Antibacterial Activity of Green Tea Kombucha-based Liquid Soap Against Staphylococcus epidermidis

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Abstracts

Body odor is commonly associated with bacterial activity on sweat, particularly that of S. epidermidis. Kombucha, a fermented tea containing organic acids and polyphenols, has potential as a natural antibacterial ingredient. This study aimed to formulate green tea kombucha-based liquid soap and evaluate its physicochemical properties and antibacterial activity against S. epidermidis according to SNI 4085:2017. Green tea was fermented for 14 days using SCOBY and sugar, followed by liquid soap preparation using the cold-process method. The product was evaluated for organoleptic properties, pH value, free fatty acids, free alkali, and antibacterial activity using the disk diffusion method. The resulting soap exhibited a yellow color, mild aroma, high viscosity, and a pH value of 10, meeting the specified quality requirements. Free fatty acid and free alkali contents were 0.15% and 0.05%, respectively, indicating effective saponification. However, no inhibition zone was observed against S. epidermidis, whereas commercial soap showed antibacterial activity at higher dilution levels. This lack of activity may be related to degradation of kombucha bioactive compounds during formulation or their limited diffusion in agar. Further formulation optimization is needed to improve antibacterial efficacy.

Keywords: Antibacterial activity, green tea, kombucha, liquid soap, *Staphylococcus epidermidis*

Introduction

Body odor is a common concern associated with the presence and activity of microorganisms on the skin. One of the microorganisms associated with body odor is Staphylococcus epidermidis (S. epidermidis). Therefore, the development of antibacterial personal-care products can be considered as one approach to reducing the microbial factors associated with body odor [1].

Liquid soap is widely used as a personal-care product and can serve as a medium for incorporating natural active ingredients. The use of natural materials with antibacterial potential has attracted attention to the development of personal-care products. Kombucha is a fermented beverage containing various bioactive compounds and has potential for application in natural care products [2]. During fermentation, organic acids, particularly acetic acid, are produced and contribute to the antibacterial properties of kombucha. The production of these organic acids can decrease the pH value of the fermented product, which may inhibit microbial growth [3] .

Green tea was selected as the substrate for kombucha fermentation because it contains bioactive phenolic compounds associated with antioxidant activity [4]. In addition, sugar serves as a nutrient source for the microorganisms present in the Symbiotic Culture of Bacteria and Yeasts (SCOBY) during fermentation [5]. The

resulting kombucha can subsequently be incorporated into the aqueous phase of liquid soap formulations. The antibacterial activity of kombucha is associated with its organic acids and polyphenolic compounds [6].

Recent studies have further investigated the antimicrobial potential of kombucha. Nyiew et al. reviewed evidence demonstrating that kombucha exhibits antimicrobial activity against a broad range of bacteria and fungi. This activity is associated with bioactive compounds produced during fermentation, particularly organic acids such as acetic acid and various polyphenols [7]. The application of kombucha in antibacterial soap formulations has also been investigated. Rezaldi et al. developed liquid bath soap containing butterfly pea flower (Clitoria ternatea L.) kombucha and evaluated its antibacterial activity against Staphylococcus epidermidis and Pseudomonas aeruginosa. Their results showed that the formulation containing 40% kombucha produced inhibition zones of 10 mm against S. epidermidis and 7 mm against P. aeruginosa [8]. This study demonstrates the potential of kombucha as an active ingredient in antibacterial soap formulations. However, the antibacterial performance of kombucha may vary depending on the fermentation substrate and formulation composition. Therefore, the present study investigates green tea kombucha-based liquid soap against S. epidermidis and evaluates its physicochemical and organoleptic characteristics in accordance with SNI 4085:2017.

Material and Methods

Materials

Materials used in this research are coconut oil and olive oil; an aqueous phase consisting of technical-grade KOH and distilled water; and Kombucha comprising a commercial SCOBY as the primary bacterial culture), commercial green tea (as the medium), and sugar (as the nutrient source) which served as an antibacterial agent against S. epidermidis. The SCOBY from this research in general comprises a complex microbial community dominated by Komagataeibacter xylinus, Gluconacetobacter oxydans, and Brettanomyces spp. These microorganisms function synergistically, with yeasts fermenting sugars into ethanol and organic acids, which are then utilized by acetic acid bacteria to produce bacterial cellulose [9]. The additives include technical-grade Na-CMC (thickener and emulsion stabilizer), SLS (surfactant and foaming agent), sodium lactate (humectant and soap hardener), phenoxyethanol (preservative), kaolin clay (oil and impurity absorber), glycerin (natural moisturizer), and essential oils (for fragrance and added relaxing effects). For the alkalinity and free fatty acid tests, analytical-grade HCl and NaOH (Merck KGaA) were used as titrants; acetic acid (Merck KGaA) and Na2CO3 (Merck KGaA) served as standard solutions, while a commercial antibacterial soap was used as a comparative control. Antibacterial activity testing employed Nutrient Agar and Nutrient Broth media (Merck KGaA), with S. epidermidis as the test bacterium.

Kombucha Fermentation

Kombucha fermentation was performed following the procedure described in Dartora et al. with modifications to the ingredient proportions [10]. Briefly, 25 g of tea leaves and 1 kg of granulated sugar were added to 2.5 L of water and brought to a boil. The mixture was then cooled to room temperature, after which previously fermented kombucha liquid was added and thoroughly mixed. The solution was transferred into a sterilized glass jar, and SCOBY was added. Fermentation was carried out for 14 days in a dry location protected from direct sunlight.

Kombucha Soap

Kombucha soap was prepared using a modified soap base using the cold process method [11]. The modification involved incorporating 8 mL of kombucha solution into the aqueous phase of the soap formulation. Briefly, 20 g of coconut oil and 30 g of olive oil were weighed and mixed to form the oil phase. Separately, 12 g of KOH was dissolved in 20 mL of water, followed by the addition of the kombucha solution. The resulting aqueous phase was then slowly incorporated into the oil

phase under continuous stirring until a homogeneous mixture was obtained. Subsequently, the additives listed in Table 1 were incorporated and mixed thoroughly to ensure uniform distribution throughout the formulation. This modification was intended to incorporate kombucha into the soap formulation while maintaining the basic procedure of soap base.

Table 1. Composition of Additives

Additive Material	amount	Additive Material	Amount
Na-CMC	0.19 g	Phenoxy ethanol	0.19 g
Aquades	10 ml	Kaolin clay	0.19 g
SLS	0.38 g	glycerin	0.19 g
Sodium Lactate	0.38 g	Essential Oil	Optional

Organoleptic Test

An organoleptic test was conducted to evaluate the physical characteristics of the soap using the senses, pH value, color, odor, and viscosity. The pH test was performed using universal pH paper, with the results compared against the color chart provided with the paper. The color test involved visual inspection of the kombucha soap. The odor test was conducted by smelling the finished kombucha soap. The viscosity test involved assessing the soap's texture on the skin.

Free Alkali Test

The procedure of free alkali test begins with standardization using 0.05 g of $Na2CO3$ dissolved in 25 mL of water with added methyl red indicator in an Erlenmeyer flask; this is titrated with 0.1 N HCl until a reddish-orange endpoint is reached, the volume is recorded, and the process is duplicate. Subsequently, 2.5 g of soap is weighed into a separate Erlenmeyer flask and dissolved in 50 mL of neutral ethanol prepared by adding 2-3 drops of phenolphthalein (PP) indicator and titrating with 0.1 N NaOH in ethanol until a faint pink color appears. The mixture is stirred thoroughly and heated to boil until the solution turns purple. Finally, the solution is titrated with 0.1 N HCl until the purple color disappears, the volume of HCl is recorded, and the procedure is performed in duplicate [12].

Free Fatty Acid Test

The procedure of free fatty acid test begins with standardization using 0.126 g of oxalic acid in 25 mL of water and adding phenolphthalein (PP) indicator in an Erlenmeyer flask; the solution was then titrated with 0.1 N NaOH until the endpoint marked by the appearance of a faint pink color was reached, and the volume of NaOH used was recorded (performed in duplicate). Subsequently, 2.5 g of soap was dissolved in 50 mL of neutral ethanol in a beaker; the neutral ethanol was prepared by adding 2–3 drops of PP indicator and titrating with 0.1 N NaOH in ethanol until a faint pink color appeared. This neutral ethanol solution was added to the Erlenmeyer flask containing the soap and mixed thoroughly, then titrated with 0.1 N NaOH to the endpoint, and the volume of NaOH was recorded (performed in duplicate) [13].

Bacterial Inhibition Test

The antibacterial activity of the kombucha soap was evaluated using the disc diffusion method to assess its ability to inhibit bacterial growth. Commercial soap and kombucha samples were used as comparative samples to evaluate their respective antibacterial effects. Commercial soap and kombucha samples were serially diluted up to 100,000-fold, and sterile paper discs were then soaked in each solution for 15 minutes. A 50 µL aliquot of bacterial suspension was applied to sterile Nutrient Agar medium and spread evenly using an L-shaped spreader. The medium was divided into four sections, and a paper disc was placed in the center of each section. The plates were incubated at 37°C for 24 hours to observe antibacterial activity [12]

Results and Discussion

Kombucha Fermentation

In kombucha fermentation, is shown in **Figure 1**, used as an ingredient in soap making; green tea and commercial granulated sugar are used as the main ingredients. Green tea is chosen because of its high content of antioxidants and antimicrobial compounds, which enable it to inhibit the growth of bacteria that cause body odors and make it effective for use in anti-body odor soap [10].

Meanwhile, sugar serves as the primary nutrient source for the *SCOBY* used in the fermentation process, enabling the formation of a kombucha solution with the desired characteristics [14]. The fermentation process produces kombucha with a level of acidity that is not too high yet remains effective enough to be used as the primary active ingredient in soap formulations. In soap formulation, kombucha is incorporated into the aqueous phase, which is one of the main components that plays a crucial role in blending the other ingredients and supports the overall stability and effectiveness of the soap.

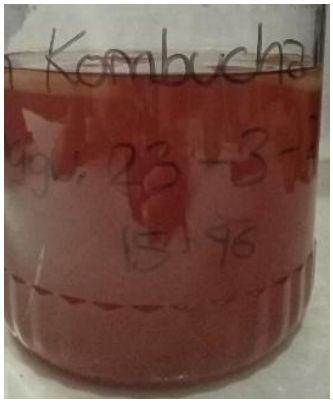


Figure 1. Kombucha fermentation

Kombucha Soap

The main active ingredient in kombucha soap, shown at **Figure 2**, is kombucha fermentation solution. Kombucha's antibacterial properties against *S. epidermidis* are attributed to active ingredients such as organic acids and polyphenols [14]. This soap is produced using the cold process method. This method involves mixing oils and a base (KOH) at room temperature, which is 25°C. The oil phase, which is olive oil and coconut oil will be mixed with the water, which is KOH and kombucha until the mixture reaches the trace stage and cures (the saponification reaction has occurred).



Figure 2. Kombucha soap

The saponification process takes 1–2 weeks to complete [10]. The additives that are added serve to provide fragrance, texture, preservation, and thickening. The reason for saponification of soap is shown at **Figure 3**.

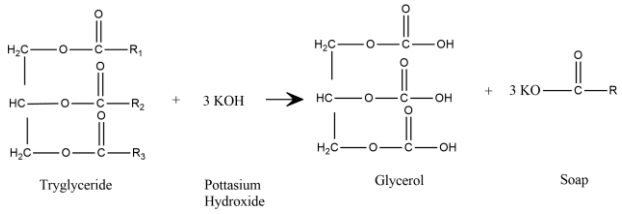


Figure 3. Soap saponification reaction [10]

Organoleptic and pH Test

Table 2. Results of the Comparison Between Kombucha Soap and Commercial Soap

Types of Soap	Observation			
	Color	Viscosity	Odor	Appearance

Commercial soap	Pink	++	++	
Kombucha soap	Yellow	++	+	

Description: Mild or low (+), Strong or high (++)

Organoleptic testing was conducted to evaluate the physical characteristics of the liquid soap, including color, viscosity, and odor, using sensory observation [15]. As shown in **Table 2**, the commercial liquid soap was pink, exhibited high viscosity, and had a strong odor. In comparison, the kombucha-based liquid soap was yellow, exhibited similarly high viscosity, and had a milder odor. These observations indicate that both liquid soaps had similar viscosity, while their color and odor differed.

Table 3. pH value of the Comparison Between Kombucha Soap and Commercial Soap

Types of Soap	pH value	Reference
Commercial soap	9	This study
Kombucha soap	10	This study
Liquid soap	4-10	SNI 4085:2017

A pH test is a quality requirement for liquid soap, since liquid soap is applied directly to the skin; if the pH value does not match the pH of the skin, it can cause irritation [16]. According to SNI Standard 4085:2017, the permissible pH range for liquid soap is between 4 and 10. Based on **Table 3**, the pH value of the commercial soap was found to be 9, while the pH value of the kombucha soap was 10, as measured using universal pH paper. When comparing the pH of kombucha soap to that of commercial soap, it can be concluded that kombucha soap meets the requirements of SNI 4085:2017 because the pH value falls within the range specified by the SNI standard.

Free Fatty Acid Test

The free fatty acid test is an important parameter in assessing the quality of liquid soap, as it indicates the amount of free fatty acids remaining due to incomplete saponification reactions or fat hydrolysis during storage. High free fatty acid levels can cause the product to go rancid, reduce shelf life, and pose a risk of skin irritation. In this study, kombucha-based liquid soaps showed excellent results with free fatty acid levels of 0.11% and 0.18%, respectively, for an average of 0.15%. This value is significantly lower than the commercial soaps tested, which showed levels of 1.62% and 1.63%. These results indicate that the kombucha soap-making process is quite efficient, with optimal saponification reactions. As shown in **Table 3**, the FFA content of the kombucha-based liquid soap was also lower than the value reported for liquid soap containing betel leaf and garlic extracts (0.21%) [17]. These findings indicate that the kombucha-based liquid soap had a relatively low FFA content compared with the commercial product and the previously reported formulation. The results were also compared with the applicable quality requirements of SNI 4085:2017. Therefore, this group of kombucha liquid soaps meets good quality standards, even below the average values from several studies, making them safer and more stable during storage. The kombucha-based liquid soap samples before and after titration with NaOH are shown in **Figures 4** and **Figure 5**, respectively.

Table 3. Free Fatty Acid Content of Kombucha-Based and Other Liquid Soap Formulations

Type of Soap	Free Fatty Acid Content	Reference
Kombucha soap	0.11 %	This study
Commercial soap	0.18 %	This study
Liquid soap containing betel leaf and garlic extracts	0.21%	[17]
Liquid soap	Max. 4%	SNI 4085:2017

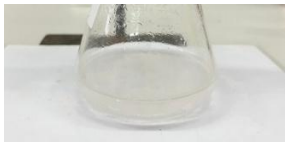


Figure 4. Kombucha Soap before titration with NaOH

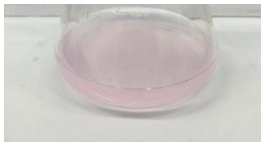


Figure 5. Kombucha Soap after titration with NaOH

Free Alkali Test

The Free Alkali Test (FAT) aims to identify strong residual bases, such as sodium hydroxide (NaOH), that do not react during the saponification process. High free alkali content in liquid soap can cause the pH value of the product is too high, making it irritating and unsuitable for long-term use on the skin. Based on the results of this study, kombucha liquid soap has a free alkali content of 0.05%, meaning that almost all the base has completely reacted with the fatty acids. As shown in Table 4, this value was lower than those reported for liquid soap containing betel leaf and garlic extracts (0.07%) [17]. The measured free alkali content was also below the maximum limit of 0.1% specified in SNI 4085:2017, provided that this limit is applicable to the parameter and calculation basis used in this study. The relatively low free alkali content suggests that limited residual alkali remained in the formulated soap after saponification. However, further testing is required to establish the product's overall safety and suitability for skin application. The kombucha-based liquid soap samples before and after titration are shown in **Figures 6** and **Figure 7**, respectively. The low free alkali content in this kombucha soap can be attributed to a well-developed formulation process and the use of natural ingredients like kombucha, which has a natural acidity, which helps facilitate the thorough saponification reaction. Therefore, the resulting kombucha liquid soap not only meets safety and skin comfort standards but also reflects optimal formulation quality.

Table 4. Free Alkali Content of Kombucha-Based and Other Liquid Soap Formulations

Type of Soap	Free Alkali Content	Reference
Kombucha soap	0.05 %	This study
Liquid soap containing betel leaf and garlic extracts	0.07%	[17]
Liquid soap	Max. 0.1%	SNI 4085:2017



Figure 6. Kombucha Soap before titration with HCl

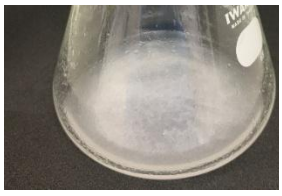


Figure 7. Kombucha Soap after titration with HCl

Bacterial Inhibitory Test

The antibacterial test results showed that an inhibition zone was formed only by the commercial soap at S5, corresponding to a 100,000× dilution, while none of the kombucha soap concentrations exhibited inhibitory activity against *S. epidermidis*. The formation of an inhibition zone by the commercial soap at S5 indicates that its active compounds have good stability and diffusion ability even at high dilution. At higher dilution, the viscosity of the solution decreases, allowing the active

compounds to diffuse more easily into the agar medium and demonstrate their antibacterial effectiveness [18] . This also suggests that the commercial soap formulation may contain stable synthetic compounds with an antibacterial spectrum effective against *S. epidermidis*. In contrast, the absence of inhibition zones at all kombucha soap concentrations may be attributed to several factors, including the possible inactivation of bioactive compounds in kombucha, particularly acetic acid and phenolic compounds, due to interactions with surfactant or alkaline components in the soap, resulting in a substantial reduction in antibacterial activity [8]. Furthermore, the physicochemical properties of these compounds may not be favorable for optimal diffusion through the agar medium, particularly if the solution is too viscous or has an unstable pH value. The absence of inhibition zones may also have been influenced by technical factors, such as inadequate pre-diffusion time, uneven bacterial distribution, or inaccurate disc placement [19]. Therefore, the difference in antibacterial effectiveness between the commercial and kombucha soaps was likely influenced by a combination of active compound stability, diffusion ability, and environmental conditions during the testing process. **Figure 8** shows the results of the inhibitory activity test for *S. epidermidis*.

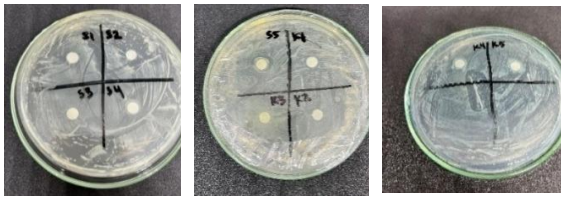


Figure 8. Results of the inhibitory activity test for *S. Epidermidis*

Description:
S1: Commercial soap 10x dilution
K1: Commercial soap 10x dilution
S2: Commercial soap 100x dilution
K2: Commercial soap 100x dilution
S3: Commercial soap 1,000x dilution
K3: Commercial soap 1,000x dilution
S4: Commercial soap 10,000x dilution
K4: Commercial soap 10,000x dilution
S5: Commercial soap 100,000x dilution
K5: Commercial soap 100,000x dilution

Conclusion

The kombucha-based liquid soap showed no antibacterial activity against S. epidermidis, as indicated by the absence of an inhibition zone in the disk diffusion assay. The formulation met the quality requirements of SNI 4085:2017, with free fatty acid and free alkali contents of 0.15% and 0.05%, respectively, indicating effective saponification. The product exhibited a yellow color, high viscosity, mild odor, and a pH value of 10, all of which complied with the specified quality standards.

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