other fermentation times. Based on the results of measuring the diameter of the inhibition zone, it was found that the average diameter of the inhibition zone increased at each level of fermentation time. The difference in the diameter of the inhibition zone is influenced by the length of fermentation time, where the longer the fermentation time, the secondary metabolite content and the acid content produced increases.

CONCLUSION(S)

There is antibacterial activity of Rosella Tea Kombucha based on variations in fermentation time 6, 8, 10, 12 and 14 days with respective inhibition zone diameters of 7.27 mm; 7.97mm; 8.43mm; 9.57mm; and 12.73 mm. There are differences in the inhibition zone diameter from variations in the fermentation time of Rosella Tea Kombucha on the growth of Escherichia coli bacteria with the largest diameter of the inhibition zone shown in the variation in fermentation time of 14 days (12.73 mm) with strong category.

Conflict of Interest

There is no conflict of interest in this research.

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