BIOLOGY, MEDICINE, & NATURAL PRODUCT CHEMISTRY
Volume 14, Number 1, April 2025 | Pages: 453-463 | DOI: 10.14421/biomedich.2025.141.453-463
ISSN 2089-6514 (paper)
ISSN 2540-9328 (online)

Potential of Kedabu (*Sonneratia ovata* Backer) Fruit Juice Supplemented in Kombucha Beverage as Antibacterial Agent

Musyirna Rahmah Nasution*, Clara Nabilah, Meiriza Djohari, Nurhapipah
Departement of Pharmacy, Sekolah Tinggi Ilmu Farmasi Riau, Pekanbaru, Riau, Indonesia.

Corresponding author*
musyirnarahmah@stifar-riau.ac.id

Manuscript received: 03 May, 2025. Revision accepted: 15 July, 2025. Published: 18 July, 2025.

Abstract

Riau Province has abundant natural resources of mangrove forests almost along the coast of Riau. Mangrove fruit can be developed into various processed foods, but processed foods derived from mangroves have not been widely developed and are of interest to coastal communities. Kombucha tea is a probiotic beverage produced through the fermentation of tea and sugar using a kombucha starter known as SCOBY (Symbiotic Culture of Bacteria and Yeast). The presence of sugar in kombucha colonies is a vital source of nutrition. Additionally, fruit juice can be incorporated to create variations of kombucha tea. Kedabu fruit (*Sonneratia ovata* B.), a native fruit of Riau Province, is recognized for its high carbohydrate content and secondary metabolites, which exhibit potential antibacterial properties. This research developed four formulations of kombucha tea, each with different concentrations of kedabu fruit juice (0%, 10%, 20%, and 30%). The study's objectives were to formulate, evaluate the physicochemical properties and antibacterial activity of these formulations against *Escherichia coli* and *Staphylococcus aureus*. The organoleptic properties, alcohol content, pH, %TTA (Total Acidity Titratable), total LAB (Lactic Acid Bacteria), and yeast count were assessed during a 14-day fermentation period. The assay results indicated that the kombucha fruit kedabu tea formulation exhibited favorable organoleptic characteristics, a decrease in pH, an increase in %TTA, total LAB, and yeast count within the acceptable range defined by standards. Moreover, it displayed inhibitory activity against *Escherichia coli* and S*taphylococcus aureus* bacteria, falling within the weak to moderate categories.

Keywords: Kombucha; Kedabu; Antibacterial; *Escherichia coli*; *Staphylococcus aureus*.

INTRODUCTION

Riau Province, located in Indonesia, is known for its abundant natural resources including land, mining, agriculture, forestry, marine, and industry. With a coastline stretching over 2,078 kilometers, the province boasts extensive mangrove forests along its shores. Mangroves offer a wide range of valuable resources that have been extensively utilized by coastal communities, both in the form of timber forest products and non-timber forest products. However, the development of processed foods derived from mangroves remains limited, despite the potential demand from these communities. Mangroves are a type of plant that thrives in tidal areas, particularly along protected tropical and sub-tropical coasts as well as sheltered sub-tropical regions. The mangrove plant encompasses various tree species, including *Avicennia, Sonneratia, Rhizophora, Bruguiera, Ceriops, Lumnitzera, Excoecaria, Xylocarpus, Aegiceras, Scyphyphora,* and Nypai (Kathiresan *et al,* 2001). One of the mangrove plants is Kedabu (*Sonneratia ovata* Backer) which is utilized in food and health.

Gastrointestinal tract infections pose a significant health concern due to the digestive system's vulnerability to bacterial invasion. As the primary entry point for food and drinks, the gastrointestinal tract risks contamination by pathogenic bacteria. Traditionally, antibiotics have been utilized to treat bacterial infections. However, the indiscriminate use of antibiotics can result in bacterial resistance, necessitating the exploration of alternative treatment options (Aminov, 2010).

One such approach to prevent gastrointestinal tract infections is using kombucha tea. Kombucha tea is a fermented beverage produced by the fermentation of black tea and sugar using a symbiotic culture of bacteria and yeast (SCOBY). The fermentation process typically takes place over 7-14 days. SCOBY is composed of various microorganisms, including acetic acid bacteria (such as *Acetobacter xylinum, Acetobacter acetic,* and *Gluconobacter oxydans*), yeast (such as *Saccharomyces cerevisiae, Zygosaccharomyces sp,* and *Torulopsis sp*), and lactic acid bacteria (such as *Lactobacillus sp* and *Lactococcus sp*) (Laureys *et al.*, 2020).

Kombucha is known to contain a diverse array of chemical compounds, including acetic acid, gluconic acid, lactic acid, vitamins (B1, B2, B6, B12, C), ethanol, carbon dioxide, polyphenols, and others (Herwin, 2022). These compounds contribute to the pharmacological effects of kombucha tea, which include improved digestion, antibacterial properties, antioxidant activity, and more. The presence of secondary metabolite compounds such as triterpenoids, alkaloids, phenolics, saponins, and flavonoids. These compounds are known for their potential health benefits and contribute to the overall bioactive profile (Chandraekara *et al.*, 2018).

Furthermore, a study revealed that kombucha tea exhibited inhibitory effects on *Escherichia coli* and *Staphylococcus aureus*, with inhibition diameters of 20.5 mm and 20.15 mm, respectively, after a 14-day fermentation period (Bhattacharya *et al.*, 2016). *Escherichia coli* and *Staphylococcus aureus* were the most susceptible bacteria to the inhibitory action of kombucha tea, resulting in inhibition diameters of 19 mm and 18 mm, respectively. Increased fermentation time of kombucha tea led to an enhanced antibacterial activity (Al-Mohammadi *et al.*, 2021).

One of the crucial factors influencing kombucha production is the presence of sugar. Sugar plays a vital role as an energy source for yeast in the SCOBY (Miranda *et al.*, 2016). Within the SCOBY, yeast metabolizes sugar by converting it into glucose and fructose, which are subsequently fermented into ethanol. *Acetobacter sp*. then utilizes ethanol to produce acetic acid (Laureys *et al.*, 2020). In kombucha tea, it is possible to substitute sugar with fruit juice as an alternative sweetener.

Sonneratia ovata Backer, commonly known as kedabu or pedada plant, is a mangrove species frequently found along the coastlines of Riau Province, Indonesia. The fruit of the kedabu plant is a significant source of nutrients. It can be processed into various products, including candy, jam, syrup, and dodol. Importantly, the kedabu fruit is non-toxic and can be consumed directly. Analysis conducted by Astuti et al. revealed that Sonneratia ovata Backer fruit contains approximately 2.19% carbohydrates. Furthermore, phytochemical screening demonstrated the presence of flavonoids, phenolics, and saponins and triterpenoids (such as oleanolic acid and maslinic acid) in the extracts and fractions of Sonneratia ovata fruit (Wu *et al.*, 2009).

Research exploring the utilization of kedabu fruit in functional food products remains limited. This is notable given the fruit's potential as a source of secondary metabolite compounds with antibacterial properties, as well as its high nutritional content. Moreover, no existing publications specifically focused on kombucha tea made from kedabu fruit, which presents an interesting opportunity for further investigation. Thus, this study aimed to formulate kombucha tea by incorporating kedabu fruit juice and evaluate its antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* bacteria. By developing kombucha tea using kedabu fruit juice, researchers aim to highlight the potential of this fruit in Riau Province, Indonesia, and promote its utilization as an alternative antibacterial remedy

MATERIALS AND METHODS

Sampling and Sample Identification

The samples were collected from West Dumai District, Dumai City, Riau, Indonesia. The samples were then taken to the Botanical Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, University of Riau, Pekanbaru, for identification. The sample identification was conducted under the reference number 611/UN19.5.1.1.3-4.1/EP/2021 (Table 2).

Kedabu Fruit Juice Preparation

Fresh Kedabu fruit weighing 500 grams was thoroughly washed. The fruit flesh was then finely chopped into small pieces and mixed with water at 1:2 (w/v). The mixture was blended using a blender to obtain a homogeneous fruit pulp. Subsequently, the kedabu fruit pulp was filtered to obtain pure kedabu fruit juice. The kedabu fruit juice was further prepared in concentrations of 10%, 20%, and 30% by dilution (Chan *et al.*, 2021).

Formulation of Kombucha

Formulation details can be found in Table 1. 250 mL of distilled water was boiled in a stainless steel pot to prepare the kombucha tea. Black tea, sugar, and kedabu juice were added to the boiling water. The mixture was then filtered and transferred to a glass jar. The kombucha tea mixture was allowed to cool down to 25°C. Next, 10% (v/v) of kombucha starter culture was added to the mixture, and distilled water was added to bring the total brew volume to 500 mL. The mixture was homogenized thoroughly. The glass jar was covered with a clean cloth or tissue and securely tied. Fermentation was conducted at a temperature of 25°C for 14 days. Samples were taken for antibacterial and chemical analysis during the fermentation process.

Table 1. Formulation of kombucha tea with kedabu fruit juice.

No	*Composition*	**F1*	*F2*	*F3*	*F4*
1	Black Tea	1,6%	1,6%	1,6%	1,6%
2	Sugar	10%	10%	10%	10%
3	Kombucha Starter	10%	10%	10%	10%
4	Kedabu Fruit Juice	0%	10%	20%	30%
5	Aquadest	ad 500 mL	ad 500 mL	ad 500 mL	ad 500 mL

Note : Formula (*F)

Evaluation of chemical changes and antibacterial activity

Some parameters used in evaluating the quality of kombuca tea supplemented with kedabu fruit juice as a fermented beverage were organoleptic test, phytochemical screening test, determination of pH with pH meter, alcohol content with alcohol meter. The total tritabel acidity (TTA) was determined using the alkalimetric method. Determination of total lactic acid bacteria (LAB) and total yeast in kombucha tea is done by using the standar plate count/SPC method (Pour plate method). As a functional beverage, one pharmacological effect test was antibacterial activity against *E coli* and *S aureus* bacteria using the disc diffusion method. Evaluation of all parameters was made before fermentation and after fermentation on the 7th and 14th day.

Data analysis

The data obtained from the antibacterial activity assay of kombucha tea supplemented with kedabu fruit juice against *Escherichia coli* and *Staphylococcus aureus* bacteria, collected at different time points (before fermentation, 7th day, and 14th day of fermentation), were analyzed using the One Way ANOVA statistical test. The analysis was performed using the SPSS application to determine if there were any significant differences among the groups.

To further determine which formula group exhibited the best antibacterial activity, a post-hoc analysis was conducted using the Tukey test. This test allows for a pairwise comparison between groups to identify significant differences.

Additionally, the data obtained from the evaluation of the kedabu fruit kombucha tea formula, which included organoleptic test, pH test, total lactic acid bacteria (LAB) test, total titratable acid (TTA) test, total yeast test, and alcohol test, were analyzed descriptively. Descriptive analysis provides a comprehensive summary of the data, including measures of central tendency and variability, to describe the characteristics of the kedabu fruit kombucha tea formula.

These data analyses provide valuable insights into the antibacterial activity and overall quality of the kedabu fruit kombucha tea formula, allowing for an objective assessment of its effectiveness as a functional beverage.

RESULTS AND DISCUSSION

Sample Identification

The sample identification was conducted under the reference number 611/UN19.5.1.1.3-4.1/EP/2021 (Table 2). The results of sample identification showed that the sample had the regional name Kedabu and the species name *Sonneratia ovata* Backer.

Table 2. The result of the identification sampel

Sample Identification	*Sample*
Kingdom	Plantae
Phylum	Magnoliophyta
Classis	Magnoliosida
Ordo	Myrtales
Family	Lythraceae
Genus	Sonneratia
Species	*Sonneratia ovata* Backer
Regional Name	Kedabu

Figure 1. Kedabu Plant (a); Kedabu Fruit (a and c).

Kedabu or pedada plants (*Sonneratia sp.*) are a species of mangrove plants commonly found along the coastlines of Riau Province, Indonesia. The fruit of the kedabu plant has gained attention due to its nutritional composition and bioactive compounds (Halifah *et al*, 2019). Exploiting natural resources to develop functional food products is an important aspect of utilizing traditional medicinal plants.

In this study, the focus was on the production of kombucha tea using kedabu fruit juice as an ingredient. Kombucha tea is a fermented beverage known for its potential health benefits and is typically prepared using black tea and sugar fermented by a symbiotic culture of bacteria and yeast (SCOBY) (Chakravorty *et al*, 2016). By incorporating kedabu fruit into the kombucha tea formulation, it is possible to enhance the nutritional and bioactive properties of the final product.

The results of the sample identification confirmed that it was *Sonneratia ovata Backer*, commonly known as kedabu fruit. The phytochemical screening test indicated that the fermentation time and addition of kedabu fruit juice significantly effected the content of secondary metabolites, increasing their levels. Kombucha tea enriched with kedabu fruit juice exhibited higher secondary metabolites, including flavonoids, phenolics, terpenoids, and saponins (Table 3). These secondary metabolites are well-known for their pharmacological effects, such as antimicrobial, antioxidant, and antidiabetic activities (Herwin, 2022).

Table 3. The results of screening phytochemical of kombuca tea with or without kedabu fruit juice.

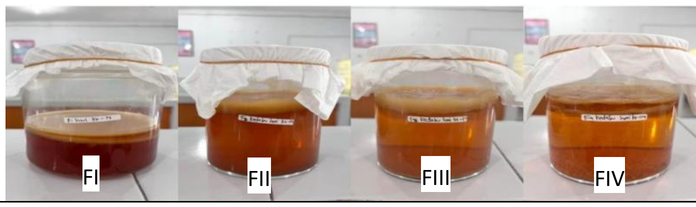
Secondary Metabolite	Reagent	Before Fermentation		After fermentation on 14 days	
		Kombucha Tea	Kombucha Tea + Kedabu Juice	Kombucha Tea	Kombucha Tea + Kedabu Juice
Alkaloid	Mayer	-	-	-	-
Fenolik	Logam Mg + $HCl_{(P)}$	+	+	+	+
Flavonoid	$FeCl_3$ 1 %	+	+	+	+
Terpenoid	Lieberman-Bouchard	-	+	+	+
Steroid	Lieberman-Bouchard	-	-	-	-
Saponin	Air	-	+	-	+

Evaluation of chemical changes and antibacterial activity.

The evaluation of kombucha tea's quality was conducted based on the standard parameters outlined in SNI 7552-2018 for fermented beverages. The organoleptic test was performed after a fermentation period of 14-days. The results demonstrated that an extended fermentation time led to a lighter color of kedabu fruit kombucha tea (Table 4). This color change can be attributed to the activity of microorganisms responsible for color degradation. Bacteria utilize total soluble solids as an energy source, resulting in a depletion of solvents in the media and a clearer liquid (Jayabalan *et al.*, 2014).

Regarding the aroma, the observations of kombucha tea indicated that a longer fermentation time resulted in a more distinct aroma in the kedabu fruit kombucha. Furthermore, increasing the concentration of kedabu fruit led to a more pronounced sour taste. The sour taste in kedabu fruit kombucha tea is attributed to the production of lactic acid and acetic acid during the fermentation process (Wang *et al.*, 2022)

Table 4. Observation of kedabu fruit kombucha tea before and after fermentation for 14 days

Description	The Color of Kedabu Fruit Kombucha Tea
Before Fermentation (0-day)	
After Fermentation on 14-day	

Note : F1: Kombucha tea without kedabu juice ;
F2,F3, F4 : Kombucha tea+kedabu juice (10%, 20% ,30%)

The subsequent test involved measuring the alcohol content using an alcohol meter. Alcohol measurement is a crucial aspect in assessing the quality and safety of kombucha tea. As fermented beverages, kombucha is known to contain alcohol. In Indonesia, where the majority of consumers are Muslims, it is essential for these beverages to comply with the halal food standards established by the Indonesian Ulama Council (MUI). According to MUI (Fatwa No. 10 of 2018) (FMUIN, 2018). fermented beverage products containing less than 0.5% alcohol/ethanol are considered halal if they are medically harmless (Pauzi *et al.*, 2019)

In this study, the obtained results revealed that the alcohol content in all formulations of kombucha tea was 0% throughout the fermentation process from day-0 to day-14 (Table 5). It is worth noting that previous research has indicated that the alcohol content in kombucha generally ranges from 0.1% to 1%.

Table 5. Evaluation of organoleptic test, alcohol content, %tta, total lab, total yeast and antibacterial activity of kedabu fruit kombucha tea formulations

Formula	**Parameters**	**Observation Results**		
		0-day	**7-day**	**14- day**
FI	Taste	Sweet	Sour	Sour
	Flavor	typical kombucha falvor	typical kombucha falvor	typical kombucha falvor
	Color	dark brown	dark brown	brown
	pH	3,37	2,94	2,92
	Alcohol	0%	0%	0%
	%TTA	0,086%	0,33%	0,51%
	Total LAB	9,9 x 10^7 *cfu*/mL	18,1 x 10^7 *cfu*/mL	16 x 10^7 *cfu*/mL
	Total Yeast	2,32 x 10^6 *cfu*/mL	1,56 x 10^6 *cfu*/mL	2,47 x 10^6 *cfu*/mL
	Antibacterial Activity *Escherichia coli*	6,8 mm	7,8 mm	8,2 mm
	Antibacterial Activity *Staphylococcus aureus*	7,2 mm	8,6 mm	8,3 mm
FII	Taste	Sweet	Sour	Sour
	Flavor	typical kombucha falvor	typical kombucha falvor	typical kombucha falvor
	Color	Dark brown	light brown	golden brown
	pH	3,25	2,86	2,72
	Alcohol	0%	0%	0%
	%TTA	0,16%	0,35%	0,75%
	Total LAB	6,5 x 10^7 *cfu*/mL	16,2 x 10^7 *cfu*/mL	13,4 x 10^7 *cfu*/mL
	Total Yeast	1,98 x 10^6 *cfu*/mL	1,3 x 10^6 *cfu*/mL	2,29 x 10^6 *cfu*/mL
	Antibacterial Activity *Escherichia coli*	7,2 mm	8,03 mm	9 mm
	Antibacterial Activity *Staphylococcus aureus*	7,6 mm	8,8 mm	10,2 mm
FIII	Taste	Sweet	Sour	Sour
	Flavor	Kedabu flavor	typical kombucha falvor	typical kombucha falvor
	Color	brown	light brown	golden brown
	pH	3,25	2,92	2,61
	Alcohol	0%	0%	0%
	%TTA	0,16%	0,58%	0,99%
	Total LAB	5,1 x 10^7 cfu/mL	9,95 x 10^7 cfu/mL	9,9 x 10^7 cfu/mL
	Total Yeast	1,53 x 10^6 cfu/mL	1,19 x 10^6 cfu/mL	1,9 x 10^6 cfu/mL
	Antibacterial Activity *Escherichia coli*	7,4 mm	8,6 mm	9,96 mm
	Antibacterial Activity *Staphylococcus aureus*	7,9 mm	9,2 mm	10,93 mm
FIV	Taste	Sour	Sour	Sour
	Flavor	Kedabu flavor	typical kombucha falvor	typical kombucha falvor
	Color	brown	light brown	golden brown
	pH	3,18	2,8	2,5
	Alcohol	0%	0%	0%
	%TTA	0,17%	0,58%	1,02%
	Total LAB	6,15 x 10^7 cfu/mL	6,95 x 10^7 cfu/mL	8,55 x 10^7 cfu/mL
	Total Yeast	1,3 x 10^6 cfu/mL	0,92 x 10^6 cfu/mL	1,68 x 10^6 cfu/mL
	Antibacterial Activity *Escherichia coli*	7,8 mm	10,5 mm	11,8 mm
	Antibacterial Activity *Staphylococcus aureus*	7,93 mm	10,3 mm	15 mm

The pH measurements of all formulations revealed a decrease in pH values ranging from 3.37 to 2.5 (Table 5). As the concentration of kedabu juice in kombucha tea increased, the pH value decreased. The duration of fermentation also influenced the pH value. The highest pH value was observed on the 0th day. The fermentation time increases, the pH value decreases (Nurikasari *et al.*, 2017). The decrease in pH value in kedabu fruit kombucha tea is attributed to the conversion of glucose into organic acids by microorganisms. The concentration of organic acids increases with prolonged fermentation (Laureys *et al.,* 2020). The pH of kombucha tea typically ranges from 2.5 to 4.2 (Murphy *et al.,* 2018). Overall, the pH of the produced kedabu fruit kombucha tea meets the general pH requirements for kombucha tea.

The total titratable acid (TTA) values were measured using the alkalimetric titration method. All formulas exhibited changes in TTA values ranging from 0.086% to 1.02% (Table 5). The maximum % total acid in kombucha tea is 3% (w/v) (Greenwalt *et al.*, 2000). Thus, the overall results obtained in this study meet the standard % total acid requirement for kombucha tea. The %TTA increases with the addition of kedabu juice in kombucha tea and the duration of fermentation. The increase in %TTA is associated with the enhanced activity of lactic acid bacteria in metabolizing sucrose into organic acids (Nguyen *et al.,* 2015).

The relationship between total titratable acid and pH is inversely proportional, meaning that as the %TTA value increases, the pH value decreases and vice versa. This observation is consistent with the findings of previous research on kombucha tea (Laureys *et al.,* 2020). The acidic nature of kombucha tea, attributed to the presence of organic acids such as acetic acid and lactic acid, contributes to its characteristic taste and potential health benefits.

Acetic acid is the primary organic acid produced during kombucha fermentation. It plays a crucial role in inhibiting the growth of pathogenic bacteria. The formation of acetic acid in kombucha fermentation has been found to have potential antibacterial properties (Herwin, 2022). As acetic acid is produced in kombucha, it undergoes decomposition, releasing free protons that lead to a decrease in pH (Hamed *et al.,* 2012).

Acetic acid in kombucha can disrupt the lipid bilayer structure of bacterial cells by introducing protons into the cytoplasm. This accumulation of intracellular protons results in acidic conditions, leading to protein denaturation and energy loss within the bacterial cells. The higher the content of organic acids, particularly acetic acid, the greater the potential for inhibiting the growth of pathogenic bacteria. The ideal formation of organic acids in kombucha reduces pH, creating highly acidic conditions that significantly affect bacterial growth. This acidic environment inactivates the cytoplasm of pathogenic bacteria and causes severe damage to their cells (Herwin, 2022).

These findings highlight the antimicrobial potential of acetic acid in kombucha and its ability to create an unfavorable environment for pathogenic bacteria. The presence of acetic acid, along with other organic acids formed during kombucha fermentation, contributes to its overall antimicrobial activity and potential health benefits.

The results of the total lactic acid bacteria (LAB) testing indicate that all treatments kedabu fruit kombucha tea contained a sufficient amount of LAB, meeting the minimum requirements for probiotic beverage quality standards, which is at least 10^7 cfu/mL (Nyanzi *et al.,* 2021). The total LAB count increased from the 0th day to the 7th day of fermentation. This increase can be attributed to the availability of an adequate nutrient source, promoting the growth of LAB. However, from the 7th day until the 14th day, there was a decrease in the total LAB count (Table 5). This decline may be attributed to the depletion of nutrients in the kedabu fruit kombucha tea medium.

At the initial stages of fermentation, there is an abundance of nutrients that actively support the division and growth of bacteria. However, as fermentation progresses, the available nutrients decrease, reducing in bacterial activity and reproduction. This decline in LAB count indicates that the bacteria have surpassed their logarithmic growth phase and are entering a phase of decreased reproduction (Villarreal *et al.,* 2018).

These findings highlight the dynamic nature of LAB growth during the fermentation of kedabu fruit kombucha tea. The initial increase in LAB count indicates active fermentation and bacterial proliferation, while the subsequent decline can be attributed to nutrient depletion. It is essential to carefully monitor the fermentation process to ensure optimal LAB growth and maintain the probiotic qualities of kombucha tea.

The minimum requirement for total yeast in kombucha tea is at least 10^4 cfu/mL (Nyhan *et al.,* 2022). The study results indicate that all kedabu juice kombucha tea treatments met this requirement, with yeast counts exceeding 10^4 cfu/mL. The findings demonstrate that the length of fermentation time has an impact on the total yeast count. A decrease in yeast count was observed from the 0th day to the 7th day of fermentation, followed by an increase from the 7th day to the 14th day.

The initial decrease in yeast count during the early stages of fermentation can be attributed to the yeast undergoing a lag phase or an adaptation phase with its environment. During this phase, some enzymes may not have been synthesized yet, and metabolic processes are still slow, resulting in a lack of cell division. As a result, the yeast count may remain stable or even decrease during this phase.

However, as fermentation progresses, the yeast adapts to its environment, and the growth rate increases. The growth rate of yeast is influenced by the availability of substrates and the pH of the kombucha medium, which provides an ideal environment for yeast growth and the conversion of glucose into alcohol and organic acids (De Miranda *et al.,* 2022). In the case of kedabu juice kombucha tea, the substrate for yeast growth is obtained from the kedabu juice.

These findings emphasize the dynamic nature of yeast growth during kombucha fermentation. The initial decrease in yeast count is followed by an increase as the yeast adapts to the fermentation environment and utilizes the available substrates. Monitoring and understanding the yeast population dynamics during fermentation is crucial to ensure the desired quality and characteristics of kombucha tea.

Table 6. The result of antibacterial activity of kedabu juice kombucha tea before fermentation.

Formula	Diameter of Inhibition (mm) → Eschericia coli			Average ± SD
	I	II	III	
K (-)	6	6	6	6 ± 0[a]
K (+)	21	24	22	22,3 ± 1,53[b]
F1	7,1	6,2	7,0	6,8 ± 0,49 [a]
F2	7,2	6,9	7,4	7,2 ± 0,25 [a]
F3	7,5	6,8	8,0	7,4 ± 0,6 [a]
F4	7,8	7,0	8,5	7,8 ± 0,75 [a]
Formula	**Diameter of Inhibition (mm)→ Staphylococcus aureus**			**Average ± SD**
	I	**II**	**III**	
K (-)	6	6	6	6 ± 0[a]
K (+)	23,3	25,1	26	24,8 ± 1,37[b]
F1	7,8	7,1	6,8	7,2 ± 0,51[a]
F2	8,5	7,1	7,4	7,6 ± 0,74 [a]
F3	8,8	7,4	7,5	7,9 ± 0,78 [a]
F4	8,7	7,6	7,5	7,93 ± 0,66 [a]

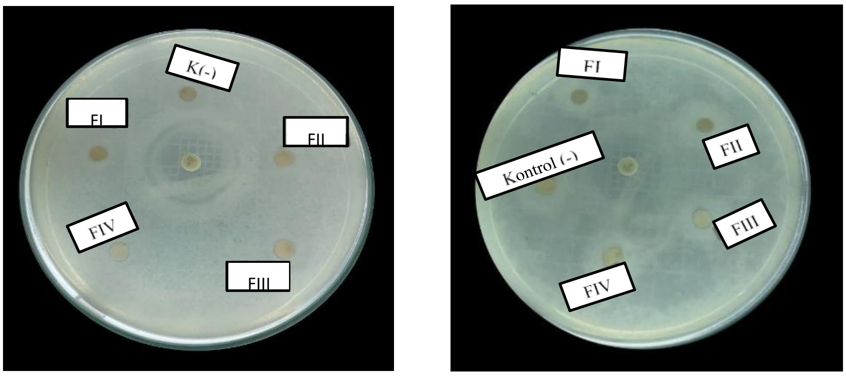


Figure 2. Antibacterial Activity of Kedabu Fruit Kombucha Tea against bacteria *Escherichia coli* and *Staphylococcus aureus* before fermentation (on 0th day).

Table 7. The result of antibacterial activity of kedabu juice kombucha tea after fermentation 7-day.

Formula	Diameter of Inhibition (mm) → Eschericia coli			Average ± SD
	I	II	III	
K (-)	6	6	6	6 ± 0[a]
K (+)	22,1	21,7	21	21,6 ± 0,56[c]
F1	9,2	7,2	7,0	7,8 ± 1,22[ab]
F2	9,5	7,4	7,2	8,03 ± 1,27 [ab]
F3	10	7,6	8,1	8,6 ± 1,26 [ab]
F4	11	7.8	10	10,5 ± 0,7[b]
Formula	**Diameter of Inhibition (mm) → Staphylococcus aureus**			**Average ± SD**
	I	**II**	**III**	
K (-)	6	6	6	6 ± 0[a]
K (+)	21,6	22,1	23,5	22,4 ± 0,98[c]
F1	8,7	9,1	8,0	8,6 ± 0,56 [b]
F2	9	9,4	7,9	8,8 ± 0,78 [b]
F3	9,1	9,6	9	9,2 ± 0,32 [b]
F4	9,6	11	10,2	10,3 ± 0,7 [b]

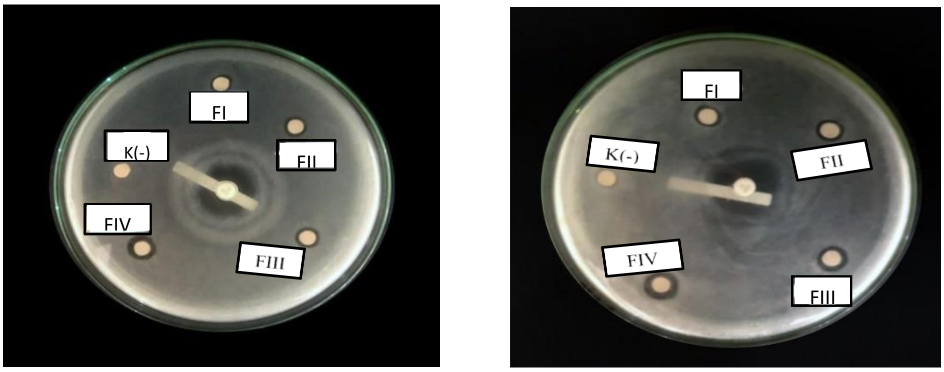


Figure 3. Antibacterial Activity of Kedabu Fruit Kombucha Tea against *Escherichia coli* and *Staphylococcus aureus* on 7-day.

Table 8. The result of antibacterial activity of kedabu juice kombucha tea after fermentation day-14.

Formula	Diameter of Inhibition (mm) → Eschericia coli			Average ± SD
	I	II	III	
K (-)	6	6	6	6 ± 0^{a}
K (+)	21,5	22,3	24	$22{,}6 \pm 1{,}27^{d}$
F1	7,8	8,3	8,4	$8{,}2 \pm 0{,}32^{ab}$
F2	8,2	9,9	8,9	$9 \pm 0{,}85^{b}$
F3	10,5	9	10,4	$9{,}96 \pm 0{,}84^{bc}$
F4	11	12	12,5	$11{,}8 \pm 0{,}7^{c}$
Formula	**Diameter of Inhibition (mm) → Staphylococcus aureus**			**Average ± SD**
	I	**II**	**III**	
K (-)	6	6	6	6 ± 0^{a}
K (+)	22,4	23,4	23	$22{,}93 \pm 0{,}5^{e}$
F1	8,7	8,3	7,9	$8{,}3 \pm 0{,}4^{b}$
F2	11,3	10,2	9	$10{,}2 \pm 1{,}15^{bc}$
F3	12,4	10,5	9,9	$10{,}93 \pm 1{,}3^{c}$
F4	15,5	15,2	14,4	$15 \pm 0{,}56^{d}$

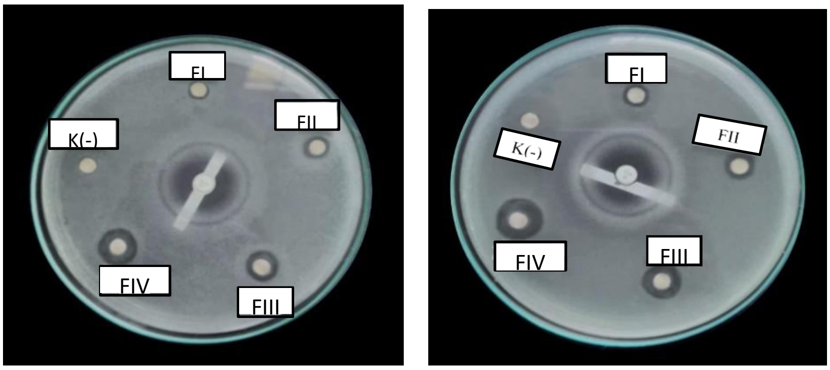


Figure 4. Antibacterial Activity of Kedabu Fruit Kombucha Tea against *Escherichia coli* and *Staphylococcus aureus* on 14th day.

The antibacterial activity test was conducted to evaluate the effectiveness of kombucha tea with and without the addition of kedabu fruit juice against *Escherichia coli* and *Staphylococcus aureus* bacteria. Four different formulations of kedabu fruit juice kombucha tea (designated as F1, F2, F3, and F4) were tested, and their inhibitory effects were categorized as weak to moderate.

Statistical analysis using the One Way ANOVA test revealed a significant difference in the diameter of the inhibition zones among the various kombucha tea formulas containing kedabu fruit juice. Further analysis using the Tukey test indicated that the formula labeled F4 exhibited a significant difference in inhibiting *Escherichia coli* and *Staphylococcus aureus* bacteria. These results suggest that increasing the concentration of kedabu fruit juice in kombucha tea leads to a greater diameter of inhibition zone formation.

The observed antibacterial activity can be attributed to bioactive compounds in kombucha tea, such as organic acids, phenolic compounds, and other secondary metabolites. The concentration of these bioactive compounds is known to increase during the fermentation process, especially with prolonged fermentation time. The elevated levels of organic acids, including lactic acid and acetic acid, contribute to the stronger antibacterial activity of the kombucha tea. This finding is consistent with previous research demonstrating that organic acid content increases with prolonged fermentation time (Jayabalan *et al.,* 2007).

It is important to note that the antibacterial activity of kombucha tea is influenced by various factors, including the type and concentration of bioactive compounds, fermentation conditions, and the targeted bacteria. Further studies are needed to elucidate the specific mechanisms underlying the antibacterial effects of kombucha tea and to identify the key bioactive components responsible for its activity against *Escherichia coli* and *Staphylococcus aureus.*

The inhibitory effects of kedabu fruit kombucha tea against *Escherichia coli* and *Staphylococcus aureus* bacteria can be attributed to the presence of lactic acid and acetic acid, which are produced by lactic acid bacteria (LAB) and acetic acid bacteria (AAB). These organic acids have been recognized as significant antibacterial agents in kombucha tea.

The antibacterial activity of organic acids is closely associated with the pH of the medium. As the pH decreases, the antibacterial activity of the organic acids increases. Lactic acid and acetic acid have the ability to disrupt the metabolic processes of pathogenic bacteria by damaging enzyme activity, as well as the structure and function of cell walls and membranes. This disruption inhibits nutrient absorption and protein synthesis in the bacteria. Furthermore, LAB-derived bacteriocin compounds can also contribute to the antibacterial activity by disrupting the membranes of pathogenic bacteria. These compounds damage essential components such as potassium ions and ATP, leading to intracellular pH imbalance and ultimately cell failure.

The findings mentioned above are supported previous research who investigated the antibacterial mechanisms of organic acids and bacteriocins produced by LAB. Their study demonstrated the damaging effects of these compounds on the cell membranes and intracellular components of bacteria, leading to the inhibition of growth and survival (Hou *et al.,* 2021).

It is important to note that the antibacterial activity of kedabu fruit kombucha tea is a complex interplay of various factors, including the specific composition of organic acids, the presence of other bioactive compounds, and the targeted bacteria strains. Further research is needed to explore the detailed mechanisms of action and the specific interactions between the organic acids and pathogenic bacteria.

The kedabu fruit kombucha tea has been found to contain various secondary metabolites as indicated by the results of phytochemical screening, including flavonoids, phenolics, terpenoids, and saponins (Table 3). Phenolic compounds identified in kombucha tea include epicatechin gallate, epigallocatechin, catechin, epicatechin, and epigallocatechin gallate. These compounds have been associated with pharmacological activities (Kaewkod *et al.,* 2019).

Flavonoid compounds present in kedabu fruit kombucha tea have been shown to exhibit inhibitory effects on nucleic acid synthesis during DNA and RNA formation. Additionally, flavonoids have the potential to disrupt bacterial cell wall permeability, microsomes, and lysosomes through interactions with bacterial DNA (Cushnie *et al.,* 2005).

The findings mentioned above highlight the presence of various bioactive compounds in kedabu fruit kombucha tea, particularly phenolic and flavonoid compounds. These compounds have been extensively studied for their potential therapeutic properties and antimicrobial activities. Further investigations are necessary to explore the specific mechanisms by which these secondary metabolites interact with bacterial DNA and exert their pharmacological effects.

The inhibitory effect of phenolic compounds on bacterial cell walls is achieved by interfering with forming the cell wall peptidoglycan layer, disrupting it (González *et al.,* 2009). Terpenoids, on the other hand, act as antibacterial agents by damaging transmembrane proteins, which serve as pathways for the entry and exit of compounds, thereby reducing the permeability of bacterial cell walls. Consequently, the lack of nutrients in the bacterial cells leads to their death (Cowan, 1999).

Saponins exert their antibacterial activity through a mechanism involving the diffusion of saponins through the outer membrane and cell wall. Once inside the bacterial cell, saponins bind to the cytoplasmic membrane, destabilizing it and causing the release of cytoplasmic contents. Ultimately, this disruption leads to cell death (Pandey *et al.,* 2011).

The mechanisms described above illustrate how phenolic compounds, terpenoids, and saponins exhibit antibacterial effects by targeting different components of bacterial cells. These mechanisms contribute to the understanding of the potential antimicrobial activities of these secondary metabolites found in kedabu fruit kombucha tea.

CONCLUSIONS

Kombucha tea supplemented with kedabu fruit juice complies with the quality standards of fermented beverages and exhibits antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*. The Kombucha tea made from kedabu fruit juice demonstrates favorable results in various quality parameters, including organoleptic evaluation, pH value, total titratable acidity, and the counts of lactic acid bacteria (LAB) and yeast, all of which meet the established standards. Additionally, the kombucha tea exhibits inhibitory effects against *Escherichia coli* and *Staphylococcus aureus* bacteria, falling within the weak to moderate categories. Statistical analysis using One Way ANOVA reveals a significant variation in the diameter of the inhibition zone among the different formulas of kedabu fruit kombucha tea. Further analysis using the Tukey test demonstrates that formula 4 significantly differs in its inhibitory effects on *Escherichia coli* and *Staphylococcus aureus* bacteria. These findings suggest that the addition of kedabu fruit juice to kombucha tea enhances its overall quality attributes and confers antibacterial properties against *Escherichia coli* and *Staphylococcus aureus.*

Acknowledgements: We thank the management of Riau College of Pharmaceutical Sciences for the enabling environment for the research for the use of their facilities for laboratory work.

Competing Interests: The authors declare no conflict of interest.

Funding: This study did not receive any funding

REFERENCES

Al-Mohammadi AR, Ismaiel AA, Ibrahim RA, Moustafa AH, Abou Zeid A, Enan G. (2021). Chemical constitution and antimicrobial activity of kombucha fermented beverage. Molecules. 26(16):5026.

Aminov RI. (2010). A brief history of the antibiotic era: lessons learned and challenges for the future. Front Microbiol. 1:134.

Bhattacharya D, Bhattacharya S, Patra MM, Chakravorty S, Sarkar S, Chakraborty W, et al. (2016). Antibacterial activity of polyphenolic fraction of kombucha against enteric bacterial pathogens. Curr Microbiol. 73:885–96.

Chan M, Sy H, Finley J, Robertson J, Brown PN. Determination of ethanol content in kombucha using headspace gas chromatography with mass spectrometry detection: Single-laboratory validation. J AOAC Int. 2021;104(1):122–8.

Chakravorty S, Bhattacharya S, Hatzinotas A, Chakraborty W, Bhattacharya D, Gachhui R. (2016). Kombucha tea fermentation: Microbial and biochemical dynamics. Int J Food Microbiol. 220:63–72.

Chandrasekara A, Shahidi F. (2018). Herbal beverages: Bioactive compounds and their role in disease risk reduction-A review. J Tradit Complement Med. 8(4):451–8.

Cowan MM. (1999). Plant products as antimicrobial agents. Clin Microbiol Rev. 12(4):564–82.

Cushnie TT, Lamb AJ. (2005). Antimicrobial activity of flavonoids. Int J Antimicrob Agents. 26(5):343–56.

De Miranda JF, Ruiz LF, Silva CB, Uekane TM, Silva KA, Gonzalez, A. G. M., et al. (2022). Kombucha: A review of substrates, regulations, composition, and biological properties. J Food Sci. 87(2):503–27.

FMUIN. (2018). 10 T. Tentang Produk Makanan Dan Minuman Yang Mengandung Alkohol/Etanol, https://halalmui.org/.

González-Lamothe R, Mitchell G, Gattuso M, Diarra MS, Malouin F, Bouarab K. (2009). Plant antimicrobial agents and their effects on plant and human pathogens. Int J Mol Sci. 10(8):3400–19.

Greenwalt CJ, Steinkraus KH, Ledford RA. (2000). Kombucha, the fermented tea: microbiology, composition, and claimed health effects. J Food Prot. 63(7):976–81.

Halifah P, Hartati H, Rachmawaty R, Yusminah H, Roshanida AR. (2019). Phytochemical screening and antimicrobial activity from Sonneratia caseolaris fruit extract. In Materials science forum. Mater Sci forum Trans Tech Publ. 967:28–33.

Hamed SF, Wagdy SM, Megahed MG. (2012). Chemical characteristics and antioxidant capacity of Egyptian and Chinese sunflower seeds: A case study. Life Sci J. 9(2):320–8.

Herwin H. (2022). Production of kombucha from Muntingia calabura L. leaves and evaluation of its antibacterial activity and total flavonoid content. J Appl Pharm Sci.12(8):187–92.

Hou J, Luo R, Ni H, Li K, Mgomi FC, Fan L, et al. (2021). Antimicrobial Potential of Kombucha Against Foodborne Pathogens: A Review. Qual Assur Saf Crop Foods.13(3):53–61.

Jayabalan R, Malbaša R V., Lončar ES, Vitas JS, Sathishkumar M. (2014). A review on kombucha tea—microbiology, composition, fermentation, beneficial effects, toxicity, and tea fungus. Compr Rev food Sci food Saf. 13(4):538–50.

Jayabalan R, Marimuthu S, Swaminathan K. (2007). Changes in content of organic acids and tea polyphenols during kombucha tea fermentation. Food Chem. 102(1):392–8.

Kaewkod T, Bovonsombut S, Tragoolpua Y. (2019). Efficacy of kombucha obtained from green, oolong, and black teas on inhibition of pathogenic bacteria, antioxidation, and toxicity on colorectal cancer cell line. Microorganisms. 7(12):700.

Kathiresan K, Bingham BL. (2001). Biology of mangroves and mangrove ecosystems. Adv Mar Biol. 40:81–251.

Laureys D, Britton SJ, De Clippeleer J. (2020). Kombucha tea fermentation: A review. J Am Soc Brew Chem. 78(3):165–74.

Miranda B, Kawtib NM, Tachibana SR, Swartz NA, Hall WP. (2016). Titration and HPLC Characterization of Kombucha Fermentation: A Laboratory Experiment in Food Analysis. J Chem Educ Publ.

Nurikasari M, Puspitasari Y, Siwi RPY. (2017). Characterization and analysis kombucha tea antioxidant activity based on long fermentation as a beverage functional. J Glob Res Public Heal. 2(2):90–6.

Murphy TE, Walia K, Farber JM. (2018). Safety aspects and guidance for consumerson the safe preparation, handling and storage of kombucha—a fermented tea beverage. Food Prot Trends. 38:329–37.

Nguyen NK, Dong NTN, Nguyen HT, Le PH. (2015). Lactic acid bacteria: promising supplements for enhancing the biological activities of kombucha. Springerplus. 4:1–6.

Nyanzi R, Jooste PJ, Buys EM. (2021). Invited review: Probiotic yogurt quality criteria, regulatory framework, clinical evidence, and analytical aspects. J Dairy Sci. 104(1):1–19.

Nyhan LM, Lynch KM, Sahin AW, Arendt EK. Advances in kombucha tea fermentation: A review. Appl Microbiol. 2022;2(1):73–103.

Pauzi N, Man S, Nawawi MSAM, Abu-Hussin MF. (2019). Ethanol standard in halal dietary product among Southeast Asian halal governing bodies. Trends Food Sci Technol. 86:375–80.

Pandey A, Kaushik A, Tiwari SK. (2011). Evaluation of antimicrobial activity and phytochemical analysis of Citrus limon. J Pharm Biomed Sci. 13(13).

Villarreal-Soto SA, Beaufort S, Bouajila J, Souchard JP, Taillandier P. (2018). Understanding kombucha tea fermentation: a review. J Food Sci. 83(3):580–8.

Wu SB, Wen Y, Li XW, Zhao Y, Zhao Z, Hu JF. (2009). Chemical constituents from the fruits of Sonneratia caseolaris and Sonneratia ovata (Sonneratiaceae). Biochem Syst Ecol. 37(1):1–5.

Wang B, Rutherfurd-Markwick K, Zhang XX, Mutukumira AN. Kombucha: Production and Microbiological Research. Foods. 11(21):3456.