



Antibacterial Potential of *Curcuma Mangga* Kombucha: The Effect of Fermentation Duration on Activity Against *Escherichia coli* and *Salmonella typhi*

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Abstract: Kombucha is a fermented beverage known for its various health benefits, including antibacterial activity. This study aims to evaluate the antibacterial potential of mango ginger (*Curcuma mangga*)-based kombucha against *Escherichia coli* and *Salmonella typhi*, as well as to analyze the effect of fermentation duration on the physicochemical properties of kombucha. Fermentation was conducted for 5, 7, 10, and 14 days, with total acidity, pH, and antibacterial activity measurements using the well diffusion method. The results showed total acidity increased as fermentation progressed, while pH decreased. Antibacterial tests demonstrated that mango ginger kombucha exhibited inhibitory activity against *Escherichia coli* and *Salmonella typhi*, with the largest inhibition zones observed on day 10 of fermentation (8.2 mm for *Escherichia coli* and 9.2 mm for *Salmonella typhi*). Although its inhibitory effect was moderate compared to the positive control (ciprofloxacin), these findings suggest the potential of mango ginger kombucha as a functional beverage with antibacterial properties. Further research is needed to identify the active compounds responsible for its antimicrobial activity and to optimize the fermentation process to enhance its effectiveness.

Keywords: Antibacterial; *Curcuma mangga*; *Escherichia coli*; Fermentation; Kombucha; *Salmonella typhi*.

INTRODUCTION

Gastroenteritis is a serious health issue worldwide that causes inflammation in the digestive tract. Gastroenteritis, with clinical manifestations such as diarrhoea, is a contagious disease transmitted through direct contact with infected individuals or consumption of food contaminated with viruses and bacteria. It has high morbidity and mortality rates, especially in developing countries. Bacteria commonly causing gastroenteritis primarily originate from the Enterobacteriaceae family, found in the human colon (Subagya et al., 2020). Most of these bacteria are part of the normal gut microflora but can become (Saffarian et al., 2024; Tandirogang et al., 2022; Villarreal-Soto et al., 2018) pathogenic under certain conditions, leading to infections. *Escherichia coli* and *Salmonella typhi* are among the types of bacteria from the Enterobacteriaceae family that cause gastrointestinal infections. One effort to maintain digestive health can be achieved through consuming functional foods or beverages (Sintyadewi et al., 2023).

Public interest in healthy lifestyles and functional food products is increasing because these products positively affect health. Functional foods provide basic nutrition and bioactive components that can enhance health, including digestive

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health. One of the most commonly found forms of functional food is fermented food products (Julia et al., 2016).

One functional food product that has recently gained significant popularity is kombucha. Kombucha is a fermented beverage made using a symbiotic culture of bacteria and yeast, known as SCOBY (Symbiotic Cultures of Bacteria and Yeasts) (Zofia et al., 2020). Scientific research on the benefits of kombucha shows that it possesses antioxidant, antimicrobial, anti-inflammatory, and antidiabetic properties (Ivanišová et al., 2020; Saffarian et al., 2024; Villarreal-Soto et al., 2018). Consuming kombucha can also contribute to lowering cholesterol, stimulating liver detoxification processes, and supporting the functioning of the immune system (Leal et al., 2018; Selvaraj & Gurumurthy, 2023). Various chemical compounds in kombucha have been identified, including organic acids, water-soluble vitamins, amino acids, proteins, hydrolytic enzymes, ethanol, carbon dioxide, polyphenols, and minerals (de Miranda et al., 2022; Júnior et al., 2022; Kluz et al., 2022; Villarreal-Soto et al., 2018).

Although kombucha traditionally refers to fermentation made from various types of tea, many studies have now explored biofermentation using SCOBY with other plant-based materials. This aims to create kombucha with more appealing sensory qualities, enhance health-promoting properties, and develop new products. Innovative alternative ingredients such as coffee (Afifah, 2019; Julia et al., 2019; Zofia et al., 2020), soursop leaves (Barkah, Muhammad Habib, Dini Sri Damayanti, 2020), lemons (Susanti et al., 2023; Velićanski et al., 2014), fruit juices (Al-Mohammadi et al., 2021), and others have been utilized.

The production of kombucha from natural ingredients has become an alternative approach since its materials are easily found in the surrounding environment. One of Indonesia's prominent spices is turmeric, which belongs to the Zingiberaceae family. Commonly, people are familiar with *Curcuma domestica* (yellow turmeric), recognized by its orange-colored rhizome, as it is frequently used in kitchens as a food seasoning and natural dye. In contrast, white turmeric remains underutilized. There are three types of white turmeric: *Kaempferia rotunda* (gomyok or pepet white turmeric), *Curcuma zedoaria*, and *Curcuma mangga* (Rahmawati et al., 2023).

Curcuma mangga is a medicinal plant from Indonesia widely used as an ingredient in traditional herbal medicine (jamu). Its rhizome has a white outer layer, while the inside ranges from lemon yellow to sulfur yellow, with a distinctive mango-like aroma (Dwiyati Pujimulyani, 2023). One way to utilize *Curcuma mangga* is by using it as a base ingredient for functional food production, as it has antibacterial properties. Compounds found in *Curcuma mangga* with antibacterial potential include essential oils, alkaloids, flavonoids, tannins, curcuminoids, and terpenoids (Putri et al., 2023; Rahmawati et al., 2023; Sugita et al., 2022).

According to Kamazeri et al. (2012), several compounds are present in the essential oil of *Curcuma mangga*, with two being the most abundant: 12.69% caryophyllene and 18.71% caryophyllene oxide. The essential oil of mango ginger can inhibit the growth of *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, as well as the fungi *Candida albicans* and *Cryptococcus neoformans* (Kamazeri et al., 2012). Mango ginger rhizomes containing curcumin are safer to use as natural antimicrobial compounds than synthetic materials. However, mango ginger remains underutilized (Sarjono & Mulyani, 2007).

Several studies have reported the antibacterial properties of kombucha tea. Kaewkod et al. (2019) found that kombucha made from various types of tea (green, oolong, and black) after 15 days of fermentation exhibited inhibitory activity against all

tested pathogenic enteric bacteria, including *Escherichia coli*, *Shigella dysenteriae*, *Salmonella typhi*, and *Vibrio cholerae* (Kaewkod et al., 2019).

Research on kombucha tea by Al Mohammad et al. (2021) showed inhibition zone diameters against tested bacterial strains, with *Escherichia coli* measuring 18 mm and *Salmonella typhi* measuring 14 mm (Al-Mohammadi et al., 2021). Similarly, a study by Bhattacharya et al. (2016) demonstrated that kombucha fermented for 21 days exhibited inhibition zones of 20.67 mm against *Escherichia coli* and 15.5 mm against *Salmonella typhi* (Bhattacharya et al., 2016).

In addition, several studies have reported the antibacterial properties of *Curcuma mangga*. Research conducted by Adila et al. (2013) showed that *Curcuma mangga* rhizomes could inhibit the growth of *Escherichia coli* with an inhibition zone width of 10.47 mm (Adila & Agustien, 2013). Similarly, Cahyaningrum et al. (2018) found that combining *Curcuma mangga* powder and anting-anting leaves could inhibit *Escherichia coli* with an inhibition zone of 14 mm (Cahyaningrum & Artini, 2018).

Research on kombucha made from red galangal (Budiari et al., 2023), temulawak (*Javanese ginger*) (Zubaidah et al., 2021), and white turmeric (Zubaidah et al., 2022) has been conducted previously. However, as far as the researcher knows, no research explores the potential antibacterial activity of kombucha made from *Curcuma mangga*. Therefore, this research aims to explore the potential antibacterial activity of kombucha made from *Curcuma mangga*, which is expected to provide new insights into the development of spice-based functional foods.

MATERIALS AND METHODS

Preparing kombucha

The research samples included *Curcuma mangga*, water, sucrose, kombucha vinegar, and SCOBY. *Curcuma mangga* with rhizomes in fresh condition, white on the outside and lemon yellow to sulfur yellow on the inside, having a distinctive mango-like aroma, and free from decay or physical damage was obtained from a supermarket in Semarang City, while the SCOBY which was in fresh condition, light brown *Curcuma mangga* in color, gelatinous, and free from contamination, was purchased from an online shop. In this study, the formula included 0.7% from 1 litre of water, 10% sugar, previously fermented kombucha vinegar, and SCOBY. The fermentation duration of *Curcuma mangga* kombucha was 5 days (T1), 7 days (T2), 10 days (T3), and 14 days (T4) as presented in Table 1. The fermentation was conducted at room temperature (25–30°C) in glass jars covered with a sterile cloth to allow airflow. Each treatment was repeated three times, resulting in 14 samples. (Budiari et al., 2023).

Table 1. Kombucha *Curcuma mangga* Formulation

Replications (U)	Fermentation Duration			
	T1	T2	T3	T4
1	T1U1	T2U1	T3U1	T4U1
2	T1U2	T2U2	T3U2	T4U2
3	T1U3	T2U3	T3U3	T4U3

Note: T1= Kombucha fermentation duration of 5 days; T2= Kombucha fermentation duration of 7 days; T3= Kombucha fermentation duration of 10 days; T4= Kombucha fermentation duration of 14 days.

Total Acidity and pH of Kombucha

The titratable acidity was measured by titrating the sample with 0.1 M NaOH, delivered from a 50 mL burette (precision ±0.1 mL), and phenolphthalein (PP) indicator

was added until a pink colour change occurred, and the result was expressed in acetic acid percentage. The formula for calculating acid content:

$$\% \text{Total Acid} = \frac{\text{Volume of NaOH} \times \text{Normality of NaOH} \times \text{Equivalent Weight of Acetic Acid}}{\text{Sample Volume}} \times 100$$

V1 = NaOH volume (mL)

V2 = Sample volume (mL)

N = NaOH Normality (0.1 N)

B = Molecular weight of acetic acid (Budiari et al., 2023)

The pH of the samples was tested using a digital pH meter. This analysis was carried out with three replications (Zubaidah et al., 2021).

Preparation of Test Bacteria

Escherichia coli and *Salmonella typhi* bacteria, obtained from the Microbiology Laboratory of the Faculty of Medicine, Diponegoro University, were first rejuvenated by inoculating a loopful of pure bacterial culture onto MacConkey agar (MAC) in a zigzag pattern. The plates were then incubated at 37°C for 24 hours (Putri et al., 2023).

Preparation of test bacterial suspension

The test bacterial suspension of *Escherichia coli* and *Salmonella typhi* was prepared by taking a loopful of colonies from solid MacConkey agar and transferring them into a test tube containing 10 mL of 0.9% NaCl. The turbidity of the bacterial suspension was then standardized to the 0.5 McFarland standard (approximately 10⁸ CFU/mL) (Nurhayati et al., 2020).

Antibacterial Activity Test

The antibacterial activity test of *Curcuma mangga* kombucha was conducted using the agar well diffusion method. Liquid Mueller Hinton Agar (MHA) was poured into a petri dish and allowed to solidify. 0.1 mL of the test bacterial suspension was inoculated onto the MHA medium, spread evenly using an L-shaped spreader, and left to dry. Wells were made using the sterile tip of a pipette. Then, according to the treatment, 0.1 mL of the *Curcuma mangga* kombucha sample was introduced into each well. The plates were incubated at 37°C for 24 hours. Antibacterial activity was observed by measuring the inhibition zone, indicated by a transparent or translucent area around the well, using a ruler (modified from Nurhayati et al., 2020).

Data analysis

The data were analyzed using SPSS software version 25.0. ANOVA (Analysis of Variance) was used to determine if there were significant differences among the treatment groups. If the test results indicated significant differences ($p < 0.05$), a post hoc Tukey test was conducted to identify which groups differed. The Kruskal-Wallis test was applied to data that were not normally distributed.

RESULTS AND DISCUSSION

Total titratable acid of *Curcuma mangga* kombucha

The total acid content of *Curcuma mangga* kombucha in this study was measured based on the fermentation duration, as shown in Table 2. Table 2 shows that total titratable acid increased as the fermentation time passed. The one-way ANOVA analysis showed a p-value of 0.095, indicating that the difference in total acid content across various fermentation durations was insignificant ($p > 0.05$). This may be due to the small differences in total acid values between samples. However, the

study results indicate that the total acid content increases as the fermentation duration lengthens.

The increase in total acid occurs during fermentation because yeast synthesizes sugar into alcohol, and acetic acid bacteria break down the alcohol into organic acids such as acetic acid, gluconic acid, and glucuronic acid (de Miranda et al., 2022). In this study, the average total acid value of kombucha fermentation on day 5 was 0.99%, increasing to 1.42% by day 14 of fermentation. Fauzi et al. (2023) also observed that kombucha made from temulawak, which was fermented for 15 days, increased the average total acid value to 1.06% (Fauzi et al., 2023). Similarly, in the study by Zubaidah et.al (2022), the total acid content in kombucha made from temulawak increased after fermentation for 12 days.

Table 2. Total Acid Content and Average pH of *Curcuma mangga* Kombucha.

	Fermentation Duration				p < 0,05
	5 days (T1)	7 days (T2)	10 days (T3)	14 days (T4)	
% Total Acid	0,99±0,01	0,90±0,02	1,05±0,11	1,42±0,18	0,095*
pH	4,2±0,58	3,7±0,00	3,7±0,00	3,6±0,58	0,095**

Data are presented as the mean of three repetitions ± standard deviation (SD).

*Statistical analysis was performed using one-way ANOVA with a significance level of p<0,05. ** Statistical analysis was performed using Kruskal-Wallis with a significance level of p<0,05.

pH of *Curcuma mangga* kombucha

The pH of *Curcuma mangga* kombucha decreased during fermentation. This decrease can be seen in Table 2. Fermentation duration affects the pH of kombucha. *Curcuma mangga* kombucha on day 5 showed the highest pH, which then decreased on day 14. This result is consistent with a study on *Curcuma longa* kombucha, where the initial fermentation pH was 4.03, which then decreased to 3.02 after 12 days of fermentation. Similarly, the total acidity increased at the end of fermentation, reaching 0.28% (Zubaidah et al., 2022). This is in line with the increase in total acids that occurred during fermentation until day 14.

During fermentation, the pH tends to decrease as the total acidity increases because the environment becomes more acidic. During fermentation, bacteria and yeast metabolise sucrose to produce organic acids such as acetic acid, gluconic acid, and glucuronic acid. The conversion of glucose into gluconic acid and other organic acids by acetobacter causes a decrease in pH because dissolved organic acids can release protons (H⁺), which lowers the pH value (Jayabalan, R., Malbaša, R. V., Lončar, E. S., Vitas, J. S., & Sathishkumar, 2014). According to existing regulations, to be considered safe, kombucha drink must have a pH range between 2.5 and 4.2 (de Miranda et al., 2022).

The antibacterial activity of *Curcuma mangga* kombucha

This study evaluates the antibacterial effectiveness of *Curcuma mangga* kombucha based on the inhibition zone diameter against *Escherichia coli* and *Salmonella typhi* at different fermentation durations (5, 7, 10, and 14 days). Based on the research results, the average inhibition zone diameters of *Curcuma mangga* kombucha fermentation at different fermentation durations against *Escherichia coli* and *Salmonella typhi* can be seen in Table 3.

Table 3. Average Inhibition Zone of Antibacterial Activity of *Curcuma mangga* Kombucha Fermentation Based on Fermentation Duration.

Strain	Inhibition Zone Diameter of <i>Curcuma mangga</i> Kombucha (Fermentation Duration)				Positive Control	p < 0,05
	5 Days (T1)	7 Days (T2)	10 Days (T3)	14 Days (T4)		
<i>Escherichia coli</i>	7,5±1,0 ^a	6,8±0,3 ^a	8,2±0,8 ^a	7,8±1,5 ^a	30±0,0 ^b	0,000
<i>Salmonella typhi</i>	6,7±1,2 ^{ab}	5,0±0,0 ^a	9,2±1,5 ^b	7,3±1,0 ^{ab}	30,5±0,0 ^c	0,000

Data presented as the mean of three repetitions ± standard deviation (SD). Positive control: Ciprofloxacin. Statistical analysis was performed using one-way ANOVA with a significance level of p<0.05. Letters (a, b, c) indicate significant differences.

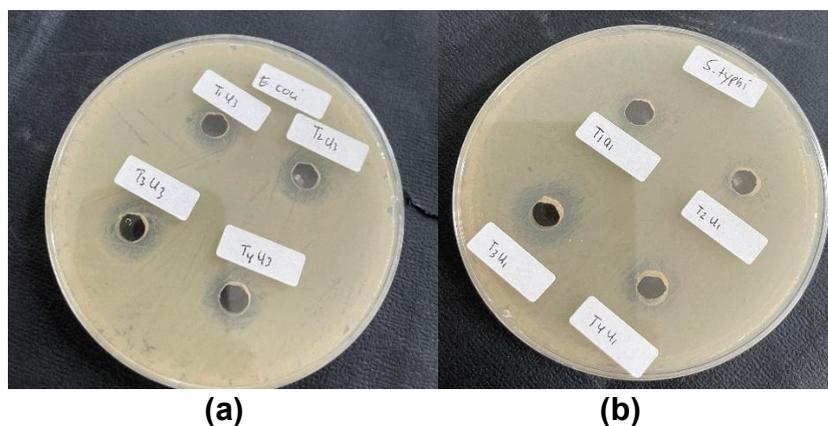


Figure 1. Inhibition zone of *Curcuma mangga* kombucha on *Escherichia coli* bacteria (a) and *Salmonella typhi* bacteria (b)

The antibacterial activity was tested using two types of bacteria, *Escherichia coli* and *Salmonella typhi*, both of which are Gram-negative bacteria. Based on the results of the analysis of variance (ANOVA), it was found that fermentation duration significantly affected ($P < 0.01$) the activity against the pathogenic bacteria *Escherichia coli* and *Salmonella typhi*.

Based on the results obtained, the inhibition zone diameter of *Curcuma mangga* kombucha against *Escherichia coli* ranged from 6.8 mm to 8.2 mm. In contrast, against *Salmonella typhi*, it ranged from 5.0 mm to 9.2 mm. The largest average inhibition zone diameter against *Escherichia coli* was observed in *Curcuma mangga* kombucha fermented on day 10 (T3). Similarly, the largest average inhibition zone diameter against *Salmonella typhi* was also seen in *Curcuma mangga* kombucha fermented on day 10 (T3).

The positive control was 500 mg of Ciprofloxacin, with an inhibition zone diameter of 30 mm against *Escherichia coli* and 30.5 mm against *Salmonella typhi*. Ciprofloxacin was selected because it is a bactericidal antibiotic with a broad spectrum that can kill Gram-negative bacteria. *Escherichia coli* and *Salmonella typhi* possess DNA gyrase and topoisomerase IV enzymes, which are the primary targets of Ciprofloxacin, making this antibiotic highly specific and effective (Budi & Sembiring, 2022; Massoud & Jafari, 2024).

The interpretation of the inhibition zone according to CLSI (Clinical and Laboratory Standards Institute) is that if the diameter of the inhibition zone is ≤ 15 mm,

it is categorised as Resistant; 16-20 mm is categorised as Intermediate; and >21 mm is categorised as Susceptible (CLSI, 2020). Based on these criteria, the inhibitory effect of *Curcuma mangga* kombucha against *Escherichia coli* and *Salmonella typhi* falls into the Resistant category. In contrast, Ciprofloxacin, as the control, falls into the Susceptible category.

This study found that the highest average inhibition was observed on day 10 of *Curcuma mangga* kombucha fermentation, with an inhibition zone of 8.2 mm against *Escherichia coli* and 9.2 mm against *Salmonella typhi*. These results are similar to those of Battikh et al. (2013), where the antimicrobial activity of black tea kombucha against *Escherichia coli* was detected with an inhibition zone of 10.5 mm, and against *Salmonella typhi*, it was 14 mm (Battikh et al., 2013). Sintyadewi et al. (2023) also reported that kombucha from kecombrang flowers fermented for 9 days had inhibition zones of 12.0 mm and 11.5 mm against *Escherichia coli* and *Salmonella typhi*, respectively, placing it in the intermediate/moderate category (Sintyadewi et al., 2023). Meanwhile, Zubaidah et al. (2021) reported an inhibition zone of 2.25 mm for *Curcuma longa* kombucha against *Escherichia coli* (Zubaidah et al., 2021).

This indicates that *Curcuma mangga* kombucha is not yet effective in inhibiting the growth of these two bacteria. The bacteria used in the study are Gram-negative, with a more complex cell wall structure, making them more resistant to antibacterial agents. The cell wall of these bacteria consists of three layers: the outer layer made of lipoproteins, the middle layer containing lipopolysaccharides that act as a barrier to the entry of bioactive antibacterial compounds, and the inner layer made of peptidoglycan with a high lipid content. This structure makes Gram-negative bacteria more difficult to destroy than other types of bacteria (Mughtaromah et al., 2020).

Although *Curcuma mangga* kombucha is not yet effective in killing pathogenic bacteria, the inhibition zone indicates that this kombucha still has antibacterial potential. Although classified as resistant according to CLSI standards, the inhibition zone formed shows that *Curcuma mangga* kombucha can somewhat inhibit bacterial growth. Recent studies have shown that the antimicrobial effects of kombucha against pathogenic bacteria are primarily due to the low pH, caused by organic acids such as acetic acid and lactic acid. Acetic acid at a concentration of 1 g/L effectively inhibits the growth of pathogenic and spore-forming bacteria (Ivanišová et al., 2020).

Organic acids, especially acetic acid, can influence antimicrobial activity. Acetic acid has lipophilic properties, allowing it to penetrate the cell walls of Gram-negative bacteria. Once inside the cell, acetic acid dissociates and releases free proton ions, which lowers the internal pH. The increased concentration of protons can disrupt the function of the microbial cell membrane, causing changes in membrane permeability. As a result, the membrane becomes unstable, eventually leading to cell death (Velićanski et al., 2014). Furthermore, undissociated acetic acid can damage the bacterial lipid bilayer structure and carry protons into the cytoplasm. The accumulation of protons inside the cell creates an acidic environment that can lead to protein denaturation, resulting in the loss of cellular energy and accelerating cellular damage (Kaewkod et al., 2019).

In addition, *Curcuma mangga* contains antimicrobial properties, as reported by Kamazeri et al. (2012), where the essential oil in *Curcuma mangga* was able to inhibit the growth of microbes such as *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans* (Kamazeri et al., 2012). Sarjono and Mulyani (2007) also reported that *Curcuma mangga* could inhibit the activity of *Escherichia coli* bacteria due to its content of curcuminoids, flavonoids, and essential oils (Sarjono & Mulyani, 2007). The

limitation of this study is the need for further testing to identify which compounds exhibit antimicrobial properties in this *Curcuma mangga* kombucha.

CONCLUSION

The fermentation process of *Curcuma mangga* kombucha significantly influences its total acid content, pH, and antibacterial activity. As fermentation duration increased, the total acid content and the antimicrobial activity of the kombucha also increased, with the highest inhibition observed at day 10 for both *Escherichia coli* and *Salmonella typhi* (8.2 mm for *Escherichia coli* and 9.2 mm for *Salmonella typhi*). The pH of the kombucha decreased as fermentation progressed, which is consistent with the production of organic acids such as acetic acid, gluconic acid, and glucuronic acid. Although *Curcuma mangga* kombucha is not yet effective in inhibiting the growth of Gram-negative bacteria (*Escherichia coli* and *Salmonella typhi*) observed during the fermentation process, it still holds potential as a functional beverage. This study highlights the potential of *Curcuma mangga* kombucha as a functional beverage with antimicrobial properties. The combination of antimicrobial compounds from both the fermentation process and the *Curcuma mangga* itself contributes to the inhibition of bacterial growth. However, further studies are needed to identify the specific compounds responsible for this antimicrobial activity and to optimise the fermentation process for maximum health benefits.

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CONFLICT OF INTEREST

There is no conflict of interest in this research.

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