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ANTIMICROBIAL ACTIVITY OF KOMBUCHA DRINK MADE OF TORCH GINGER (*Etlingera elatior*) FLOWERS TOWARDS PATHOGENS

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ABSTRACT

Kombucha is a functional drink with benefits for human health, including antimicrobial activity. It could be made from torch ginger, an Indonesian tropical plant that is known as a vegetable and ornamental plant and has antimicrobial activity against pathogens. This study aimed to determine the effect of fermentation time and sugar content of kombucha made from torch ginger flowers on antimicrobial activity against pathogens. The research was conducted with two treatments: 20, 25, and 30% (w/v) sugar addition and 7, 14, and 21 days of fermentation. The antimicrobial activity of the kombucha drink made from 12% torch ginger was evaluated using the well diffusion method. The test pathogens were Escherichia coli ATCC 11775, *Bacillus cereus* ATCC 11778, Staphylococcus aureus ATCC 33591, and *Salmonella typhi* ATCC 14028. The results showed that torch ginger kombucha with 20% sugar and fermented for 14 days significantly inhibited Staphylococcus aureus ATCC 33591 and Bacillus cereus ATCC 11778. The Minimum Inhibitory Concentration (MIC) against both pathogens was observed in 20% kombucha. Therefore, kombucha made from torch ginger has the potential to be an antimicrobial against pathogens.

Keywords: Antimicrobial; Functional drink; fermentation time; Well diffusion Methods

INTRODUCTION

Fermentation is one of the oldest food-preservation techniques. Kombucha is a traditional fermented beverage that has been consumed since 220 BC (Wang *et al*., 2022). This beverage is fermented from a sugary tea drink through the metabolic activity of a Symbiotic Culture of Bacteria and Yeast (SCOBY), primarily characterized by a synergistic relationship between acetic acid bacteria (AAB), including *Komagataeibacter*, *Gluconobacter*, and *Acetobacter,* and lactic acid bacteria (LAB), specifically *Lactobacillus* and *Lactococcus*.

Kombucha is recognized for its potential bioactivity to enhance human health. The widespread consumption of these beverages is due to their bioactive compounds, including various essential vitamins (B1, B2, B3, B6, B12, B15, and C), organic acids such as glucuronic acid and acetic acid, polyphenols with potential antioxidant activities (Cholidah et al., 2020), essential amino acids, folic acid, and various enzymes, which can be beneficial to human health (Khamidah and Antarlina, 2020).

Furthermore, investigations have explored the utilization of soursop leaves (*Annona muricata*), rhizomes such as ginger (*Zingiber officinale*), and non-traditional bases such as bamboo shoot process water (*rebu*). Kombucha fermentation can be combined with various plants, such as rosella, butterfly pea, and torch ginger (Indonesian: *kecombrang*) flowers. Some studies have explored the combination of tea leaves with soursop leaves, ginger, and corn silk tea. It was found that rosella kombucha containing acetic acid, which is used as an antibacterial agent, had a significant effect on the antibacterial activity of *Escherichia coli* (Cholidah et al., 2020). The combination of kombucha with butterfly pea flowers has anthocyanin content that showed potential inhibition of the growth of bacteria *Salmonella typhi* and *Escherichia coli* (Sefrina, 2021).

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Torch ginger (*Etlingera elatior*), a member of the *Zingiberaceae* family, contains a wide range of bioactive compounds, including phenolics, flavonoids, alkaloids, saponins, and terpenoids, which contribute to its antibacterial and antioxidant activities (Rasyadi et al., 2022). The beneficial effects of kombucha are associated with the presence of bioactive compounds, including D-saccharic acid-1,4-lactone, gluconic acid, glucuronic acid, tea polyphenols, vitamins, amino acids, various micronutrients, and antibiotics. The antioxidant and antimicrobial activities of kombucha are mainly attributed to the presence of these bioactive compounds (Ojo and de Smidt, 2023).

Fermentation into kombucha could enhance the antimicrobial activity compared to non-fermented torch ginger, due to the increased production of organic acids and the formation of other bioactive metabolites during the fermentation process (Mizuta *et al*., 2020. Kombucha drink with torch ginger extract was able to inhibit *Escherichia coli* and *Staphylococcus aureus* at sugar concentrations of 20%, 30%, 40%, and 50% with a fermentation duration of 12 days (Fadhilah et al., 2023). Kombucha drinks made from torch ginger extract also showed antibacterial activity against *Escherichia coli*, *Salmonella thypimurium*, and *Staphylococcus aureus* after a fermentation period of 9 days and the addition of 2.5% granulated sugar. (Sintyadewi et al., 2023).

In this study, kombucha was combined with torch ginger extract and used as a fermented beverage. This study focused on the antimicrobial potential of kombucha torch ginger extract in inhibiting *Escherichia coli* ATCC 11775, *Bacillus cereus* ATCC 11778, *Staphylococcus aureus* ATCC 33591, and *Salmonella thypi* ATCC 14028, considering the duration of kombucha fermentation and the addition of different palm sugar concentrations.

MATERIALS AND METHOD

Material

The materials used in this study were torch ginger (*Etlingera elatior*) flowers obtained from the Beluluq Lingau market on Jalan PM Noor, Samarinda City, SCOBY obtained from an online shop, Tuana Tuha palm sugar under the Gulaku Premium Sugar brand obtained from local shop in Samarinda.

Experimental Design and Sample Preparation

The experimental design used in this study was a completely randomized design consisting of two factors, each with three levels of treatment. The first factor was fermentation time with three treatment levels: 7, 14, and 21 d. The second factor was the addition of sugar at different concentrations, which had three treatment levels, namely 20, 25, and 30%, as well as positive and negative controls with three replicates.

In the first stage of the research, the results were in the form of inhibition zones resulting from antimicrobial activity tests based on different sugar concentrations and fermentation periods. The best treatment was indicated by the diameter of the inhibition zone formed around the well. The diameter of the inhibition zone was measured using a caliper in mm.

Data Analysis

The data obtained in this study were analyzed using Analysis of Variance continued by Tukey test using SigmaPlot 14.0 data-processing application. The results of the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) tests are presented in a descriptive form.

Procedure for Making Torch ginger Extract

The first step was to cut the flowers from the stems and wash them with clean water. After washing, the samples were blended to smoothen the mixture. The next step was to weigh 250 g of torch ginger flowers in 2 liters of water and boil them for 30 min on a hotplate at the maximum temperature. The final step was to filter the torch ginger flowers from the torch ginger flower extract.

Procedure for Making Kombucha Drink from Torch ginger Extract

The production of torch ginger extract kombucha refers to the procedure (Abdilah et al., 2022). Two liters of boiled torch ginger extract was divided into three parts for nine

jars with the addition of sugar according to the treatment, namely 20, 25, and 30% (b/v) for one repetition. Next, the boiled torch ginger extract was cooled to ± 25 °C, and a kombucha starter, namely a 9 cm diameter SCOBY, was added to each treatment at 25% (v/v). The glass jars were then tightly sealed using their lids and fermented according to the treatment for 7, 14, and 21 d at room temperature away from direct sunlight.

Analysis Procedure

Preparation of Test Bacterial Culture (Ningtyas, 2015)

The test microorganisms were pure cultures of *Escherichia coli* ATCC 11775, *Staphylococcus aureus* ATCC 33591, *Bacillus cereus* ATCC 11778, and *Salmonella typhi* ATCC 14028. Before cultivation, each bacterial culture was refreshed by reinoculation in a test tube containing 10 mL of Nutrient Broth (NB) medium and incubated in an incubator at 37 °C for 24 h.

Antimicrobial Activity Test

The antimicrobial activity of torch ginger extract kombucha was tested using the well diffusion method, as described by Nikmah (2020), with slight modifications. The inoculation process was carried out by applying the bacterial suspension to the surface of the NA medium in a dish using a sterile cotton swab and then left for 5 min to allow the suspension to soak evenly. Next, five wells with a diameter of 6 mm were created in each dish. The torch ginger extract kombucha with various sugar concentrations (20, 25, and 30%) and various fermentation times (7, 14, and 21 days), and the positive control of amoxicillin 500 mg (for bacteria) as the negative control, were each poured into the wells at 40 µL (0.04 mL). All Petri dishes containing the test samples were incubated for 24 h at 37°C.

$$\text{Inhibition Zone Diameter (mm)} = \frac{D1+D2+D3}{3} - \chi$$

Description:
D1 = Vertical Diameter, D2 = Horizontal Diameter, D3 = Diagonal Diameter, χ = 6 mm Well Diameter

Determination of Minimum Inhibitory Concentration

This Minimum Inhibitory Concentration (MIC) assay used the well diffusion method, which refers to research conducted by Rahman et al. (2022). The kombucha drink from torch ginger after 14 d of fermentation and with 20% pal sugar was diluted with sterile aquadest to make solutions with varying concentrations: original (0.5 mL kombucha), 80% (0.4 mL kombucha + 0.1 mL water), 60% (0.3 mL kombucha + 0.2 mL water), 40% (0.2 mL kombucha + 0.3 mL water), and 20% (0.1 mL kombucha + 0.4 mL water). These were placed in 5 mL vials for each dilution and homogenized using a vortex mixer. The second stage involved making eight wells in each dish containing NA medium with a diameter of 6 mm, then swabbing the medium surface with *Bacillus cereus* ATCC 11778 and *Staphylococcus aureus* ATCC 33591 bacterial suspensions using a sterile cotton swab and leaving it for 5 min. Both test bacteria were chosen for their good antibacterial activities in the previous step. Amoxicillin was used as a positive control. All Petri dishes containing the test samples were incubated in an incubator for 24 h at 37 °C. The treatment was repeated thrice. After incubation for 24 h, the diameter of the inhibition zone formed on the medium in each well was measured using a caliper.

Determination of Minimum Bactericidal Concentration

The Minimum Bactericidal Concentration (MBC) assay was performed according to research conducted by Inanta (2023) using the spread plate method. Based on the results of the MIC assay, the MBC assay was conducted with the dilution that showed the best inhibition against the two bacteria, *Bacillus cereus* ATCC 11778 and *Staphylococcus aureus* ATCC 33591. Fifty microliters of each freshly cultured bacteria were inoculated into torch ginger kombucha dilution and homogenized. The tube test was incubated at 37 °C for 24 h.

From the dilution, the spread plate method was applied by taking 100 µL of each dilution and dripping it onto a Petri dish containing 20 mL of NA medium (for bacteria) and spreading it evenly using an L-rod (triangular rod). All petri dishes containing the test samples were incubated at 37 °C for 24 h. The concentration of the test material that can kill bacteria was determined

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by observing and counting the number of colonies that grew on each dish. The determination of the Minimum Bactericidal Concentration (MBC) is based on the smallest number of microbes, so it can be stated that this concentration can kill the test bacteria.

RESULTS AND DISCUSSION

Antimicrobial Activity

The antimicrobial activity of torch ginger (*Etlingera elatior*) kombucha extract against *Escherichia coli* ATCC 11775, *Staphylococcus aureus* ATCC 33591, *Bacillus cereus* ATCC 11778, and *Salmonella typhi* ATTC 14028 was assessed using the well diffusion method with sugar concentrations of 20, 25, and 30% and fermentation periods of 7, 14, and 21 d. Antimicrobial activity was measured based on the size of the inhibition zone formed around the well containing the test sample. Antimicrobial inhibition power can be classified into four categories based on the Davis and Stout standard: very strong (≥ 20 mm), strong (10–19 mm), moderate (5–10 mm), and weak (< 5 mm) (Khanifah, 2022). This antimicrobial activity can also be formed because the pH in the sample also greatly affects the formation of the inhibition zone.

The results of the antimicrobial activity test show that torch ginger extract kombucha with different sugar concentrations and fermentation times can inhibit the growth of *Escherichia coli* ATCC 11775, *Staphylococcus aureus* ATCC 33591, *Bacillus cereus* ATCC 11778, and *Salmonella typhi* ATTC 14028, as indicated by the presence of clear zones (inhibition zones) around the wells (Figure 1.).

The antimicrobial activity of torch ginger kombucha extract originates from torch ginger (*Etlingera elatior*) flowers, which contain antimicrobial compounds such as flavonoids, saponins, polyphenols, alkaloids, steroids, tannins, phytosteroids, and amino acids. (Bogoriani et al., 2022). These phytochemical compounds have the potential to act as natural antimicrobials against pathogenic bacteria, such as those tested in this study. The results show that the formation of acid during kombucha fermentation plays an effective role in suppressing the growth of microorganisms, thereby extending the shelf life and improving product safety by preventing spoilage and inhibiting pathogenic microorganisms. (Kostić et al., 2025).

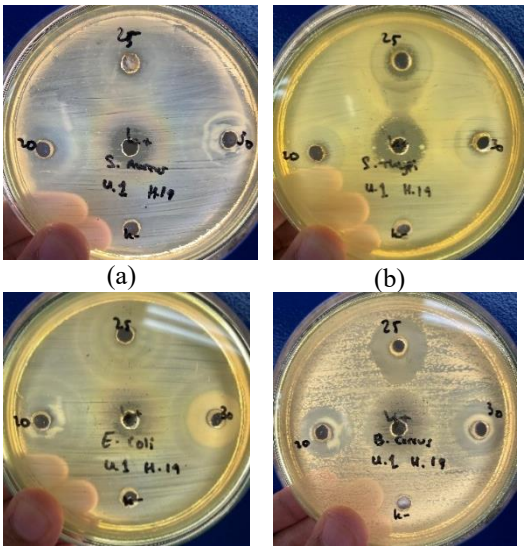

Figure 1. Inhibition Zone diameter of torch ginger Extract Kombucha after 14 d of fermentation. *Staphylococcus aureus* ATCC 33591 (a), *Salmonella typhi* ATTC 14028 (b), *Escherichia coli* ATCC 11775 (c), *Bacillus cereus* ATCC 11778 (d).

Based on the data and results of the discussion above, it can be concluded that the kombucha extract from torch ginger at 20% sugar concentration with a fermentation period of 14 days has consistent antimicrobial activity (inhibitory power) from the first to the third repetition, compared to other concentrations and fermentation periods. A fermentation period of 14 days was optimal and had the potential for strong inhibitory activity against the growth of *Bacillus cereus* ATCC 11778 and *Staphylococcus aureus* ATCC 33591 (Table 1.).

The gram-positive bacteria, *Bacillus cereus* ATCC 11778 and *Staphylococcus aureus* ATCC 33591, were the most sensitive to antimicrobial testing. Gram-positive bacteria have resistance to acidic conditions through a mechanism involving a proton pump that can balance the pH in the cell and the antimicrobial substrate. The antimicrobial activity is mainly influenced by low pH conditions due to the formation of acetic acid and the presence of catechin compounds

produced by bacteria and yeast in the SCOBY culture during the fermentation process, thereby inhibiting the growth of various gram-positive and gram-negative microorganisms (Coelho et al., 2022).

Tabel 1. The diameter (mm) of inhibition zone of Kombucha from Torch ginger Extract on Pathogenic Bacteria

Sugar Concentration (%)	Fermentation Time (days)			Average
	7	14	21	
Bacillus cereus				
20	11.74±1.92	15.44±2.40	10.74±5.67	12.64±3.87
25	7.35±5.87*	17.69±3.33	14.12±8.60	13.06±7.11
30	9.40±5.25	18.03±5.81**	14.62±8.20	14.02±6.81
Average	9.50±4.48a	17.06±3.76b	13.16±6.83a	
K+	25.55±6.31	22.61±4.87	9.91±4.56	
K-	0.00±0.00	0.00±0.00	0.00±0.00	
Escherichia coli				
20	10.63±1.21c	14.03±2.60Ab	7.37±2.10a	10.67±1.97
25	8.42±0.55a	21.93±1.69Bb	6.23±2.76a	12.19±1.67
30	9.85±1.78b	22.85±0.54Bc**	4.80±1.49Ca*	12.50±1.27
Average	7.27±1.19b	13.18±1.19c	4.43±1.63a	
K+	7.46±2.43	7.12±3.58	3.79±1.48	
K-	0.00+0.00	0.00+0.00	0.00+0.00	
Staphylococcus aureus				
20	13.56±2.25**	10.94±7.47Aa	6.13±8.65*	10.21±6.12
25	11.82±6.07	12.89±10.14Aa	8.18±7.33	10.96±7.85
30	13.28±6.01	9.82±4.33Aa	8.96±7.82	10.69±6.05
Average	12.89±2.94	11.22±4.44	7.76±4.59	
K+	8.96±4.20	11.15±1.11	6.35±1.80	
K-	0.00±0.00	0.00±0.00	0.00±0.00	
Salmonella typhi				
20	13.33±4.10	9.11±5.64*	13.61±8.69	12.01±6,14
25	12.15±5.83	14.70±10.60	15.43±9.28	14.09±8,57
30	15.25±5.50	16.07±12.97	16.11±10.60**	15.81±9,69
Average	13.58±3.01	13.29±5.89	15.05±5.52	
K+	12.00±3.56	14.37±2.89	13.05±3.52	
K-	0.00±0.00	0.00±0.00	0.00±0.00	

Note: Data (mean± SD) were obtained from three replicates. Data were analyzed using analysis of variance (ANOVA). The data in the columns and rows with a gray background are those on the effects of sugar concentration and fermentation duration. The data in the areas with a white background represents the interaction between the treatments. Interaction data in the same row followed by different capital letters indicate an effect on different sugar concentrations, whereas interaction data in the same column followed by lowercase letters indicate an effect on fermentation time (DMRTα 5% test). The asterisks in the table indicate the highest (**) and lowest (*) inhibition zone results. Amoxycillin was used as a positive control.

Antimicrobial activity testing in this study used amoxicillin as a positive control for pathogenic bacteria. The positive control in this test was used to compare the inhibitory effects of the kombucha samples. Amoxicillin is a broad-spectrum antibiotic that can inhibit and kill both gram-positive and gram-negative bacteria. Resistance may occur because this antibiotic is widely used in the medical world for treatment. Antibiotic resistance can spread among bacteria through various channels, causing previously susceptible bacteria to become resistant to antibiotics. (Halawa et al., 2023).

Minimum Inhibitory Concentration (MIC)

The determination of the inhibition zone in the previous stage of the best treatment for further MIC testing was performed on *Bacillus cereus* ATCC 11778 and *Staphylococcus aureus* ATCC 33591 bacteria with a sugar concentration of 20% and a fermentation period of 14 d, as shown in Table 2 for the data obtained in this MIC test. This MIC method uses the well-diffusion

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method, but the samples to be tested must first be diluted. The dilutions used in this MIC test were original (100%), 80%, 60%, 40%, and 20%. The inhibition zones were shown in Figure 2.

Table 2. MIC Determination of *Bacillus cereus* and *Staphylococcus aureus*

Kombucha Dilution Concentration (%)	Inhibition zone (mm)		MBC Assay (mm)	
	B. cereus ATCC 11778	*S. aureus* ATCC 33591	*B. cereus* ATCC 11778	*S. aureus* ATCC 33591
100	6.73	0.90	214.50	287.50
	1.13	0.50	89.00	75.50
	11.80	0.83	80.00	3.00
80	4.63	0.70	50.50	83.00
	0.80	0.40	54.50	398.00
	9.37	0.43	90.00	4.50
60	0.50	0.67	421.50	58.50
	0.50	0.00	286.00	0.00
	6.13	0.00	71.00	0.00
40	0.70	0.00	68.50	0.00
	0.60	0.00	171.50	0.00
	0.80	0.00	51.00	0.00
20	0.00	0.00	0.00	0.00
	0.00	0.00	0.00	0.00
	0.00	0.00	0.00	0.00
Average	2.91	0.30	109.87	60.67
SD	3.85	0.35	117.75	120.09

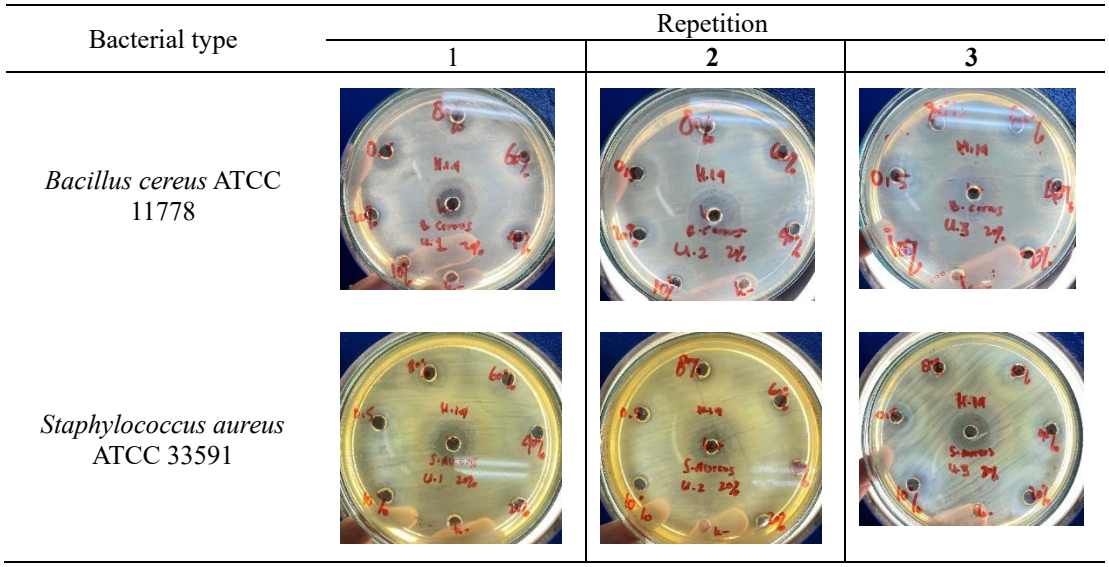

Figure 2. Width clear zones of the MIC assay on *B*. cereus and *S. aureus* ATCC 33591 at 14 d fermentation period.

Based on the data and results of the discussion in the table and figure above, *Bacillus cereus* ATCC 11778 showed no bacterial growth at 40% and 20% concentrations. *Staphylococcus aureus* ATCC 33591 at 80, 40, and 20% concentrations

showed no bacterial growth. The lowest concentration at which no bacterial growth was observed was defined as the Minimum Inhibitory Concentration (MIC). Therefore, the Minimum Inhibitory Concentration (MIC) value for the *Bacillus cereus* ATCC 11778 bacteria is at a concentration of 40%. The Minimum Inhibitory Concentration (MIC) value for *Staphylococcus aureus* ATCC 33591 bacteria is at a concentration of 80%. The Minimum Inhibitory Concentration (MIC) value is indicated by the absence of an inhibition zone around the well at the concentration that has been made. The inhibition of test microbe growth can be caused by active compounds such as tannins, essential oils, flavonoids, and anthocyanins, which are found in torch ginger extract. This can result in a reduction in the availability of nutrients for bacteria, so that bacteria cannot synthesize DNA, peptidoglycan, and other processes necessary for their growth. This can result in a decrease in the ability of bacteria to adhere to surfaces and inhibit the synthesis necessary for cell wall formation (Inanta, 2023).

Minimum Bactericidal Concentration (MBC)

The MBC was determined based on the best inhibition observed in the MIC assay. The MBC test aimed to determine the concentration that could kill the microbes being tested. The MBC method uses the spread plate method, whereby the sample to be tested is first diluted. The dilution used in this MBC test was *Staphylococcus aureus* ATCC 33591 bacteria. The solution samples selected for the MBC test were 100%, 80%, and 60% (replication 1) concentrations. For replication 2 and 3, the solution samples selected for the MBC test were 100% and 80%, respectively. For *B. cereus* ATCC 11778, the solution samples selected for the MBC test were 100, 80, 60, and 40% for replicates 1 to 3, respectively (Figure 3.).

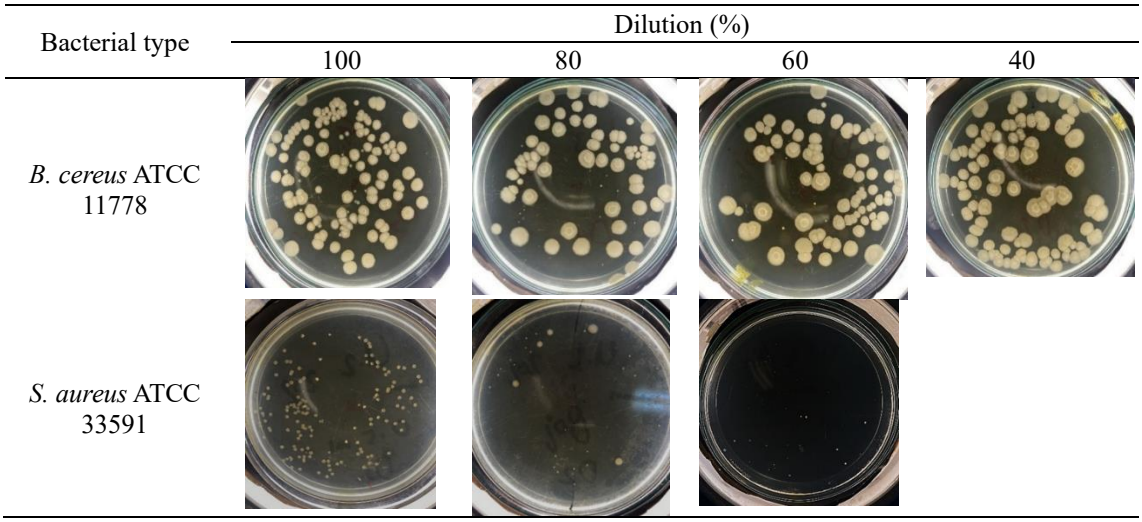

Figure 3. Colony growth in the MBC assay of *Bacillus cereus* ATCC 11778 and *Staphylococcus aureus* ATCC 33591

The data obtained showed that torch ginger leaf and flower extracts have significant antibacterial activity against gram-positive bacteria. This is in line with the results of the MIC and MBC tests on kombucha samples made from torch ginger extract, which confirmed the ability of this fermented drink to inhibit and inactivate the growth of *Bacillus cereus* and *Staphylococcus aureus*. These findings indicate that torch ginger extract kombucha has the potential to be developed as a natural antibacterial agent, with a mechanism of action that can be quantitatively measured using MIC and MBC values.

CONCLUSION

The results of the antimicrobial activity test to determine the inhibition zone showed that the effect of different sugar

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concentrations and fermentation times of torch ginger (*Etlingera elatior*) kombucha extract had a significant effect on the antimicrobial activity of torch ginger (*Etlingera elatior*) kombucha extract on Escherichia coli ATCC 11775 bacteria, and Bacillus cereus ATCC 11778, while sugar concentration and fermentation duration did not significantly affect the antimicrobial activity of torch ginger (*Etlingera elatior*) kombucha extract on Staphylococcus aureus ATCC 33591 and Salmonella typhi ATCC 14028 bacteria.

The minimum inhibitory concentration (MIC) test was conducted on Bacillus cereus ATCC 11778 and Staphylococcus aureus ATCC 33591 bacteria with a sugar concentration of 20% and a fermentation period of 14 days. The Minimum Lethal Concentration (MLC) test on Bacillus cereus ATCC 11778 bacteria yielded results at a dilution concentration of 10%. Staphylococcus aureus ATCC 33591 yielded results at a dilution of 20%.

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