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Microbiology, antioxidant, and antibacterial activity of sinom kombucha

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KEYWORDS

Fermentation
Kombucha
Sinom beverage
Tamarind leaves
Turmeric

ABSTRACT

Kombucha is a traditional drink made from a fermented sweet tea with a mixed culture known as a symbiotic culture of bacteria and yeast (or SCOBY). Although kombucha is usually made from black tea, nowadays, kombucha can be made in various variations such as sinom beverages. Sinom is a traditional Indonesian herbal drink originating from young tamarind leaves or sinom. In addition to tamarind leaves, sinom includes other ingredients such as sugar and turmeric. Turmeric contains curcumin, which is beneficial for health because it has the ability as an antioxidant, anti-inflammatory, antitumor, and lowering blood fat or cholesterol levels. Tamarind leaves contain chemical compounds of flavonoids, phenols, terpenoids, steroids/triterpenoids and are included in the group of plant extracts with antioxidant activity. These chemical compounds can be increased through the kombucha fermentation process. The concentration of turmeric rhizome used in kombucha was 0.8% (w/v), while the tamarind leaves used were 0%, 0.2%, 0.4%, 0.6%, 0.8%, and 1% (w/v). Sinom kombucha fermented for 7 days. The analysis performed included total microbes, total lactic acid bacteria (LAB), pH, total sugar, total phenol, antioxidant activity, and antibacterial activity against *S. aureus* and *E. Coli* growth. The fermentation process and various tamarind leaf concentrations have significant effects in total microbes, total LAB, total sugar, total phenol, IC_{50}. The antibacterial activity is affected by fermentation but not by the variations in tamarind leaf concentrations. The best tamarind leaves concentration of kombucha sinom beverages was at 0.6%, which has a pH value of 3.81, total sugar content of 7,58%, and total phenol content of 240.9 µg GAE/mL, IC_{50} value of 159.84 ppm, total microbial count 3.37 x 10^7 CFU/mL, total LAB of 4.63 x 10^5, antibacterial activity against *E. coli* of 3.1 mm, and antibacterial activity against *S. aureus* of 3.27 mm.

Introduction

Kombucha is a beverage made from black tea and sugar, which undergoes fermentation using a symbiotic culture of bacteria and yeast (SCOBY). Kombucha has several health benefits, such as improving gastrointestinal and glandular function, relieving joint pain, having a positive cholesterol-lowering effect, having blood detoxification capabilities, addressing aging-related issues, and possessing antibiotic properties (Dutta and Paul, 2019). Kombucha is also known to have antioxidant activity. The antioxidant activity of kombucha is associated with health benefits such as cancer prevention, immune system enhancement, and inflammation control. Studies have shown increased antioxidant activity throughout tfermentation (Jayabalan et al., 2014).

Sinom is a beverage made from turmeric and tamarind leaves (Paramita et al., 2015). Sinom is one of Indonesia's traditional beverages and it has the potential to provide excellent antioxidants for health. Sinom contains bioactive compounds from the flavonoid group with antioxidant properties. Combining turmeric rhizome and tamarind leaves as raw materials for making sinom is a natural source of antioxidants (Meis et al., 2020).

The compounds in kombucha, such as acetic acid and vitamin B, have antibacterial and antioxidant effects (Jaya, 2019). Processing substrates (tamarind leaves and turmeric) into kombucha can enhance its antibacterial activity and benefits (Widyasari, 2019). Chemical, microbiological, and antibacterial activity analysis of sinom kombucha was conducted to determine

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the influence of tamarind leaf concentration and fermentation treatment for 7 days. A research conducted by Wijayanti (2020), found that the optimal turmeric concentration in turmeric kombucha was 0.8%.

Kombucha fermentation involves microbial activities, including yeast, acetic acid bacteria, and lactic acid bacteria (Loncar et al., 2014). Microbial activities during fermentation may produce enzymes that trigger the transformation of phenolic compounds into simpler forms (Zubaidah et al., 2023). Phenolic compounds like epicatechin undergo hydrolysis, increasing total phenol concentration (Noronha et al., 2022). Phenolic compounds formed during fermentation can influence the growth of microorganisms in the fermentation medium. During fermentation, microbes uses sucrose as an energy source for metabolism and cell growth. However, the continuous increase in total phenols throughout fermentation can inhibit microbial growth. Research by Rodriguez et al. (2009) states that phenolic compounds have antimicrobial activity that can inhibit the growth of Lactobacillus.

Kombucha made from sinom can be a new breakthrough in kombucha beverage variations. The appropriate concentration of raw materials in kombucha production is crucial because the raw materials may contain compounds that can either support or inhibit microbial growth. This research aimed to determine the optimal concentration of tamarind leaf in sinom kombucha production, evaluate its impact on antioxidant and antibacterial activities, and analyze its effect on the growth of Lactic Acid Bacteria (LAB).

Research Methods

Materials and equipment

Turmeric and tamarind leaves were sourced from the Traditional Market in Malang city, while *E. coli* and *S. aureus* were obtained from the Food Microbiology Laboratory of the Faculty of Agricultural Technology and the Faculty of Medicine at the University of Brawijaya, respectively. Other materials include filter paper (Macherey-Nagel), Nutrient Broth media, Nutrient Agar media, Peptone, Plate Count Agar, de Man Rogosa Sharpe Agar, Anthrone, H_2SO_4 (Sulfuric acid), Folin-Ciocalteu, $CaCO_3$ (Calcium carbonate), Na_2CO_3 (Sodium carbonate), Methanol P.A. (Pro Analysis), and DPPH in methanol (Diphenylpicrylhydrazyl in methanol).

Production of sinom kombucha

500 mL of water was boiled, thendried tamarind leaves (i.e., 0, 1, 2, 3, 4, and 5 g), dried turmeric (0.8%), and 10% sugar were added. Themixture was strained and stored in a sterilized glass jar. 10% starter was added at room temperature, and the jar was covered with a sterile cloth, followed by fermentation fermented for 7 days at room temperature (Wijayanti, 2020).

Total microbes

The method for calculating total microbes was Total Plate Count (TPC) (Fardiaz, 1987). The sample was diluted to a dilution level of 10^{-6}. The pour plate technique was performed for the last three dilutions. The samples were incubated at 37°C for 24-48 hours.

pH

The pH analysis was conducted using a pH meter (Apriyantono et al., 1989).

Total phenol

1 mL of the sample was diluted using methanol in a 10 ml volumetric flask, and then 0.5 ml iwas taken and placed in a test tube. Then, 2.5 mL of 10% Folin Ciocalteau reagent and 2.5 mL of 7.5% Na_2CO_3 were added, and the mixture is vortexed for 2 minutes. The sample was incubated at room temperature for 30 minutes. Absorbance was measured using a spectrophotometer at the maximum wavelength. The content of free phenolic compounds is expressed as Gallic Acid Equivalents per gram (mg GAE/g). This method was modified from Sharma et al. (2019).

Total sugar

The total sugar analysis was conducted using the anthrone method (Islam, 2013). A 10 mL of sample was taken and diluted to 100 ml, then $CaCO_3$ was added. The sample was then heated at 95°C for 30 minutes. The sample was filtered and centrifuged. 1 mL of the sample was reacted with 5 mL of Anthrone, then heated at 95°C for 12 minutes. Absorbance was measured using a spectrophotometer.

Antioxidant activity

The antioxidant activity was measured using the DPPH method, modified from Vitas et al. (2020). Sample solutions were prepared in methanol with concentrations of 100, 200, 300, 400, and 500 ppm. For each concentration, 1 mL was taken and mixed with 1 mL of a 0.16% DPPH solution in methanol. The samples were then incubated in a dark room at room temperature for 30 minutes. After incubation, the solutions' absorbance was measured at a wavelength of 517 nm. The IC_{50} value was calculated.

Antibacterial activity

The antibacterial activity was measured using a paper disc diffusion method (Kholia 2017). Paper discs were placed on the surface of bacterial isolates (*E. coli* and *S. aureus*). 20 µL of the sample was added onto the paper disc, and then incubated for 6 hours at 37°C. After incubation, the diameter of the inhibition zone was measured.

Total LAB

A 1 ml sample of sinom kombucha is mixed with 9 ml of peptone and diluted from 10^-1 to 10^-4. Then, 1 ml is taken from the 10^-2, 10^-3, and 10^-4 dilutions and placed into sterilized Petri dishes. Next, MRSA (de Man Rogosa Sharpe Agar) with 1% CaCO3 (calcium carbonate) is added, homogenized, and incubated at 37°C for 48 hours. The number of microbial colonies that grow is counted to determine the total LAB. This method was modified from Wang et al. (2016).

Statistical analysis

The data was statistically analyzed using analysis of variance (ANOVA) and Tukey's test at a significance level of 5% using Minitab 2019.

Best treatment selection

The best treatment was determined using the Multiple Criteria Decision Making (MCDM) method with the Simple Additive Weighting (SAW) technique. Parameters of selection were total microorganisms, pH, total sugar, total phenol, IC_{50} (or antioxidant activity), antibacterial activity, and the total LAB.

Results and Discussion

Total microbes

The total microbial count in sinom kombucha increased significantly during the 7-day fermentation period (Figure 1a). The increase occurred in tamarind leaf concentrations from 0% to 0.6%, while a decrease was observed at 0.8%. This indicates that variation in tamarind leaf concentrations affects the total microbial count during fermentation.

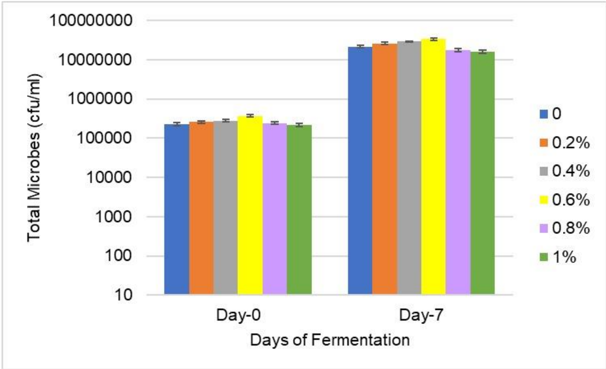
The total microbes in turmeric sinom kombucha increased because, during fermentation, the microbes utilize the sugar content in the substrate as an energy source for metabolism and cell synthesis. According to Jayabalan et al. (2014), sugar in kombucha is a good energy source for microorganisms. Sucrose is used as the primary substrate by bacteria and yeast during the fermentation. As the concentration of tamarind leaves increased, the total microbes in each sample increased, and at a certain point, the value tended to decrease. Tamarind leaves contain nutrients and vitamins that can support the growth of microorganisms. Siddig et al. (2006) mentioned that tamarind leaves contain fiber, fat, protein, tartaric acid, alkaloids, flavonoids, tannins, saponins, and minerals (i.e., potassium, sodium, magnesium, sulfur, phosphorus, and calcium). In addition, tamarind leaves also contain various vitamins such as vitamin B1 (thiamine), vitamin B2 (riboflavin), vitamin B3 or B complex (niacin), Vitamin C (ascorbic acid), and vitamin A (β-carotene).

A decrease in the total microbes at tamarind leaves concentration of 0.8% was likely due to its phenolic and organic acid content. Increasing the concentration of tamarind leaves is parallel to an increase in the phenolic and organic acid content. This may exhibit antimicrobial properties, which could eradicate microorganisms or inhibit the growth of bacteria, fungi, and protozoa. The inhibition mechanism of bacterial growth includes permeabilization and stabilization of the plasma membrane and inhibition of extracellular microbial enzymes (Sabel et al., 2017). Phenolic and organic acid components play a role in antibacterial and antifungal activities (Kitwetcharoen et al., 2023).

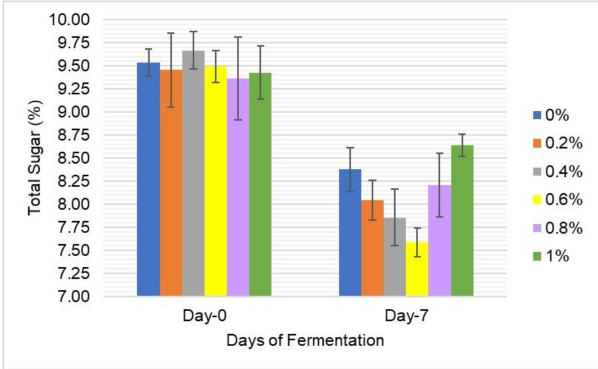
pH

The pH value of sinom kombucha decreased after the fermentation. The pH value of sinom kombucha on day 0 ranged from 4.06 to 5.24, while on day 7 ranged from 3.64 to 4.66 (Figure 1b). This occurs due to an increase in the concentration of organic acids during fermentation (Bishop et al., 2022). According to Neffe-Skocińska et al. (2017), some organic acids were produced during fermentation, including glucuronic acid, gluconic acid, lactic acid, malic acid, citric acid, tartaric acid, folate, malonic acid, oxalic acid, succinic acid, pyruvic acid, and usnic acid.

The results show that the pH value decreases with the increasing concentration of tamarind leaves. Tamarind leaves contain several types of organic acids, such as L-malic acid (Katsayal et al., 2019) and a relatively high tartaric acid content (Deepak et al., 2016). Syed et al. (2014) showed that tamarind leaf extracts were identified to contain various types of organic acids, including oxalic acid, tartaric acid, citric acid, and malic acid.



(a)



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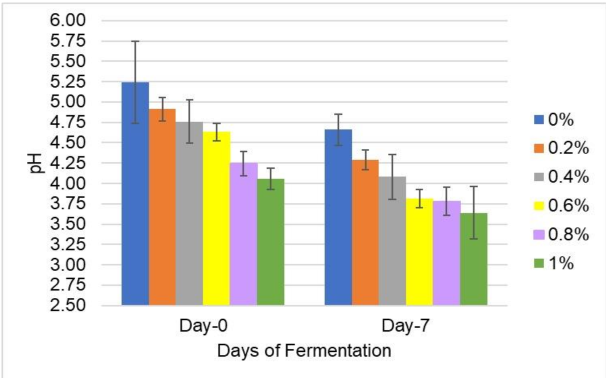

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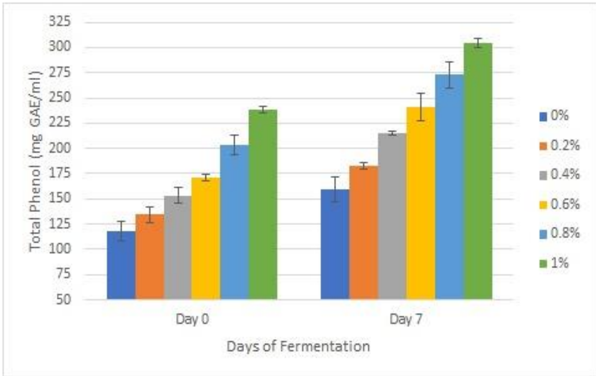

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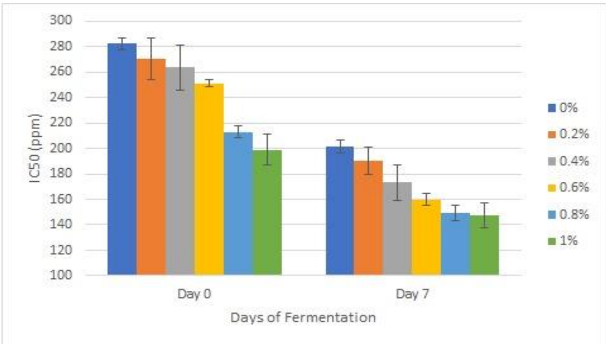


(e)

Figure 1. Total microbes (a), pH (b), total sugar (c), total phenol (d), and IC_{50} values (e) in sinom kombucha at various concentrations of tamarind leaves on day 0 and day 7 of fermentation. Error bars represents standard deviation from three measurements.

Total sugar

A decrease in total sugar was observed from day 0 to day 7 of fermentation (Figure 1c). This occurred because sucrose was added to the sinom beverage, which was utilized by microorganisms during fermentation. Yeast cells hydrolyze sucrose into glucose and fructose, and then through the glycolysis process using yeast invertase enzyme to produce ethanol (Sutthiphatkul et al., 2023). Acetic acid bacteria use glucose to produce gluconic acid and ethanol to produce acetic acid (Jayabalan et al., 2014), while lactic acid bacteria use sugar as an energy source and convert it into lactic acid through metabolic processes (Widowati and Misyogarta, 2003).

At each concentration of tamarind leaves added, there was a significant difference in the decrease in total sugar. This indicates that the tamarind leaf content affects the fermentation rate and the substrate's sugar content reduction. At a certain concentration threshold, the tamarind leaf content can inhibit the growth of microorganisms. According to Abdallah and Muhammad (2018), tamarind leaves contain alkaloids, saponins, flavonoids, steroids, phenols, terpenoids, tannins, and anthraquinones. The flavonoid and polyphenol content in tamarind leaves has antibacterial properties.

Total phenol

The total phenol content in sinom kombucha increased after 7 days of fermentation (Figure 1d). The results also demonstrated that increasing concentration of tamarind leaves increases the total phenols in sinom kombucha. This indicates that the fermentation influences the total phenolic compound content in kombucha. Breakdown of complex molecules during fermentation occurs when enzymes are released by bacteria and yeast, which affects the content of phenolic compounds. Therefore, phenolic compounds increase as fermentation progresses (Massoud et al., 2022). Microorganisms in kombucha could break down plant cell walls, releasing phenolates and flavonoid compounds (Zhang et al., 2020). During the fermentation stage, there is biotransformation of compounds (i.e., catechin and other polyphenolic compounds) by enzymes from the kombucha culture (i.e., invertase, cellulase, and amylase. These enzymes break the complex bonds between phenolic compounds and tissue structures, leading to an increase in the total phenolic compounds in kombucha beverages (Essawet et al., 2015).

IC_{50}

Sinom kombucha experiences a decrease in IC_{50} values during fermentation. The IC_{50} values of sinom kombucha on day 0 of fermentation ranged from 282.10 to 199.11 ppm, while on day 7 ranged from 147.52 to 201.38 ppm (Figure 1e).

The change in IC_{50} values in sinom kombucha indicates an increase in antioxidant activity after fermentation. This mechanism can occur when complex phenolic compounds are in an acidic environment or when enzymes are released by bacteria and yeast in the tea fungus. As a result, complex molecules were degraded into smaller molecules. This degradation increases the total phenolic compounds available in the kombucha (Srihari and Satyanarayana, 2012).

Phenolic compounds reduce free radicals by releasing hydrogen atoms and donating them to free radical compounds, making initially highly reactive free radicals more stable and less reactive (Hartati, 2010). A higher number of hydroxyl groups in a phenolic compound equates to stronger antioxidant activity, due to more hydrogen atoms are available to neutralize free radicals.

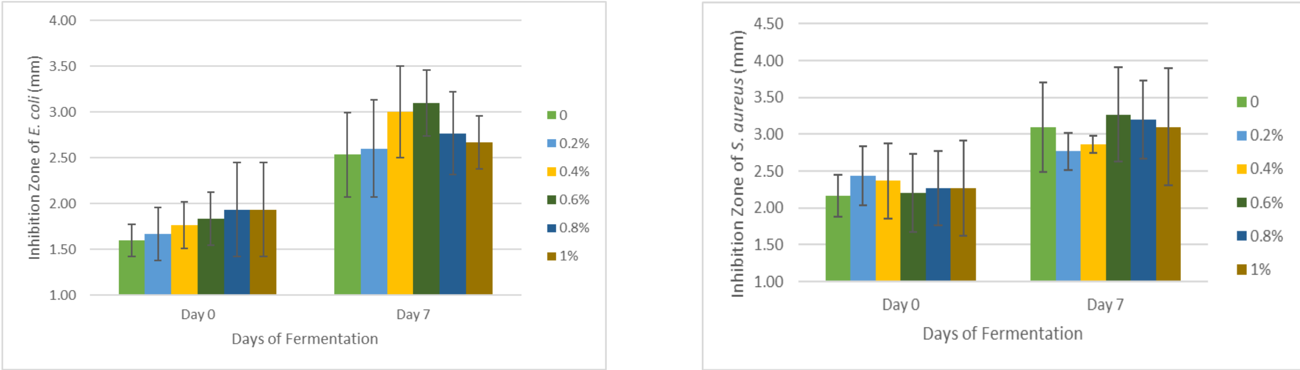
Antibacterial activity

Figure 2a shows that the antibacterial activity against *E. coli* in turmeric sinom kombucha on day 0 tends to increase with the increasing concentration of tamarind leaves. Detailed values of the antibacterial activity of turmeric sinom kombucha against *E. coli* can be seen in Table 1.

Table 1 shows that fermentation treatment affects the clear zone diameter of turmeric sinom kombucha against *E. coli* bacteria. There was no significant difference in the clear zone diameter of turmeric sinom kombucha in all treatments. This is likely because the range of tamarind leaf concentrations was not wide enough. The antibacterial activity of turmeric sinom kombucha on day 0 is derived from the chemical compounds in the raw materials. According to Abdallah and Muhammad (2018), tamarind leaves contain alkaloids, saponins, flavonoids, steroids, phenols, terpenoids, tannins, and anthraquinones. The flavonoid and polyphenol content in tamarind leaves have antibacterial properties against gram-negative bacteria, including *E. coli*. This study aligns with Adeniyi et al. (2017), who stated that tamarind leaves have antibacterial capabilities against *Escherichia coli, Enterobacter Gergovia, Hafnia alvei, S. aureus, Bacillus subtillis*, and *Salmonella typhi*.

Based on Figure 2b, the antibacterial activity of turmeric sinom kombucha against *Staphylococcus aureus* was affected by fermentation, with the values shown in Table 2. However, the variation in tamarind leaf concentration did not influence the diameter of the inhibition zones against *S. aureus,* as indicated by no significant difference at $P < 0.05$. This is likely due to the limited range of tamarind leaf concentrations used. On the other hand, fermentation significantly affected the diameter of the inhibition zones against *S. aureus*. The antibacterial activity of turmeric sinom kombucha on day 0 was likely influenced by the chemical compounds in the raw materials.

The fermentation process for 7 days significantly affected the diameter of the inhibition zones of turmeric sinom kombucha against both *E. coli* and *S. aureus*. During fermentation, sugar content in the substrate is broken down into glucose and fructose by yeast, producing carbon dioxide (CO_2) and ethanol. Sugar is a source of nutrition for the bacteria and yeast in the kombucha culture. Acetic acid bacteria convert ethanol into acetic acid and gluconic acid. On the other hand, fructose transforms into organic acids through the process carried out by microorganisms in kombucha. The ethanol produced by yeast can stimulate acetic acid bacteria to produce acetic acid. Acetic acid can penetrate bacterial cell walls and cause structural changes, disrupting the function of plasma cell proteins. Additionally, it inhibits the growth of bacteria (Bhandari et al., 2022).



(a) (b)

Figure 2. Changes in antibacterial activity of turmeric ginger kombucha against *Escherichia coli* (a) and *Staphylococcus aureus* (b) due to different concentrations of tamarind leaves during fermentation**.** Error bars represents standard deviation from three measurements.

Table 1. The antibacterial activity value of sinom kombucha due to different concentrations of tamarind leaves during fermentation against *Escherichia coli*

Tamarind Leaves Concentration (%)	**Zone of Inhibition Diameter (mm)**	
	***E. coli* Day 0**	***E. coli* Day 7**
0	1.60±0.17[a]	2.53±0.46[a]
0,2	1.67±0.29[a]	2.60±0.53[a]
0,4	1.77±0.25[a]	3.00±0.50[a]
0,6	1.83±0.29[a]	3.10±0.36[a]
0,8	1.93±0.51[a]	2.77±0.45[a]
1	1.93±0.51[a]	2.67±0.29[a]

Note: * Different notation letters indicate a significant difference at the significance level (P<0.05). ± is the standard deviation from three measurements

Table 2. The values of antibacterial activity of sinom kombucha due to different concentrations of tamarind leaves during fermentation on *Staphylococcus aureus*

Tamarind Leaves Concentration (%)	**Zone of Inhibition Diameter (mm)**	
	***S. aureus* Day 0**	***S. aureus* Day 7**
0	2.17±0.29[a]	3.10±0.61[a]
0,2	2.43±0.40[a]	2.77±0.25[a]
0,4	2.37±0.51[a]	2.87±0.12[a]
0,6	2.20±0.53[a]	3.27±0.64[a]
0,8	2.27±0.50[a]	3.20±0.53[a]
1	2.27±0.64[a]	3.10±0.79[a]

Note: * Different notation letters indicate a significant difference at the significance level (P<0.05). ± is the standard deviation from three measurements

Acetic acid produced during fermentation has antibacterial properties against various types of bacteria, including both gram-positive and gram-negative bacteria. Acetic acid can penetrate bacterial cell walls, causing changes in their structure and disrupting plasma cell protein function (Bhandari et al., 2022). The increased total phenol content also contributes to the antibacterial activity of kombucha. Hydroxyl groups (OH) in phenolic compounds can inhibit bacterial growth. The number and position of hydroxyl groups (OH) in phenolic molecules affect their toxicity to microorganisms. The more phenolic compounds are oxidized, the more they inhibit the growth of microorganisms. Furthermore, kombucha contains antibiotic compounds that can inhibit the growth of pathogenic microorganisms (Jayabalan et al., 2014).

As a raw material for making turmeric sinom kombucha, tamarind leaves contain various organic acids (Syed et al., 2014). Organic acids exhibit microbicidal properties through a mechanism in which non-dissociated molecules flow through the microorganism's cell membrane and ionize internally. Hydrogen ions are released to maintain pH balance inside the cell, decreasing intracellular pH. A decrease in pH can cause deformation and damage to enzymatic activity, proteins, and DNA structure, ultimately damaging the extracellular membrane (Kong-López et al., 2011).

The antibacterial activity of turmeric sinom kombucha against *S. aureus* appears higher than against *E. coli*. This difference can be explained by the differences in the cell wall structures of these two bacteria. *S. aureus* is a gram-positive bacterium with a single plasma membrane surrounded by a cell wall made of peptidoglycan with a rigid structure. The cell wall of gram-positive bacteria consists of teichoic acid containing alcohol and phosphate. Teichoic acid is a negatively charged molecule that binds cations in the cell wall, regulating the movement of these cations inside or outside the cell wall. In contrast, gram-negative bacteria have a cell wall with fewer peptidoglycan layers and are bound to lipoproteins in the outer cell membrane, with no teichoic acid (Sharma et al., 2022). The secondary metabolite compounds produced by microorganisms in kombucha can damage the peptidoglycan components in bacterial cell walls. Because gram-positive bacteria have more peptidoglycan components, their structure becomes more susceptible to damage by these compounds (Sreeramulu et al., 2000). Additionally, *Escherichia coli* bacteria have a protective capsule could resistant to antibacterial compounds, making it less susceptible to these antibacterial compounds (Kohanski et al., 2010).

These findings align with research conducted by Borkani et al. (2016), who showed that kombucha has higher antibacterial activity against gram-positive bacteria, especially *S. aureus*. This research also aligns Yanti et al. (2020), who reported that kombucha derived from soursop leaves exhibited higher antimicrobial activity against *S. aureus* than *E. coli*. This strengthens the fact that kombucha has the potential for stronger antibacterial activity against gram-positive than gram-negative bacteria.

Total lactic acid bacteria (LAB)

Figure 3 shows that the total LAB count in turmeric sinom kombucha increased after 7 days of fermentation. An increase in the total LAB count was found at the tamarind leaves concentration ranged from 0% to 0.6%. The highest increase in the total LAB count occurs at a concentration of 0.6%, reaching 4.89 x 105 CFU/mL. This is because the addition of tamarind leaves provides more nutrients that can support the growth of LAB. LAB cells can grow to a maximum number in a medium where the availability of nutrients is sufficient (Hidayat et al., 2013).

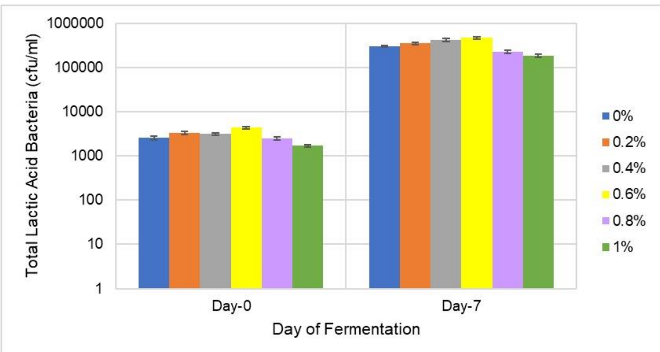


Figure 3. Total LAB in sinom kombucha at various concentrations of tamarind leaves on day 0 and day 7 of fermentation. Error bars represents standard deviation from three measurements.

Table 3. The chemical characteristics of the best-treated sinom kombucha

Parameters	Sinom Kombucha 0.6%
Total Microbes (CFU/mL)	3.37×10^7
pH	3.81
Total Sugar (%)	7.58
Total Phenol (mg GAE/mL)	204.90
IC_{50} (ppm)	159.84
Antibacterial Activity *E. coli* (mm)	3.1
Antibacterial Activity *S. aureus* (mm)	3.27
Total LAB (cfu/mL)	4.63×10^3

The nutrients needed by LAB to grow optimally include sugar, protein, fat, and vitamins, which are present in tamarind leaves (Teusink and Molenaar, 2017; Deepak et al., 2016). The protein in tamarind leaves will be hydrolyzed by LAB, beneficial for its growth. Tamarind leaves contain several vitamins (i.e., vitamin B1, vitamin C, vitamin B3, vitamin B2, and vitamin A), needed by most microbes for growth (Fakhrurrazi et al., 2016). Vitamins are essential factors for microbial growth, acting as coenzymes during cellular metabolism (LeBlanc et al., 2013; Miret and Munne-Bosch, 2014). LAB require sugar for metabolic activities and reproduction, and one of the secondary metabolites being lactic acid (Yin et al., 1998). Providing sugar as a substrate in the medium can increase the viability of LAB. During fermentation, LAB may reach the optimum limit in utilizing sugar as an energy source (Dante et al., 2016).

The increase in total LAB after fermentation decreases at a concentration of 0.8%. Tamarind leaves contain polyphenolic compounds such as apigenin, catechin, epicatechin, and procyanidin B2. The higher the polyphenol content in a substance, the higher the total phenol content during fermentation (Arshad et al., 2019). Phenolic compounds have high antibacterial activity (Cardoso et al., 2020). Tamarind leaves also contain major organic acids including tartaric acid, malic acid, citric acid, and oxalic acid (Syed et al., 2014). High organic acid content can lower the pH of the substrate, affecting the bacterial growth rate. A low pH value in a medium can cause severe cell damage because the bacterial cytoplasm becomes acidic (Kumar and Joshi, 2016).

Best Treatment

The selected best treatment was sinom kombucha with 0.6% tamarind leaves, with the chemical characteristics as shown in Table 3. Compared to other treatment, the selected best treatment had the highest antibacterial activity against *E. coli* and *S. aureus*, as well as the total LAB content. Sinom kombucha with such characteristics is potential for healthy drink.

Conclusions

The concentration of tamarind leaves in the production of sinom kombucha affects the total microbe count, total LAB, pH, total sugar, total phenols, and IC_{50} value. However, variations in tamarind leaves concentration have no significant difference in antibacterial activity. Fermentation treatment significantly influences the characteristics of sinom kombucha, including total microbe count, total LAB count, pH value, total phenols, total sugar, IC_{50} value, and antibacterial activity. The best chemical characteristics for sinom kombucha were achieved with addition 0.6% of tamarind leaves

Declarations

Conflict of interests The authors declare no competing interests.

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