https://doi.org/10.1051/bioconf/202520301004

Antibacterial test of fermented kombucha green tea (*Camellia sinensis* L.) facial wash soap preparation against *Cutibacterium acnes*

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Abstract. *Acne vulgaris* is a common inflammatory skin condition influenced by psychological factors and infection by *Cutibacterium acnes*. Long-term antibiotic use can cause resistance and disrupt the skin microbiome, creating a need for safe and effective alternatives. Green tea kombucha, a fermented product of bacteria and yeast (SCOBY), contains bioactive compounds such as polyphenols, organic acids, and probiotics with antimicrobial and anti-inflammatory properties. This study evaluated the antibacterial activity of facial cleansers formulated with green tea kombucha against *Cutibacterium acnes* and assessed user preferences for each formula. Antibacterial testing used the well diffusion method, supported by statistical analysis to compare the effectiveness of the formulations with a positive control. All preparations demonstrated antibacterial activity, with formula P1 producing the largest inhibition zone (28.46 ± 0.70 mm), comparable to clindamycin (31.53 ± 0.70 mm). However, formula P2 was most preferred by panelists based on texture, colour, and aroma. These findings show that green tea kombucha has strong potential as a natural active ingredient in facial cleansers to inhibit *Cutibacterium acnes*.
Keywords: facial cleanser, natural soap, probiotic soap, kombucha, green tea, *Cutibacterium acnes.*

1 Introduction

Acne vulgaris is a common skin inflammation in adolescents to adults, influenced by psychological factors such as depression and anxiety. Its pathophysiology involves *Cutibacterium acnes* bacteria, excess sebum production, and follicular keratinisation. Factors in the pathogenesis of acne vulgaris include the skin microbiome, which consists of various microorganisms such as *Cutibacterium acnes* and *Malassezia* spp. These microorganisms contribute to the development of acne through their influence on sebum secretion, comedone formation, and inflammatory response [1]. Antibiotics have been used as the primary treatment option for acne for decades, but their effectiveness is now being questioned due to increasing antimicrobial resistance. Research in Indonesia has revealed a fairly high level of resistance, especially in the macrolide group, with *Cutibacterium acnes* showing resistance reaching 60.1% [2]. This condition is a serious concern because *Cutibacterium acnes* plays an important role in maintaining the balance of the skin microbiome.

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In recent years, probiotics have been recognised as beneficial for gut and skin health, possessing antimicrobial properties and the ability to prevent inflammation. With the rise in antibiotic resistance, probiotics have emerged as a promising therapeutic alternative. Probiotics are microorganisms that are beneficial to health when used appropriately. One of their mechanisms is by reducing sebum production, thereby suppressing the growth of *Cutibacterium acnes* [3]. Kombucha is a beverage made from tea that undergoes fermentation by a colony of bacteria and yeast, known as Symbiotic Culture of Bacteria and Yeast (SCOBY), containing probiotics that can help maintain the balance of skin microbiota. By maintaining healthy bacterial populations on the skin, kombucha can help reduce the growth of bacteria that cause acne, such as *Cutibacterium acnes* [4]. Several studies have shown that components in kombucha are anti-inflammatory, helping to reduce inflammation in acne, such as redness and swelling [5].

Green tea (*Camellia sinensis* L.) contains polyphenolic compounds such as catechins, which have antibacterial and antioxidant activities. Alkaloids and flavonoids in tea can inhibit the growth of pathogenic bacteria by disrupting protein synthesis and damaging cell membranes [6]. Through the fermentation process, the content of bioactive compounds in green tea can be increased and produce new compounds that have stronger biological activity [7]. Green tea contains catechins such as epigallocatechin-3-gallate (EGCG), which possess strong antimicrobial and antioxidant properties. These compounds help stabilise free radicals, prevent damage to collagen and hyaluronic acid, and slow down skin ageing [8].

Green tea kombucha exhibits antimicrobial properties against various pathogens, including bacteria and fungi. Green tea and black tea kombucha demonstrate antibacterial activity, with green tea kombucha generally showing higher potential. The antimicrobial effects are associated with the presence of phenolic compounds, particularly flavonoids, phenolic acids, and other polyphenols. These compounds can disrupt bacterial cell walls, interfere with peptidoglycan synthesis, and cause cell death [9]. Fermentation process increases the phenolic content in kombucha, enhancing its antioxidant and anti-inflammatory effects [10].

Based on these findings, this study tested the antibacterial activity of facial cleansers containing green tea kombucha, referring to previous research [14], which demonstrated that kombucha possesses strong antimicrobial properties due to the presence of acetic acid, gluconic acid, and polyphenols. The combination of green tea and kombucha in the facial cleanser formulation is expected to increase its effectiveness in reducing acne-causing bacteria, such as *Cutibacterium acnes*. This study [12], evaluated green tea dregs extract against *Cutibacterium acnes*, total phenolic content (catechins), and tested its antibacterial activity using the microdilution method. Extraction under brewing conditions of 2 minutes and the "ultrasonic-assisted extraction" method showed an MIC_{50} value of 8.586 mg/mL against *Cutibacterium acnes*. This indicates that green tea extract with a sufficiently high phenolic content has the ability to inhibit the growth of *Cutibacterium acnes*. Therefore, this study aims to develop and evaluate the effectiveness of a facial cleanser containing a combination of these natural antibacterial ingredients in combating the growth of *Cutibacterium acnes* and to determine its antibacterial potential through direct testing on this acne-causing microorganism.

2 Methods

This study was conducted over four months at the University of 'Aisyiyah Yogyakarta Research Laboratory. The treatments consisted of four variations of green tea kombucha concentration, namely P1 (40%), P2 (30%), P3 (20%), and P4 (0%). Medi-Klin Clindamycin Phosphate Gel 1% was used as a positive control, while Menbox Black AHA BHA *Facial Wash* was used as a comparison.

2.1 Tool preparation

The equipment to be used was first washed thoroughly and dried. Glassware such as Petri dishes, beakers, and measuring cups were wrapped in paper and plastic before being sterilised. Other equipment such as tips and L-shaped rods were wrapped in plastic, then sterilised using an autoclave at a temperature of 121°C and a pressure of 1 atm for 15 minutes. Ose is sterilised by soaking it in 70% alcohol for 5 minutes, then flaming it over a Bunsen burner. Tweezers are also sterilised by soaking them in 70% alcohol for 5 minutes [6].

2.2 Preparation of agar media

A total of 3.8 g of Mueller Hinton Agar (MHA) medium was weighed and placed in a 250 mL Erlenmeyer flask, then 100 mL of distilled water was added. The mixture was heated over a Bunsen burner until it dissolved homogeneously, then covered with cotton wool. Next, the medium was sterilised in an autoclave at 121°C for 15 minutes. After the sterilisation process was complete, the pressure and temperature were reduced until the medium was no longer too hot. The medium was then removed from the autoclave and 20 mL was poured into each sterile Petri dish, then left to solidify [13].

2.3 Reculture *Cutibacterium acnes* bacteria

Re-culturing *Cutibacterium acnes* bacteria is done by taking a single colony from the stock culture using an ose needle that has been heated until red-hot and then cooled. The colony is streaked onto the surface of solid NA medium using the streak plate method to obtain a single colony. The Petridish is then covered, labelled, and incubated at 37 °C under anaerobic (anaerobic jar) conditions for 72 hours [13].

2.4 Preparation of green tea kombucha

900 mL of water was boiled, then steeped with 12 g of green tea for 10 minutes. After filtering and adding 50 g of sugar, the solution is cooled and then mixed with 100 mL of kombucha starter and SCOBY. Fermentation is carried out for 10 days at 25°C in a dark room. This method refers to research [14].

2.5 Facial Wash Soap Formulation

This facial wash soap formulation consists of four treatments with varying concentrations of green tea kombucha. The formulation was adjusted based on a saponification calculator to obtain a balanced composition of oil and alkali. Each formula has a different composition of ingredients as shown in **Table 1**.

Table 1. Tea Kombucha Facial Wash Formulation

Ingredients	P1	P2	P3	P4
Green Tea Kombucha (ml)	40	30	20	-
Olive Oil (ml)	15	15	15	15
Canola Oil (ml)	10	10	10	10
KOH (g)	6	6	6	6
Glycerin (ml)	10	10	10	10
Asam Sitrat (ml)	Adj pH 6	Adj pH 6	Adj pH 6	Adj pH 6
Phenoxyethanol (ml)	1	1	1	1
Akuades (ml)	Ad 100	Ad 100	Ad 100	Ad 100

The process of making facial soap begins by heating olive oil and canola oil to a temperature of 50°C. Separately, KOH is dissolved in 10 mL of distilled water. Once both solutions have reached the same temperature, the KOH solution is slowly added to the oil mixture while stirring until it reaches the trace phase. Next, green tea kombucha and distilled water are added to the mixture and stirred until evenly mixed. After that, glycerine is slowly added to the mixture and stirred again. Citric acid is slowly added while adjusting the pH. In the final stage, phenoxyethanol is added as a preservative and stirred until the mixture is homogeneous [14].

2.6 Organoleptic Test

Organoleptic testing was conducted on facial wash formulations containing active ingredients derived from green tea kombucha fermentation solution, using samples P1, P2, P3, and P4. The evaluation was carried out by 15 panelists using a hedonic scale to assess ten sensory parameters, namely: colour, consistency, visual appearance, aroma, aroma intensity, texture, foam, ease of rinsing, skin sensation, overall impression, and favourite sample choice [18].

2.7 pH test

One gram of soap was dissolved in 10 mL of distilled water, and the pH was measured using a pH meter with three replications [14].

2.8 Kombucha antibacterial test

200 µL of *Cutibacterium acnes* suspension was spread on the surface of MHA, then 4 wells were made on the medium. Kombucha 40% (K1), 30% (K2), 20% (K3) and positive control were added, each 75 µL. Incubated for 24 hours at 37°C. The diameter of the inhibition zone was measured with three replications [14].

2.9 Antibacterial test of kombucha soap

The steps were the same as for the kombucha antibacterial test, with 6 wells filled with soaps P1–P4, positive control (Medi-Klin Clindamycin phosphate GEL 1%), and commercial soap (0.5 g). Incubate for 24 hours, then measure the inhibition zone with three replications [14].

3 Results and Discussion

3.1 Organoleptic test

Organoleptic testing is conducted to assess the physical characteristics of liquid soap, such as shape, colour, smell, consistency, texture, and sensation of use through sensory observation. This evaluation aims to ensure product quality meets standards, assess attractiveness and comfort during use, and stability during storage. In addition, sensory evaluation is used to determine consumer preferences, where panellists are asked to give a final assessment of each sample. A high score indicates a greater level of preference for the liquid soap formulation being tested. The assessment results are presented in the following figure.

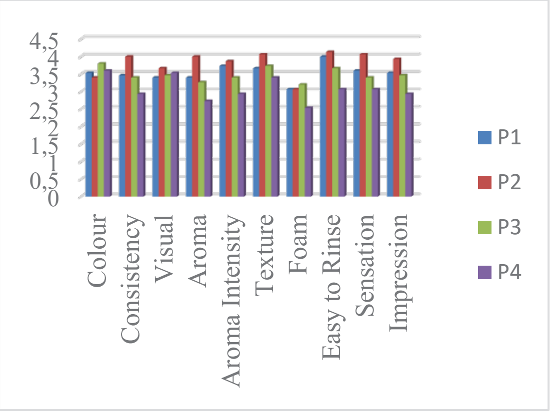

Fig.1. Histogram of organoleptic test

Based on **Figure 1**, sample P2 showed the best performance compared to the other three formulations. P2 obtained the highest scores on most parameters, such as texture, foam, rinse, skin sensation, and final impression, indicating a high level of panelist preference for this formulation. In terms of colour and appearance, the four samples did not show any striking differences; all had a natural appearance. However, there were clear variations in consistency. The P2 formulation was considered the most balanced, while P4 (without kombucha) appeared too thin, and P1 (40%) tended to be thicker. The thickness of the soap was closely related to the amount of biomass formed. Kombucha contains fermented microorganisms that produce metabolites and increase the total amount of solids. The higher the concentration of kombucha added, the greater the accumulation of biomass, thereby increasing the viscosity of the soap. This is in line with the opinion [14], that an increase in biomass in the fermentation medium has an impact on the increase in total solids, which affects the viscosity of the preparation.

In terms of pH, the addition of kombucha also causes a decrease in the pH value of the preparation. This decrease in pH affects the characteristics of the soap, one of which is a more acidic aroma. According to [15], this is because microbial metabolic activity during fermentation produces organic acids, such as acetic acid, which results in a more acidic environment.

In terms of aroma, P2 received the highest rating because its balance was considered the most appropriate. In contrast, P3 was considered to have a weaker aroma, while P1 was considered too pungent due to its dominant sour aroma. As for P3, there was no significant difference in the soap's aroma. Differences were also apparent in the ability to form foam and ease of rinsing. Soap without added surfactants produces little foam but is easy to rinse off, yet it does not meet the preferences of the panelists who desire more abundant foam. In this regard, P2 received the highest score, indicating optimal performance. Although P1 scored well in terms of ease of use, this formulation also caused skin irritation after use, which reduced its overall score. P3 stood out visually, but did not excel in other parameters. Meanwhile, P4 received the lowest score, particularly in terms of aroma and consistency. Overall, P2 is considered the most balanced and preferred formulation, making it the primary sample in this testing, with P1, P3, and P4 as comparators.

3.2 pH test

The pH test is one of the important parameters in evaluating the quality of liquid facial cleansers. The pH of the formulation must match the pH of the skin to avoid irritation and maintain the skin's natural balance.

Table 2. pH Test of Green Tea Kombucha Facial Wash Preparation

Sample	pH value
P1	5.65 ± 0.041
P2	5.81 ± 0.036
P3	5.84 ± 0.021
P4	5.97 ± 0.040

Table 2 shows that the four green tea kombucha facial cleanser formulations have an average pH ranging from 5.45 to 5.52, which is still within the ideal range for facial skin care products. According to [15], this indicates that all soap formulations meet the pH safety standards for skin. According to the National Standards Agency, a good liquid facial soap should have a pH between 4.5 and 6.5. The pH stability of each preparation is achieved through gradual adjustment using citric acid during the formulation process. Citric acid is added slowly until the pH reaches a range of 5.5 ± 0.5, so that the final product remains safe and comfortable to use on the skin, without causing irritation. The relatively consistent pH value in each repetition also reflects the reliability of the formulation process and the product's potential to maintain its stability during storage.

3.3 Antibacterial test using well diffusion method

The results of antibacterial activity tests on green tea kombucha preparations against *Cutibacterium acnes* bacteria were obtained by observing bacterial colony growth after incubation for 48 hours. The observation was conducted to evaluate the presence of an inhibition zone around the area of application of the soap preparation on the culture medium, which is an indicator of antibacterial effectiveness. After 48 hours of incubation under anaerobic conditions, the results showed that several soap preparations provided varying responses to inhibit the growth *of Cutibacterium acnes*, as indicated by the presence of a clear zone around the application point, as shown in Figures 4 and 5. The size and clarity of the inhibition zones were then measured using a digital caliper and compared between formulations to assess the relative effectiveness of each soap formulation.

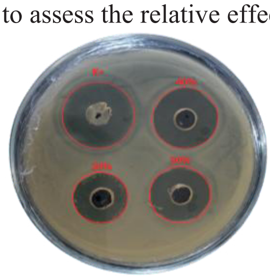

Fig.2. Antibacterial Test on Green Tea Kombucha Preparation

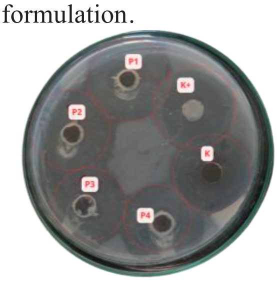

Fig.3. Facial Wash Preparation

Table 3. Clear zone diameter of green tea kombucha preparation on *Cutibacterium acnes Bacteria*

Sample	Clear Zone Diameter (mm)
P1	28.73 ± 0.67 [b]
P2	25.47 ± 0.55[c]
P3	23.57 ± 0.60 [d]
P4	20.47 ± 0.42[a]
[a, b, c, d] in the same column show a real difference based on the statistical test used (p < 0.05).	

The results of testing the antibacterial activity of green tea kombucha preparations against *Cutibacterium acnes* are shown in **Table 3**. It can be seen that an increase in kombucha concentration is directly proportional to an increase in the diameter of the inhibition zone. A concentration of 40% produced the largest inhibition zone diameter (28.73 ± 0.67 mm), which was significantly different ($p < 0.05$) from all other concentrations. The 20% concentration showed the lowest inhibition zone (23.57 ± 0.60 mm), while the 30% concentration produced a diameter of 25.47 ± 0.55 mm. The positive control (Medi-Klin Clindamycin Phosphate Gel 1%) provided the largest inhibition zone compared to all treatments, while the negative control showed no inhibition zone.

Antibacterial activity testing showed that P1 (40% kombucha) had the highest inhibition zone compared to other formulations. This was due to the higher biomass concentration, which resulted in a higher yield of bioactive metabolites, such as organic acids and polyphenols. These bioactive compounds were able to damage the bacterial cell membrane and inhibit the growth of *Cutibacterium acnes*. These results are supported by the research [15], which states that the antimicrobial activity of kombucha increases with the concentration of fermented tea extract used.

Table 4. Kombucha facial soap preparation against *Cutibacterium acnes*

Sample	Clear Zone Diameter (mm)
P1	28.46 ± 0.70[a]
P2	28.06 ± 0.45[a]
P3	26.13 ± 0.67[b]
P4	18.96 ± 0.60 [c]
Commercial Soap	23.46 ± 1.19[d]
Positive Control	31.53 ± 0.38[e]
[a, b, c, d] in the same column show a real difference based on the statistical test used (p < 0.05).	

The ANOVA test showed that variations in kombucha concentration in the soap had a significant effect on the diameter of the inhibition zone against *Cutibacterium acnes* ($p < 0.05$). The highest average inhibition zone diameter was obtained in the positive control group (315.33 ± 3.79 mm), followed by P1 with 284.67 ± 7.02 mm, and P2 with 280.67 ± 4.51 mm. P3 had an inhibition zone diameter of 261.33 ± 6.66 mm, while P4 showed the lowest value of 189.67 ± 6.03 mm. Commercial soap had an inhibition zone diameter of 234.67 ± 11.93 mm. The Bonferroni post-hoc test showed that all groups were significantly different, except for P1 and P2, which were not significantly different ($p > 0.05$).

The antibacterial activity test on green tea kombucha soap was carried out by observing the inhibition zone after 48 hours of anaerobic incubation. The positive control produced the largest inhibition zone, while the formula without active ingredients (P4) showed the smallest (18.97 mm). Formulas containing kombucha demonstrated higher activity, with P3 at 26.13 mm, P2 at 28.07 mm, and P1 at 28.47 mm. These results show that increasing kombucha concentration increases biomass and active metabolite content, which enhances antibacterial activity against *Cutibacterium acnes*.

The increased concentration of kombucha in the formulation results in a greater inhibition zone. The concentration of 20% showed the lowest inhibition (20.47 ° 0.42 mm), while the concentrations of 30% (25.47 ° 0.55 mm) and 40% (28.73 ° 0.67 mm) produced larger zones. The difference between 30% and 40% is insignificant, indicating the saturation of the active compound. This occurs when the target bacteria or their inhibitory mechanisms reach their maximum capacity. Concentrations of 30% and 40% provide equally strong antibacterial activity, suggesting that 30% is the optimal point. Further increase does not significantly enhance the effect due to the saturation of polyphenols, organic acids and other metabolites. Study by [8] states that the antibacterial effect of kombucha results from the synergy of bioactive compounds. EGCG from green tea damages bacterial membranes and interferes with enzymes, while fermentation products such as acetic acid and gluconic acid lower the pH, creating an unfavorable environment for anaerobic bacteria. Ethanol formed during subsequent fermentation contributes by denaturing bacterial proteins.

In soap formulations, surfactants improve the distribution and penetration of active compounds into bacterial biofilms, resulting in a stronger antibacterial effect than kombucha alone. Report by [15] said that the existence of synergistic interactions between kombucha metabolites and surfactants. Overall, green tea kombucha soap shows strong potential as a natural antibacterial cleanser. Its effectiveness is supported by surfactants that facilitate penetration and glycerin, which improves moisture retention, foam stability and distribution of active ingredients. Study by [15] confirmed that glycerin improves the physical quality and stability of liquid soap formulations.

The limitations of this study include antibacterial testing only performed in vitro against *Cutibacterium acnes*, without direct testing on human skin, and the variability of phenolic content in the soap due to the non-standardized kombucha fermentation process. Additionally, the study has not yet evaluated the stability of the formulation during storage or its potential for skin irritation. Future research potential includes clinical trials on humans to assess the effectiveness and safety of the soap, standardization of kombucha fermentation to maintain optimal active compound content, development of other cosmetic formulations such as gels or toners, and studies on the mechanism of action of phenolics against *Cutibacterium acnes* and their effect on skin microbiota balance.

4 Conclusion

Based on the research results, facial soap with green tea kombucha extract has proven antibacterial activity against *Cutibacterium acnes*. Formula P1 (40%) showed the largest inhibition zone (28.47 mm), comparable to the positive control clindamycin (31.53 mm). However, formula P2 was preferred by panelists organoleptically despite its lower antibacterial activity, indicating the need for optimisation to achieve a balance between formulation effectiveness and comfort.

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