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Chemical Study and Evaluation of Antibacterial and Antioxidant Activities of Kombucha Variation Gel with Strawberry (*Fragaria vesca*) Extract and Ashoka Flower (*Ixora coccinea* L.) Extract

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Abstract

Kombucha is a fermented drink made from tea, sugar, yeast, and acetic acid bacteria known as SCOBY (Symbiotic Culture of Bacteria and Yeast). Ashoka flowers (*Ixora coccinea* L.) are tropical ornamental plants rich in polyphenols that have antioxidant activity. Strawberries (*Fragaria vesca*) contain flavonoids that can inhibit bacterial growth by disrupting DNA processes, damaging cell membranes, and denaturing proteins in bacteria. This research was making gel preparations that actively inhibit bacterial growth. Examples of pathogenic bacteria include *S. aureus* and *S. epidermidis* which generally attack the skin, and its antioxidant activity. The research was making kombucha tea with a combination of strawberries and Ashoka flowers, making gel preparations, testing activity against *S. aureus*, *S. epidermidis*, and *Methilen Resistant Staphylococcus aureus* (MRSA) bacteria, and testing antioxidant activity using the FRAP method. Gel with Fermented Tea (Kombucha) Combination of Strawberries and Ashoka Flowers has antibacterial activity against *S. aureus* bacteria in the strong category in Formula F3 (MIC 10.9 mm), against *S. epidermidis* bacteria in the moderate category in Formula F3 (MIC 9.06 mm), and against MRSA bacteria in the moderate category in Formula F3 (MIC 9.51 mm). Formulation F3 has antioxidant activity in the very strong category (47.32 mgAAE/g). Kombucha contains Phenol and Acid content that can damage bacterial cell walls. The addition of Strawberries and Ashoka flowers containing phenolic and flavonoid compounds increases the antibacterial and antioxidant activity of the gel.

Keywords: Kombucha; Strawberry; Ashoka Flower; Gel; Antibacterial; Antioxidant.

Abbreviations: Symbiotic Culture of Bacteria and Yeast (SCOBY), Methilen Resistant Staphylococcus aureus (MRSA), Minimun Inhibitor Concentration (MIC), mg Ascorbic Acid Equivalent/g (mgAAE/g).

INTRODUCTION

Gel is a liquid preparation that is inserted into a semi-solid textured structure made of a series of organic particles or organic molecules. Gel is a carrier used for the purpose of administering drugs to the mucosal parts, such as the eyes, nose, vagina, and administration through the rectum. The composition of gel preparations generally consists of components of ingredients that can expand in the presence of water, humectants (skin moisturizers), and preservatives to make them susceptible to microbial contamination, sometimes ingredients are needed that can increase the penetration of efficacious ingredients (enhancers) (Wardiyah, 2015) .

The advantages of gel preparations are that they are cool on the skin when used, can produce good skin spread, good drug release, have a clear and elegant appearance, when applied they will leave a transparent, elastic, easy to wash and stable film in storage (Putri & Anindhita, 2022).

Kombucha is a fermented drink with various benefits for the body that made from tea leaves (*Camelia sinensis*) one of popular leaves from Indonesia and sugar involving the Symbiosis of Yeast and Acetic Acid Bacteria (SCOBY). In the SCOBY colony there are several bacteria including: *Acetobacter xylinum, Xylinoides gluconicum, Acetobacter ketogenium, Pichia fermentans, Torulavarietas, Lactobacillus sp, Pediococcus sp,* these bacteria produce acetic acid. The yeast contained in the Kombucha starter includes: *Saccharomyces ludwigii, S. apiculatus* and *Schizosaccharomyces pombe*. Kombucha has long been known in various European countries and Japan. Many people suspect that kombucha was first consumed by people in mainland China who have known this

fermented tea since 3000 years ago. Kombucha is believed to have health effects to help digestion, prevent microbial infections, reduce pain due to arthritis, and improve the immune system (Ita et al., 2018).

Kombucha contains various types of organic acids such as glucuronic acid, gluconic acid, acetic acid, folic acid, amino acids, vitamins, and other substances (Wistiana & Elok, 2015). The composition and concentration of secondary metabolites in kombucha depend on the source of the inoculum, the concentration of sugar and tea, the fermentation time, and the temperature used (Khanifah, 2022).

Kombucha is reported to have antimicrobial activity against gram-positive and gram-negative pathogenic bacteria. The efficacy of kombucha as an antibacterial, anticancer, and antioxidant opens up opportunities for development as an active cosmetic ingredient (Rezaldi, 2021). Several studies have shown that kombucha has antimicrobial activity against *Helicobacter pylori, Salmonella typhimurium, Staphylococcus aureus , Agrobacterium tumefaciens, Bacillus cereus, Shigella sonnei, Salmonella enteritidis*, and *Escherichia coli* (Khanifah, 2022).

Strawberries contain vitamin C and ellagic acid as antioxidants, along with vitamins B1, B2, and provitamin A (Widyastuti et al., 2016). The aroma of strawberry kombucha is more pungent. This is because strawberries contain a higher acid content compared to other fruit juices. Increasing the acidic compounds or components in kombucha will make the sour aroma more pronounced. The color of kombucha tea can be influenced by the dyes present in the fruit, and the length of fermentation also affects the color change process in kombucha tea. Longer fermentation makes the color of kombucha tea more intense.

The flavonoid content in strawberries can inhibit bacterial growth due to disruption of DNA processes, damage to cell membranes, and protein denaturation in bacteria (Parseh et al., 2012). In a study conducted by Selvia et al., (2014) in vitro using the solid dilution method, it was proven that strawberry ethanol extract was able to inhibit the growth of Staphylococcus epidermidis bacteria with a minimal bactericidal concentration (MBC) at a concentration of 1.5%. This is due to the activity of phenolic compounds, such as ellagitannin, proanthocyanidin, and anthocyanidin which work synergistically as antimicrobials (Selvia et al., 2014).

Ashoka flower (*Ixora coccinea* L.) is one of the species of the genus Ixora that grows in Indonesia. Ashoka flowers and leaves have the potential as antioxidants because they contain phenolic compounds. Many plants can be used for the welfare of society, for example *Ixora coccinea* L. is used in traditional medicine for various diseases such as gynecological problems, uterine fibroids, burns, and defecation (Tutik et al., 2021)

Ashoka is one of the plants that contains flavonoids, tannins also contain saponins. One of the flavonoid groups in the Ashoka flower is anthocyanin which gives color to plants. Anthocyanin is a compound that is good for health because it has antioxidant activity (Prasetyo et al., 2021).

Antibacterial is a substance that interferes with the growth or even kills bacteria by interfering with microbial metabolism. The antibacterial effect of Kombucha is obtained through the acid produced during fermentation and other antibacterial substances. The high amount of acid in kombucha can inhibit bacterial growth, and the increase in the number of protons in high concentrations can denature proteins and enzymes in the bacterial cell walls. In addition to the formation of acid, phenol compounds formed during fermentation can also provide antibacterial effects in kombucha (Wistiana & Elok, 2015). In addition to being made from tea leaves (*Camelia sinensis*), kombucha can also be made from fruits, one of which is strawberries. Strawberries contain vitamin C and ellagic acid as antioxidants, vitamins B1, B2, and provitamin A (Widyastuti et al., 2016). The aroma produced in the strawberry variation of kombucha is more pungent. This is because the acid component contained in strawberries is higher compared to other fruit juices. The flavonoid content in strawberries can inhibit bacterial growth due to disruption of the DNA process, damage to cell membranes and protein denaturation in bacteria (Parseh et al., 2012). In a study conducted by Selvia et al., in vitro with the solid dilution method, it can be proven that strawberry ethanol extract can inhibit the growth of *Staphylococcus epidermidis* bacteria with a minimum kill rate (MBC) at a concentration of 1.5%. This is due to the activity of phenolic compounds, such as ellagitannin, proanthocyanidin, and anthocyanidin which work synergistically as antimicrobials (Selvia et al., 2014).

The presence of antibacterials as substances that interfere with the growth or kill bacteria by disrupting the metabolism of harmful microbes. The presence of microorganisms is very dangerous because it can infect and cause disease (Retnowati, 2011). Bacteria that can cause disease in humans, for example, *Staphylococcus aureus* and *Staphylococcus epidermidis*.

Staphylococcus aureus is a gram positive bacteria that is classified as a pathogenic bacteria. Some infectious diseases caused by *Staphylococcus aureus* are boils, acne, impetigo, and wound infections. More severe infections include pneumonia, mastitis, phlebitis, meningitis, urinary tract infections, osteomyelitis, and endocarditis. *Staphylococcus epidermidis* is one of the normal flora bacteria found on the skin, respiratory tract, and digestive tract in humans. *Staphylococcus epidermidis* can generally cause swelling diseases (abscesses) such as acne, skin infections, urinary tract infections, and kidney infections (Retnowati, 2011).

MRSA is a *Staphylococcus aureus* bacterium that is resistant to several antibiotics, including methicillin. Irrational antibiotic use can cause changes in the *Staphylococcus aureus* gene, thus affecting bacterial resistance to methicillin. MRSA bacteria are resistant not only to methicillin but also to various antibiotics such as all beta-lactams and macrolides (erythromycin). The prevalence of MRSA has increased annually, resulting in increased morbidity, more difficult antibiotic management, increased hospital costs due to longer hospital stays, and an increased risk of death. Nosocomial infections due to MRSA can occur through the installation of medical devices or through transmission from healthcare workers who have been colonized by MRSA (MRSA carriers) to patients. The prevalence of MRSA has currently reached 70% in Asia and 28% in Indonesia (Oktavian et al., 2020).

The study was conducted by making fermented kombucha tea with a combination of Strawberries and Ashoka Flowers, making a gel preparation, testing activity against human patogenic skin bacteria *S. aureus, S. epidermidi*s, MRSA, and its testing antioxidant activity. The results obtained are a gel preparation that has antibacterial and antioxidant activity.

MATERIALS AND METHODS

Materials

The materials used in this study were Mueller Hinton Agar (MHA) media, Dimethyl sulfoxide (DMSO) (Merck®), Clindamycin, 70% alcohol, physiological NaCl solution, powdered tea, granulated sugar, SCOBY, kombucha starter, strawberries, Ashoka flowers obtained from Limau Manis Village, Kampar Regency, Riau, distilled water, methyl paraben, TEA, propylene glycol, glycerin, Carbopol 940, nutrient agar, NaCl, *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228, MRSA ATCC 33591, ascorbic acid, 10% trichloroacetic acid, 0.1% $FeCl_3$, phosphate buffer (0.2 M, pH 6.6), 1% potassium ferricyanide.

Tools

Glass jars, aluminum foil, petri dishes, test tubes, beakers, sterile blank disc paper, measuring pipettes, measuring cylinders, Erlenmeyer flasks, electric stoves, autoclaves, incubators, laminar air flow (LAF), UV-Vis spectrophotometers, rotary evaporators, pH meters, centrifuges, vernier calipers.

Method

Kombucha Preparations

The first step in making kombucha is to heat 1 liter of water to a temperature of 100°C. After the water boils, turn off the heat, then add 15 grams of powdered tea and stir for about 5 minutes until the tea color comes out. Add 125 grams of sugar and stir until the sugar dissolves in the water for about 3-5 minutes. The tea solution is cooled until completely cold and at room temperature (25°C). The cooled tea solution is put into a jar and strain the tea dregs so that it is not dirty during fermentation until the harvest stage. SCOBY and kombucha starter are poured into 100 ml of tea solution in the same jar. Cover the lip of the jar using a cloth and seal it with a rubber band until it is neatly closed. Store the jar for the fermentation process in a safe, clean, dry place, and not exposed to direct sunlight. The first fermentation takes 7 days. Add blended strawberries and Ashoka flower infusion (from 200 grams of Ashoka Flowers) in the second fermentation and wait for 8-14 days.

Preparation of Gel Preparation Formulation

Table 1. Formulation of fermented kombucha tea gel combined with Strawberries and Ashoka Flowers.

Materials	Formula (%)		
	F1	F2	F3
Kombucha tea combined with Strawberries and Ashoka Flowers	6,25	12,5	25
Carbopol 940	2	2	2
Glycerin	5	5	5
Propylene Glycol	10	10	10
Triethanolamine	1	1	1
Methyl Paraben	0,1	0,1	0,1
Aquadest	ad 100 mL	ad 100 mL	ad 100 mL

Carbopol is developed with hot distilled water in a mortar (W1). Methyl paraben is dissolved in glycerin, stirred until dissolved (W2). Fermented kombucha tea combined with strawberry and ethanol extract of ashoka flowers is ground by adding some propylene glycol until the texture becomes soft and homogeneous (W3). After the carbopol expands (W1), grind it first by adding TEA little by little to form a gel base (W4). A mixture of glycerin and methyl paraben (W2) is added to the gel base (W4) while grinding until homogeneous (W5). The remaining propylene glycol is added to the base mixture, grind until homogeneous. Mix fermented kombucha tea

combined with strawberries (W3) into the gel base (W5) and grind until homogeneous. Add the remaining aquadest little by little. The gel that has been formed is evaluated as a gel preparation (Tutik et al., 2021).

Gel Evaluation Test

In the gel evaluation, tests were carried out including organoleptic tests, pH tests, homogeneity tests, hedonic tests, antibacterial tests, and antioxidant tests.

Antibacterial activity test against S. *aureus, S. epidermidis*, and MRSA

All tools for testing purposes are sterilized before use by autoclaving. The sterilized MHA media is poured into each petri dish as much as 25 ml and left for a while until it solidifies. On the solid media, the bacterial suspension is spread by sweeping which has previously been adjusted to the Mc Farland standard using a sterile spreading rod until the bacterial suspension is evenly distributed over the entire surface of the 25 ml MHA media. Determination of the antibacterial activity of *Staphylococcus aureus*, *Staphylococcus epidermidis*, MRSA is carried out using the agar diffusion method by means of a well, namely by: The ose needle is dipped into a test tube containing a suspension of *Staphylococcus aureus*, *Staphylococcus epidermidis,* MRSA and smeared on the surface of the media in a zigzag pattern, using an ose needle until all the media is evenly smeared. Antibacterial test using the disc diffusion method by means of a sterile cotton swab dipped into a test tube containing the test suspension, then squeezing the cotton swab from the walls of the test tube while rotating it. After that, the test suspension was applied to the surface of the media in a zigzag manner until all media were evenly applied. Then the gentamicin disc paper was taken and placed on the surface of the media used as a positive control (+) and the gel base with a negative control (-). Then the blank disc paper was soaked with kombucha and each gel with a concentration of 6.25%, 12.5%, 25% for 30 minutes. Place the disk and each concentration on the surface of the media. Repetition was carried out 3 times. Furthermore, the cup was incubated in an incubator at a temperature of 37 ℃ for 24-48 hours and the diameter of the inhibition zone was measured using a caliper. Repetition was carried out 3 times. The inhibition zone was measured and recorded and bioactivity was assessed using a very strong (> 15 mm), strong (10-15 mm), moderate (5-10 mm), inactive (< 5 mm) scoring system (Faroliu et al., 2023).

Determination of Total Antioxidants

a. *0.2 M phosphate buffer solution pH 6.6*
   The solution is prepared by weighing 2 grams of NaOH and dissolving it with Aquadest until exactly 250 mL in a measuring flask, then 6.8 grams of KH_2PO_4 are dissolved with 250 mL Aquadest in a measuring flask. Then pipette 16.4 mL of NaOH into a measuring flask and mix 50 mL of KH_2PO_4in Aquadest and dilute it in a 200 mL measuring flask.
b. *$K_3Fe(CN)_6$ (Potassium Ferricyanide) solution. The solution is prepared by dissolving 1 gram of potassium ferricyanide in Aquadest and diluting it in a 100 mL measuring flask.*
c. *0.1% $FeCl_3$ solution*
   The solution is prepared by dissolving 0.1 grams of $FeCl_3$ in Aquadest and diluting it in a 100 mL measuring flask.
d. *Preparation of Ascorbic Acid Standard Solution*
   1000 ppm stock solution is made by dissolving 25 mg of Ascorbic Acid dissolved in 96% ethanol to the limit of a 25 mL volumetric flask. Next, from the 1000 ppm stock solution, 0.7, 0.9, 1.0 and 1.3 mL were taken in different 25 mL measuring flasks and diluted with 96% ethanol to 25 mL and homogenized, pipetted 1 mL, added 1 mL of 0.2 M phosphate buffer (pH 6.6) and 1 mL ($K_3Fe(CN)_6$ 1% after that incubated for 20 minutes at a temperature of 50℃. 1 mL of distilled water and 0.5 mL of 0.1% $FeCl_3$ were added after that incubated and the absorbance was measured at a maximum wavelength of 692 nm on a UV-Vis spectrophotometer. The concentration of the 1000 ppm ascorbic acid standard solution was 70, 90, 110, and 130 ppm.
e. *Antioxidant Activity Test by FRAP Method*
   Total of 10 mg of ethanol extract and 10 mg of ethyl acetate fraction were each dissolved in 10 mL of ethanol, then pipetted 1 mL, added 1 mL of 0.2 M phosphate buffer (pH 6.6) and 1 mL of 1% $K_3Fe(CN)_6$ after that, incubated for 20 minutes at a temperature of 50°C. After incubation, 1 mL of trichloroacetic acid was added and then centrifuged at 3000 rpm for 10 minutes. After centrifugation, 1 mL of the upper layer was pipetted into a test tube, and 1 mL of distilled water and 0.5 mL of 0.1% $FeCl_3$ were added after that incubated and the absorbance was measured at a maximum wavelength of 692 nm.

RESULTS AND DISCUSSION

Gel preparation formulation contains fermented tea (kombucha) combined with strawberries with different concentrations of 6.25%, 12.5% and 25%. The ingredients used in making the gel preparation are carbopol 940 which functions as a gelling agent which is the main ingredient in making a gel that has good stability and with a low concentration can provide high viscosity (Rahmatullah et al., 2021), glycerin can retain moisture and increase skin moisture (Rowe et al., 2020), propylene glycol as a humectant functions to maintain moisture in the gel preparation because propylene glycol has hygroscopic properties. Propylene glycol is a humectant that can prevent microbial growth so it can also be used as a preservative (Rowe et al., 2020), triethanolamine functions to produce a homogeneous and

stable emulsion, methyl paraben functions as a preservative to prevent pathogen growth and to stop unwanted chemical changes.

Table 2. Results of Organoleptic Test of Gel Preparation.

Formula	Characteristics		
	Colour	Smell	Forms
F1	pale yellow	no smell	Gel
F2	light orange	no smell	Gel
F3	orange	strawberry smell	Gel

Based on the results of organoleptic test examination on kombucha combination of strawberry and ashoka flower, it was found that F1 was pale yellow, F2 was light orange, F3 was orange. This color difference is influenced by the concentration of fermented tea (kombucha) combination of strawberry and asoka flower which varies. The higher the concentration of fermented tea (kombucha) combination of strawberry as an active substance, the more concentrated the color of the preparation. The smell in F1, F2 is odorless while in F3 there is a strawberry smell from fermented tea (kombucha) strawberry the form of the 3 formulas is gel-like.

Table 3. Results of pH Test of Gel.

Formula	pH
F1	5,2
F2	5,3
F3	5,7

The pH test was carried out using a pH meter, aiming to ensure the stability of the acidity level of the fermented tea gel preparation (kombucha) combination of strawberries and ahsoka flowers. Topical preparations with a pH that is too acidic can irritate the skin while a pH that is too alkaline can make the skin dry and scaly. The normal skin pH range is between 4.5-6.5. The results of the pH test observations of the fermented tea gel preparation (kombucha) obtained a pH range of 5.2-5.7. This shows that the strawberry fermented tea gel preparation has met the requirements.

Table 4. Results of Homogeneity Test of Gel Preparations.

Formula	Homogeneity
F1	Homogen
F2	Homogen
F3	Homogen

Homogeneity test was conducted to determine the homogeneity of fermented tea gel (kombucha) combined with strawberries by looking at the uniformity of the particles in the preparation. The three formulas of fermented tea gel (kombucha) combined with strawberries have a homogeneous composition marked by no part that is not mixed well. Thus, all gel preparations have good homogeneity when the gel is applied using a glass object and the presence or absence of coarse grains in the gel is observed. This shows that the strawberry fermented tea gel (kombucha) preparation has met the requirements.

Table 5. Hedonic Test Results of Gel.

Formula	Characteristics			
	Aroma	Texture	Comfort	Irritation
F1	Less liked	Approve	Approve	No Irritation
F2	Approve	Approve	Approve	No Irritation
F3	Approve	Approve	Approve	No Irritation

The hedonic test was conducted to determine the panelists' personal responses regarding their liking or disliking of the strawberry fermented tea gel (kombucha) preparation. Panelists were asked to fill out a questionnaire to assess the level of liking for the color, aroma, texture, comfort and irritation of the preparation when used. Based on the results of the hedonic test, each formula obtained by 10 panelists was that the aroma F1 was less liked, and F2, F3 the level of liking was liked, the texture in F1, F2, F3 the level of liking was liked, the comfort in F1, F2, F3 the level of liking was liked, the irritation in F1, F2, F3 the level of irritation was not irritated.

Table 6. Antibacterial Activity of Fermented Tea Gel Preparation (Kombucha) Combination of Strawberry and Ashoka Flower Against *Staphylococcus aureus* Bacteria.

Formula	Inhibition Zone (mm)				Category
	T1	T2	T3	Mean	
F1	7,8	7	6,3	7,03±0,61	Moderate
F2	9	11,8	9,9	10,2±1,16	Strong
F3	10,4	12,2	10,3	10,9±0,87	Strong
Kombucha	21,4	20,3	23	21,5±1,1	Very Strong
K (+)	25,3	25	26,6	25,6±0,69	Very Strong
K (-)	0	0	0	0	-

Note: K (+) : Clindamycin gel; K (-) : F0, gel base without kombucha

Antibacterial activity test using the disc method was carried out by soaking a blank disc in the gel preparation on F1, F2, F3, and fermented tea (kombucha) strawberries and ashoka flowers as a comparison at each concentration for 30 minutes on agar media that had been smeared with *Staphylococcus aureus* and *Staphylococcus epidermidis* bacteria and incubated for 24 hours. The average diameter of the inhibition zone on *Staphylococcus aureus* bacteria F1, F2, F3, fermented tea (kombucha) strawberries and ashoka flowers, the diameter of the inhibition zone F1 is 7.03 mm in the moderate category, F2 is 10.2 mm in the strong category, and F3 is 10.9 mm in the strong category, kombucha is 21.5 mm in the very strong category, and positive control is 25.6 mm in the very strong category. The higher the concentration given, the greater the antibacterial activity of inhibiting bacterial growth on *Staphylococcus aureus*.

These results align with research by Battikh et al. (2011), which demonstrated the antibacterial activity of fermented kombucha against gram-positive and gram-negative bacteria. The inhibition zone diameter of green tea was between 12 and 22 mm, while that of black tea was 10.5 and 19 mm. For *S. aureus*, the white tea treatment demonstrated strong antibacterial activity. The green, oolong, and black tea treatments were categorized as very strong. Kombucha's antibacterial activity is largely due to the presence of organic acids, particularly acetic acid, large proteins, and catechins. Acetic acid and catechins are known to inhibit a number of gram-positive and gram-negative bacteria (Sreeramulu & Knol, 2000).

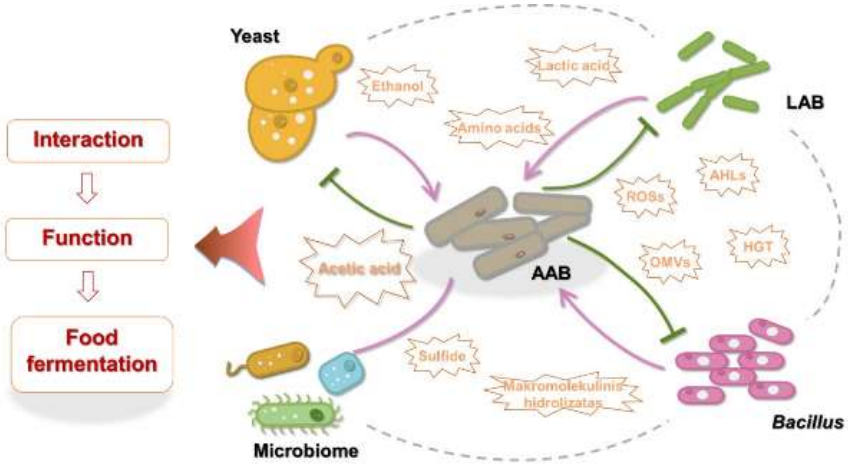

Figure 2. Mechanism of Acetic Acid Killing Bacteria (Source : Han, *et al*., 2024 (Han et al., 2024)).

Rinihapsari et al. (Rinihapsari & AR, 2008) explained that the antibacterial activity of kombucha is due to the acetic acid produced during fermentation. Kombucha tea fermented for a week or more produces a number of organic acids (Ahmed, 2003). Organic acids inhibit the growth of microorganisms by lowering the pH of their environment, which in turn increases the digestibility of the material, balances the microflora, and enhances metabolism (Rofiq, 2003).

Research conducted on the fermentation of kombucha tea from several tea varieties against *E. coli* and *S. aureus* revealed that the inhibition zone formed in *S. aureus* (gram-positive) was larger than that in *E. coli* (gram-negative). This is because Gram-negative bacteria have a more complex cell wall structure than Gram-positive bacteria. The main difference is the presence of an outer membrane layer containing peptidoglycan. This membrane causes the cell wall of Gram-negative bacteria

to contain a lipopolysaccharide layer that acts as a barrier to the entry of several substances, including antibiotics. The cell wall of Gram-positive bacteria lacks lipopolysaccharide, making the cells more susceptible to lysis (Volk, 1992).

Table 7. Antibacterial Activity of Fermented Tea Gel Preparation (Kombucha) Combination of Strawberry and Ashoka Flower Against *Staphylococcus epidermidis* Bacteria.

Formula	Inhibition Zone (mm)				Category
	T1	T2	T3	Mean	
F1	9	7,3	7,4	7,9±0,77	Moderate
F2	9	8,5	7,4	8,3±0,66	Moderate
F3	9,7	8,5	9	9,06±0,49	Moderate
Kombucha	10,9	10,4	8,9	10,0±0,84	Moderate
K (+)	22,8	20,6	21,9	21,7±0,9	Very Strong
K (-)	0	0	0	0	-

Note: K (+) : Clindamycin gel; K (-) : F0, gel base without kombucha.

The average diameter of the inhibition zone in *Staphylococcus epidermidi*s bacteria F1, F2, F3, fermented tea (kombucha) strawberries and ashoka flowers, the diameter of the inhibition zone F1 is 7.9 mm in the medium category, F2 is 8.3 mm in the medium category, and F3 is 9.06 mm in the medium category, kombucha is 10 mm in the medium category, and the positive control is 21.7 mm in the very strong category.

Table 8. Antibacterial Activity of Fermented Tea Gel Preparation (Kombucha) Combination of Strawberry and Ashoka Flower Against Methicillin Resistant *Staphylococcus aureus* (MRSA) Bacteria

Formula	Inhibition Zone (mm)				Category
	T1	T2	T3	Mean	
F1	8,75	8,05	8,05	8,29 ± 0,32	Moderate
F2	9,75	9,05	9,02	9,27 ± 0,33	Moderate
F3	9,16	10	9,37	9,51 ± 0,35	Moderate
Kombucha	8,9	10,4	10,9	10,0±0,84	Moderate
K (+)	22,7	20,7	21,9	21,8±0,9	Very Strong
K (-)	0	0	0	0	-

Note: K (+) : Clindamycin gel; K (-) : F0, gel base without kombucha.

The average diameter of the inhibition zone in MRSA bacteria F1, F2, F3, fermented tea (kombucha) strawberries and ashoka flowers, the diameter of the inhibition zone F1 is 8.29 mm in the moderate category, F2 is 9.27 mm in the moderate category, and F3 is 9.51 mm in the moderate category, kombucha is 10 mm in the moderate category, and the positive control is 21.8 mm in the very strong category.

The antibacterial activity of kombucha and fermented tea gel preparations (kombucha) strawberries and ashoka flowers occurs because the high amount of acid in kombucha can inhibit bacterial growth, and an increase in the number of protons in high concentrations can denature proteins and enzymes in bacterial cell walls. In addition to acid formation, phenolic compounds formed during fermentation can also provide antibacterial effects in kombucha.

Ashoka flowers also contain Phenolic and flavonoid substances. Phenol kills bacteria by denaturing bacterial cell proteins, destroying cell walls, and inactivating important enzymes in bacterial cells. Meanwhile, Flavonoids have the ability to kill bacteria through several mechanisms, such as inhibiting nucleic acid synthesis, inhibiting cytoplasmic membrane function, and inhibiting bacterial energy metabolism.

Table 9. Active Compouds in Strawberry.

Compounds	Pharmacology Activity
Anthocyanins	These are the pigments that give strawberries their red color and act as powerful Antioxidants, help protect against cellular damage, can lower cholesterol, and help relax blood vessels to prevent cardiovascular disease.
Ellagic Acid	A phenolic compound that is a potent antioxidant and potential anti-carcinogenic
Ascorbic acid	Immune function and acts as a vital Antioxidant
Lycopene	This carotenoid is known for its antioxidant properties and may help regulate cholesterol metabolism
Quercetin and	Antioxidant activity
Kaempferol	Antioxidant activity

Table 10. Active Compouds in Ashoka Flower.

Compounds	Pharmacology Activity
Flavonoids: Quercetin, apigenin-7-O-p-D-glucoside, kaempferol-3-O-P-D-glucoside, and quercetin-3-O-P-D-glucoside	Flavonoids contribute to the plant's antioxidant and anti-inflammatory properties
Phenolic Acids: Gallic acid and Ellagic acid	A phenolic compound that is a potent antioxidant and potential anti-carcinogenic
Fatty Acids: Oleic acid, linoleic acid, palmitic acid, linolenic acid, and stearic acid	contributing to its cardioprotective properties

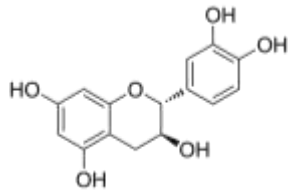

Figure 3. Catechin.

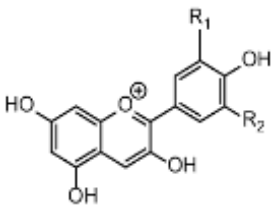

Figure 4. Anthocyanin.

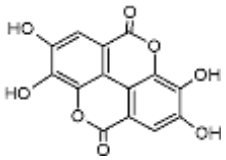

Figure 5. Ellagic Acid.

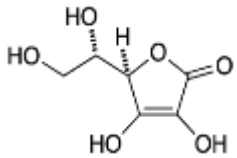

Figure 6. Ascorbic Acid.

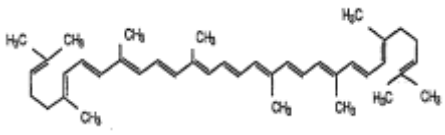

Figure 7. Lycopene.

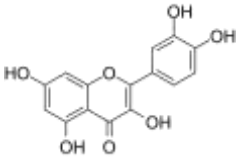

Figure 8. Quercetin.

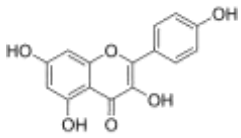

Figure 9. Kaempferol.

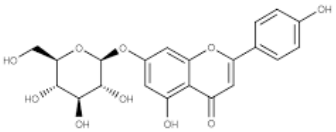

Figure 10. apigenin-7-glucoside.

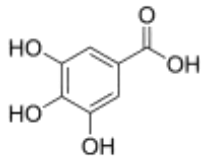

Figure 11. Gallic Acid.

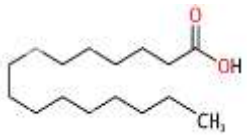

Figure 12. Palmitic Acid.

Figure 13. Stearic Acid.

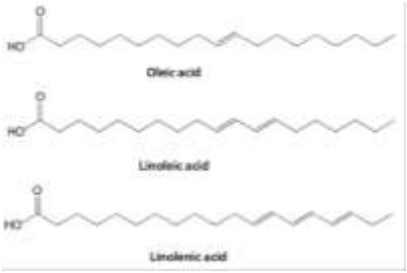

Figure 14. Oleic, Linoleic, and Linolenic Acid

Table 11. Comparison of Antioxidant Activity.

Formula	Amount	Category
F0	223,78	Moderate
F1	124,56	Moderate
F2	82,44	Strong
F3	47,32	Very Strong
Ascorbic Acid (Standard)	34	Very Strong

Antioxidant activity testing was carried out using the FRAP method. The principle of this method is to determine the total antioxidant content of a material based on the ability of antioxidant compounds to reduce Fe^{3+} ions to Fe^{2+} so that the antioxidant strength of a compound is analogous to the reducing ability of the compound.

Antioxidant activity was tested by the FRAP method measured by UV-Vis spectrophotometry at the maximum wavelength obtained in this study was 692 nm. The reference standard used in the antioxidant activity test was ascorbic acid. Standard solutions of ascorbic acid at concentrations of 70; 90; 110; and 130 µg/mL were measured for absorbance by UV-Vis spectrophotometry

at a wavelength of 692 nm. The regression equation obtained was y = 0.0028x – 0.0615 with an R^2 value of 0.9753 obtained by plotting the concentration of ascorbic acid (x-axis) against its absorbance (y-axis) and had an IC_{50} value of 34 µg/mL (Very Strong). In the results of the antioxidant activity test by conducting 3 replications each, the average antioxidant activity of Formula F3 was 47.32 mgAAE/g and the average result of Formula F2 was 82.44 mgAAE/g.

In Formulation F3, the results of the Antioxidant test were obtained with the Very Strong Category, and Formulation F2 obtained the results of the Antioxidant test with the Strong Category. This is because Strawberries and Ashoka Flowers contain secondary metabolites that have antioxidant activity such as Phenolics and Flavonoids.

The phenolic compounds found in Ashoka flowers are phenolic derivatives with antioxidant activity. Phenolic compounds work as antioxidants by preventing the formation of new free radicals, converting existing free radicals into harmless molecules, acting as free radical scavengers, and preventing chain reactions. Phenolic compounds have antioxidant potential due to the presence of hydroxyl groups in phenolic compounds. The hydroxyl group functions as a hydrogen atom donor when reacting with radicals through an electron transfer mechanism, thus inhibiting the oxidation process.

Flavonoids have three mechanisms of action to prevent free radicals: inhibiting the formation of ROS (Reactive Oxygen Species), destroying them, and regulating and protecting with antioxidants. In preventing free radicals, flavonoids can stabilize ROS by eliminating oxidizing xenobiotic compounds. In the ROS inhibition process, flavonoids activate signaling pathways from endogenous enzymes, such as Cat, SOD, and GPx, preventing the formation of hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH). Flavonoids can neutralize free radicals directly by donating H atoms. Free radicals become inactive. (Widiasriani et al., 2024)

Discussion

The antibacterial activity test using the disc diffusion method demonstrated that both kombucha and gel formulations containing fermented strawberries and ashoka flowers possess inhibitory effects against *Staphylococcus aureus*, *Staphylococcus epidermidis*, and MRSA, with activity generally increasing alongside concentration. For *S. aureus*, inhibition zones ranged from moderate (F1: 7.03 mm) to strong (F2: 10.2 mm; F3: 10.9 mm), while kombucha and the positive control showed very strong activity (21.5 mm and 25.6 mm, respectively), indicating a clear dose-dependent response. Similar trends were observed in *S. epidermidis* and MRSA, although the inhibition remained mostly in the moderate category, suggesting comparatively lower susceptibility. These findings are consistent with previous studies (Battikh et al., 2011; Sreeramulu & Knol, 2000; Rinihapsari & AR, 2008), which attribute kombucha's antibacterial effects to organic acids (especially acetic acid), catechins, and bioactive metabolites produced during fermentation that lower environmental pH and disrupt bacterial cell integrity. The stronger inhibition observed in Gram-positive bacteria compared to Gram-negative strains aligns with differences in cell wall structure, where the absence of an outer lipopolysaccharide membrane in Gram-positive bacteria increases susceptibility (Volk, 1992). Additionally, phenolic and flavonoid compounds from ashoka flowers contribute to antibacterial activity through protein denaturation, membrane disruption, and inhibition of nucleic acid synthesis and energy metabolism. In parallel, antioxidant testing using the FRAP method showed that F3 exhibited very strong antioxidant activity (47.32 mgAAE/g), while F2 was categorized as strong (82.44 mgAAE/g), supported by an ascorbic acid standard curve (R^2 = 0.9753; IC_{50} = 34 µg/mL). This antioxidant potential is attributed to phenolic and flavonoid compounds capable of donating hydrogen atoms, scavenging free radicals, inhibiting ROS formation, and enhancing endogenous antioxidant defenses, thereby confirming that the kombucha-based gel formulations not only exhibit antibacterial activity but also significant antioxidant properties.

CONCLUSIONS

Based on the results of the study, it can be concluded that gel formulations containing fermented kombucha from strawberries and ashoka flowers exhibit antibacterial activity against *Staphylococcus aureus*, *Staphylococcus epidermidis*, and MRSA, with effectiveness increasing as the concentration increases, where formula F3 showed the highest activity among the tested formulations. Pure kombucha demonstrated very strong antibacterial activity, approaching that of the positive control, which is attributed to the presence of organic acids, phenolic compounds, and flavonoids produced during fermentation that can damage bacterial cell structures. In addition, Gram-positive bacteria were found to be more susceptible than Gram-negative bacteria due to differences in cell wall structure. Antioxidant activity testing using the FRAP method indicated that the formulations, particularly F3, possess very strong antioxidant potential, which is associated with the presence of phenolic and flavonoid compounds that act as free radical scavengers and oxidation inhibitors. Therefore, the kombucha-based gel formulation of strawberries and ashoka flowers has strong potential as an effective natural antibacterial and antioxidant agent.

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Authors' Contributions: Goldha Faroliu designed this study and conducted antibacterial and antioxidant testing. Kony Putriani prepared the Ashoka flower extract and performed antibacterial testing. Yessi Rahayu performed data analysis. Nanda Rahma Tri Cahyaningrum produced kombucha, prepared the preparations, and performed antibacterial testing. Mardiatul Jannah performed antioxidant testing. Goldha Faroliu wrote the manuscript. All authors read and approved the final version of the manuscript.

Competing Interests: The authors declare that there are no financial, academic, or personal conflicts of interest that may have influenced the findings or interpretations presented in this study.

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