BIOLOGY, MEDICINE, & NATURAL PRODUCT CHEMISTRY ISSN 2089-6514 (paper)
Volume 14, Number 2, October 2025 | Pages: 1143-1150 | DOI: 10.14421/biomedich.2025.142.1143-1150 ISSN 2540-9328 (online)

Effect of Different Concentration of SCOBY Kombucha as an Anti-acne Against *Staphylococcus aureus* in The Ear of Mice (*Mus musculus*)

Anggun Putri Ferdyanti[1], Eva Agustina[2], Hanik Faizah[2], Risa Purnamasari[1]*
[1]Biology Education Department; [2]Biology Department, Faculty of Science and Technology, UIN Sunan Ampel Surabaya
Jl. Dr. Ir. H. Soekarno No.682, Gn. Anyar, Kec. Gn. Anyar, Surabaya, Jawa Timur 60294.

Corresponding author*
risauinsby@gmail.com

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

Abstract

SCOBY (*Symbiotic Culture of Bacteria and Yeast)* results from symbiosis between yeast and bacteria during fermentation. SCOBY kombucha gel combined with orange peel extract has antibacterial compounds that can inhibit the growth of *S. aureus* bacteria. The method in this study was to make variations in the concentration of SCOBY in the gel and inject *S. aureus* bacteria into the ears of mice. After 2x24 hours, the diameter of the inflammation caused was observed and SCOBY kombucha gel was applied (0%, 2%, 4%, 8%, 10%, and 12%), positive control (Mediclin 1%), and negative control (without gel application). The results showed that the higher the concentration of SCOBY, the faster the healing activity of the gel against *S. aureus*. SCOBY kombucha gel has the fastest healing activity in inhibiting acne pathogen *S. aureus* 12% SCOBY gel. Inflammation in the ears of mice treated with 12% SCOBY gel had healed by the 90th hour, while in other groups of mice it had only healed at the last observation hour, namely the 114th hour.

Keywords: Acne; Gel; *Mus musculus*; SCOBY; *S. aureus*.

INTRODUCTION

Symbiotic Culture of Bacteria and Yeast (SCOBY) results from kombucha fermentation formed from symbiosis between yeast and bacteria with the help of sugar as a substrate (Falahuddin & Apriani, 2017). The most dominant bacteria in the kombucha fermentation process are *Acetobacter sp*. and yeast which play a role in the genus *Saccharomyces sp*. The symbiosis between the two will produce polysaccharides in the form of white fibers that form a thick layer of sheet-like nata (Fajriah, 2015). During the fermentation process, the *Symbiotic Culture of Bacteria and Yeast* (SCOBY) will multiply and continue to thicken by absorbing the contents formed in the green tea kombucha solution. SCOBY kombucha is a culture used for further tea fermentation (Rommana & Hanna, 2016). Kombucha products have been widely developed as beauty products, especially as antiacne because they contain antibacterial compounds. Research by Yan *et al*. (2023) states that gels containing kombucha can have potential as antimicrobials and treat inflammation.

Gel preparations are more effective for acne treatment because they are polar, so they are easily rinsed with water and do not store oil, which is feared to aggravate acne on the skin (Sasanti *et al*., 2012). Gel preparation is a topical treatment that will be more easily absorbed by acne skin so that the drug's effectiveness can be achieved properly (Ardana *et al*., 2015). In making anti acne gel, it is necessary to add methyl paraben as a preservative that contains antibacterials so that it can inhibit the growth of acne bacteria. To minimize the irritation that may be caused by synthetic methyl parabens, natural methyl parabens that contain medicinal compounds such as oranges can be used. Not only the fruit, orange peel is also widely used as a face mask because of its antioxidant content which is quite good for the skin (Friatna *et al*., 2011).

Symbiotic Culture of Bacteria and Yeast (SCOBY) kombucha gel combined with orange peel extract is believed to have potential as an antiacne against *S. aureus*. This is because each of these ingredients has antibacterial compounds that can inhibit the growth of pathogenic bacteria, especially acne bacteria. Research by Malik *et al.* (2021) also stated in the results of their research that orange peel extract has a relatively high antimicrobial activity, especially on *S. aureus* bacteria. *S. aureus* bacteria are one of the pathogens that cause acne. According to the research of Dhillon and Varshney (2013), it was found that in acne lesions, several bacteria dominated, namely *Staphylococcus aureus*, as much as 45% and *Propiniobacterium acnes*, as much as 32%. According to research by Imasari and Emasari (2021), it was found that *S. aureus* causes acne with a percentage of 79% on human skin. *S. aureus* lives a lot on the skin's

surface, one of which is on facial skin. *S. aureus* can secrete protease, lipase, hyaluronidase, and kinase enzymes that cause tissue damage resulting in inflammation (Rosalina, 2018).

This study will be conducted in vivo by injecting *S. aureus* bacteria into the ears of mice because the ears of mice have a thin surface so that the inflammation caused is more visible. *S. aureus* is able to infect the skin by entering the stratum corneum, causing toxins that can cause skin infections. The skin will produce excess oil so that the pores are clogged by a collection of fat mixed with sweat, dust and other dirt and cause inflammation known as acne (Pratami *et al.*, 2013). Inflammation in the ears of mice will be smeared with a combination gel of orange peel extract and different concentrations of SCOBY kombucha.

MATERIALS AND METHODS

Materials

The materials needed in this study include *S. aureus* bacteria, green tea, sugar, water, xylazine, carbomer, TEA, glycerin, orange peel, ethyl acetate, distilled water, physiological solution NaCl, Na2CO3, nutrient agar media, Mediclin 1%, male mice, husks, mice feed and drink, label paper, and tissue.

The tools needed in this study include analytical scales (OHAUS PX224), 1 cc syringe, erlenmeyer (Phyrex®), plastic wrap, petri dish, beaker glass, test tube, test tube rack, ose needle, measuring cup, stirring rod, drop pipette, oven, cotton, hotplate (Jisico J-HMS), micropipette (Sorenson) Laminar Air Flow (LAF) (Robust LAF140).

Procedures

Animal Preparation

The test animals used in this study were white mice (Mus musculus) male DDY strain aged 6 months with body weight (BW) 26-32 g. The number of mice used was 24 in healthy condition obtained from the Farma Veterinary Centre (PUSVETMA) Surabaya. Prior to treatment, the mice were acclimatised for 2 months with ad libitum feeding and drinking and replacement of husks every 3 days.

Preparation of Orange Peel Extract

Extract preparation was carried out by maceration for 24 hours. The oranges used were siam oranges (Citrus nobilis) which were peeled and cut into small pieces to be put in the oven. The dried orange peel was then blended until it became smooth. Orange peels that have been finely filtered to take fine powder and weighed. In this study, 100gr of orange peel fine powder was obtained and then macerated for 24 hours using ethyl acetate which was added as much as 300ml. The selection of ethyl acetate as a solvent in orange peel because ethyl acetate can bind compounds that have potential in antibacterial activity such as polyphenols and flavonoids (Nina, 2014). The results of maceration are then evaporated using a rotary evaporator so that a thick extract of orange peel with ethyl acetate solvent will be obtained. Then calculated the yield using the following formula:

$$\%\, randemen = \frac{berat\ ekstrak\ (g)}{berat\ sampel\ (g)}\ x\ 100\%$$

Preparation of SCOBY Kombucha

Propagation of SCOBY kombucha is done by boiling 2000 ml of water until it boils, then adding sugar as much as 10% of the amount of water used (200 grams) and adding tea to 0.5% of the amount of water (10 grams). After all the ingredients were mixed, the solution was filtered and the filtrate was put in a large jar and covered with aluminium foil for a few minutes until the temperature of the solution decreased. Next, the kombucha culture or SCOBY was added to the jar to account for 10% of the water volume (200 ml) and sealed for up to 14 days. During fermentation, SCOBY will grow bigger and thicker by utilising glucose from the kombucha tea solution (Nurikasari et al., 2017).

SCOBY Kombucha Gel Preparation

Preparation of Gel Base

Preparation of gel base is done by weighing carbomer 940 as much as 10 grams using a watch glass then mixed with 500 ml of distilled water in a glass beaker and stirred quickly on a hotplate until homogeneous. After forming the gel mass, 10 drops of TEA and methyl paraben were added. Methyl paraben was dissolved first by weighing 1 gram and put in a glass beaker then dissolved in 25 ml of distilled water. After dissolving, methyl paraben was added to the first mixture then glycerin was added using a measuring cup as much as 5 ml and stirred until homogeneous while heating (Putri, 2017; Rinawati et al., 2022).

Preparation of SCOBY Gel Variations

Variations of SCOBY kombucha begin with harvesting SCOBY kombucha during the fermentation process. SCOBY kombucha on the surface of the tea is taken then blended and filtered until smooth. SCOBY kombucha gel was made with variations of 2%, 4%, 8%, 10%, and 12%. The addition of SCOBY weight is calculated based on w/w so that 2 g, 4 g, 8 g, 10 g, and 12 g are needed. Each SCOBY that has been weighed is added to the gel base up to 100 g and homogenised. The variation of kombucha SCOBY concentration added to the gel can be calculated using the following formula:

$$M1.V1 = M2.V2$$

Preparation of Bacterial Suspension

Aseptically, S. aureus bacteria were inoculated with the scratch method as much as one ose on NA media. Then incubated at 37°C for 1 x 24 hours (Yanti & Mitika,

2017). Then make a physiological solution by dissolving 0.85 grams of NaCl in 100 ml of distilled water and put in an autoclave at a temperature of 121° C. After that, it is cooled to a temperature of 45-50° C before use (Yanti & Mitika, 2017).

Bacteria rejuvenated on agar media are taken using a sterile ose heated with a bunsen burner and then suspended in a test tube containing NaCl physiological solution. Test tubes containing bacteria and physiological solution were then vortexed until homogeneous and then the absorbance value was measured using a 625 nm wavelength spectrophotometer. The turbidity measurement results must comply with the Mc. Farland standard, namely with an absorbance of 0.08-0.5 (Rosmania & Yanti-, 2020).

Injection of Bacteria into the Ear of Mice (Mus musculus)

Acne on the ears of mice is induced by injection using a 1 cc syringe. Before injection in the ears of mice, mice were anaesthetised using xylazine in doses of 0.05 ml. Furthermore, mice were injected with a suspension of S. aureus bacteria in the right ear as much as 0.2 ml and left for 2x24 hours until inflammation appeared in the ears of mice. (Fitriani et al., 2022).

Application of SCOBY Kombucha Gel Combined with Orange Peel Extract

The application of SCOBY kombucha gel was carried out after 2x24 hours of bacterial administration to the ears of mice. Gel application is given according to the concentration of each group where in this study it was divided into 8 treatment groups, namely 1 positive control group using Mediclin, 1 negative control group, and 5 treatment groups (2%, 4%, 8%, 10%, and 12%). The application was carried out 2 x a day, namely in the morning and evening as much as the tip of an aluminium spoon equally between mice.

Measurement of Inflammation Diameter

Measurement of the diameter of the inflammation was carried out after 2x24 hours of bacterial injection until the inflammation healed. Measurements were made using a calliper every 3 hours from 9am to 3pm. Inflammation of the mice's ears was measured by holding the mice's ears and then measuring the width of the acne inflammation starting from the base of the ear to the tip of the outer ear using a calliper. Parameters of healing of inflammation in the ears of mice can be seen from the shrinking of redness on the skin of the ears of mice.

Data Analys

Inflammation diameter data will be tested for Normal Distribution and Homogeneity Test with the provision of sig value >0.05 which indicates that the data is normally distributed and homogeneous (Purnomo, 2016). If the data is normally distributed and homogeneous, then the test continues with one-way analysis of variance or *One Way* ANOVA to determine the average difference between treatment groups. Data that are not normally distributed and inhomogeneous will continue with the *Kruskal Wallis* test to see significant differences between groups. If the *Kruskal wallis* test obtained significant changes, the *Mann-Whitney* test will be continued to determine significant differences between treatment groups.

RESULTS AND DISCUSSION

The right ear of the mice was injected with *S. aureus* bacteria and left for 2x24 hours. After that, the gel was applied to the inflammation caused. From the results of the average diameter of each gel application, the difference in inflammation reduction will be recorded for each observation. The results of inflammation healing at each time can be seen in Table 1.

Table 1. Average healing of inflammatory acne.

Concentration		Bacterial Growth.				Gel Application Process.						
		24 hour	42 hour	45 hour	48 hour	66 hour	69 hour	72 hour	90 hour	93 hour	96 hour	114 hour
P1	I		0.09	0.02	0.02							
	R					0.09	0.03	0.03	0.14	0.04	0.08	0.15
	D	0.42	0.52	0.54	0.56	0.47	0.44	0.41	0.27	0.23	0.15	0.00
P2	I		0.03	0.05	0.01							
	R					0.05	0.03	0.03	0.08	0.04	0.04	0.15
	D	0.35	0.38	0.43	0.44	0.39	0.36	0.33	0.25	0.22	0.17	0.00
P3	I		0.17	0.01	0.01							
	R					0.06	0.04	0.04	0.12	0.05	0.05	0.10
	D	0.32	0.49	0.51	0.54	0.45	0.41	0.37	0.28	0.19	0.14	0.00
P4	I		0.07	0.02	0.01							
	R					0.08	0.03	0.04	0.10	0.05	0.10	0.06
	D	0.40	0.47	0.48	0.50	0.42	0.39	0.34	0.25	0.20	0.10	0.00
P5	I		0.18	0.02	0.02							
	R					0.09	0.03	0.04	0.10	0.07	0.06	0.16
	D	0.36	0.54	0.56	0.58	0.49	0.45	0.41	0.26	0.24	0.18	0.00
P6	I		0.10	0.02	0.01							
	R					0.07	0.04	0.04	0.17	0.12	0.06	0.02
	D	0.39	0.49	0.50	0.52	0.45	0.41	0.37	0.21	0.08	0.02	0.00
P7	I		0.12	0.02	0.03							
	R					0.07	0.06	0.08	0.31	0.00	0.00	0.00
	D	0.35	0.47	0.49	0.52	0.44	0.38	0.31	0.00	0.00	0.00	0.00
P8	I		0.06	0.03	0.02							
	R					0.02	0.02	0.02	0.04	0.03	0.03	0.04
	D	0.39	0.45	0.48	0.49	0.48	0.46	0.44	0.40	0.36	0.34	0.30

Note: (Increased): increase in inflammatory diameter during acne bacteria growth (24-48th hour); R (Reduction): reduction/decrease in inflammatory diameter during the gel application process (48-114th hour); D (Diameter): Diameter of acne inflammation Description: P = Treatment (*) = Sign of significant difference in Mann-Whitney test (Sig.p<0.05), P1 = Mediclin 1%, P2 = SCOBY 0% Citrus Peel Extract, P3 = SCOBY 2% Citrus Peel Extract, P4 = SCOBY 4% Citrus Peel Extract, P5 = SCOBY 8% Citrus Peel Extract, P6 = SCOBY 10% Citrus Peel Extract, P7 = SCOBY 12% Citrus Peel Extract, P8 = No Gel Application.

The inflammatory diameter will get bigger for 48 hours because at this time, it will be the growth period of *S. aureus* bacteria in the right ear of mice. This happens because *S. aureus* bacteria can damage skin tissue by secreting chemical compounds that can destroy pore walls so that fatty acids and oils are clogged and harden into acne bumps (Miratunnisa *et al*., 2015). After 48 hours, the gel was applied according to each treatment group. Based on Table 1, it can be seen that the gel that has the fastest healing activity against *S. aureus* bacteria is P8 (SCOBY 12%). This is because in P8 the inflammation has healed with an average of 0.00 at the 90th hour. Whereas in the other treatment groups, the inflammation was completely healed at the 114th hour, which was the last hour of observation. To determine the differences in each treatment group, SPSS statistical analysis started with normality and homogeneity tests to determine whether the data were normally distributed.

The results of the normality test were carried out using the difference data taken from the initial data of inflammation before the gel was applied and the data when the first treatment group recovered, namely at 48 hours and 90 hours. The results can be seen in table 1 which shows that the data at the 90th hour is not normally distributed because of the treatments tested some treatments have a significant value of 0.00 <0.05. Therefore, to find out the differences in each treatment can be continued with non-parametric test analysis, namely *Kruskal Wallis* and *Mann Whitney* tests can be continued in Table 2

Table 2. Initial Healing Analysis Results (90th hour).

Treatments	48 hour	90 hour	Average Difference	% acne healing	*Kruskal Wallis*
P1	0.56	0,27	0.29	52%	.018
P2	0.44	0,25	0.19	43%	
P3	0.54	0,28	0.26	48%	
P4	0.50	0,25	0.25	50%	
P5	0.53	0,26	0.27	51%	
P6	0.52	0,21	0.31	60%	
P7	0.52	0,00	0.52	100%	
P8	0.49	0,40	0.10	20%	

Table 3. *Mann-Whitney* Test Results 90th hour.

Treatment	1	2	3	4	5	6	7
P1							
P2	0,050						
P3	0,513	0,184					
P4	0,507	0,825	0,507				
P5	0,376	0,050	0,513	0,507			
P6	0,376	0,050	0,513	0,507	0,127		
P7	0,046*	0,046*	0,046*	0,043*	0,046*	0,046*	
P8	0,050	0,050	0,050	0,050	0,050	0,050	0,046*

Based on Table 2, the significance value of the *Kruskal wallis* test results on the right ear of mice injected with *S. aureus* bacteria is 0.018 <0.05, which means that the treatment of mice's ears makes a significant difference to the healing of acne inflammation of mice injected with *S. aureus*. This is because each SCOBY gel has different healing activity. Based on the results of the *Mann-Whitney* test, it shows that all treatment groups have a significant value <0.05 against P7. This means that all treatment groups in this study have different healing activities from P7. This is by table 2 where at the 90th hour the diameter of the P7 inflammation was 0.00 while the other treatment groups still showed 0.21-0.35 cm. Table 2 shows that at the 90th hour the P7 group has the largest average difference value among others, which is 0.52 cm. This means the P7 group has the fastest healing activity compared to other treatments. P7 has a faster healing activity than P6 (10% SCOBY gel). This can be seen in Table 2 which shows the difference in the average diameter of inflammation in P6 is 0.31 cm with an inflammatory diameter of 0.21 at the 90th hour. This is consistent with the greater percentage of inflammation healing in P7, which is 100% while the percentage of P6 is 60%.

P1 (Mediclin positive control) has faster healing activity compared to SCOBY gel groups P3-P6, gel group without SCOBY (P2), and P8 (without gel administration). This can be seen in Table 2, P1 has a greater average difference in inflammation, which is 0.29 cm with an inflammation diameter of 0.27 at the 90th hour. In P2, the diameter of inflammation at the 90th hour was still greater than in P1, which was 0.25 cm. This is by the percentage in Table 2 which shows that P1 has greater healing activity, 52% while P2 is 43%. The treatment groups with various concentrations of SCOBY P3 (SCOBY 2%), P4 (SCOBY 4%), and P5 (SCOBY 8%) had faster healing activity than the gel treatment without SCOBY P2 (SCOBY 0% orange peel extract). This can be seen from Table 2 which shows that the difference in the average diameter of inflammation in the SCOBY gel treatment group at the 90th hour is greater than the gel treatment group without SCOBY. P3, P4, and P5 at the 90th hour had an inflammatory diameter in the 0.25 -0.28 cm range. While P2 still showed a larger diameter, namely 0.25. This is also in accordance with the healing percentage of SCOBY treatment groups P3, P4, and P5 which is greater than P2. P3, P4, and P5 had healing percentages of around 48-51%, while P2 only reached 43%. This shows that the addition of SCOBY extract has an effect on the healing process of inflammatory acne.

The negative control in this study was P8 (without gel application). Table 1 shows that the average difference in inflammatory diameter from the beginning of gel application to the 90th hour is 0.10 cm. This means that P8 has a healing activity that tends to be longer than the other treatment groups. At the 90th hour, P8 had a larger inflammatory diameter than the other treatment groups, which was 0.40 cm with a healing percentage that only reached 20%. In addition to being seen from the difference in inflammatory diameter healing and the percentage of healing, the effectiveness of the gel in healing can be seen in the mean rank between treatments as shown in Figure 1

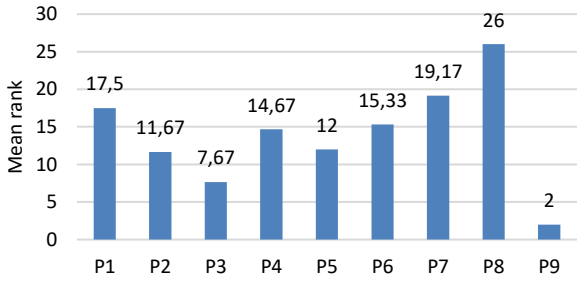

Figure 1. Effect of Gel Administration on *S. aureus* Inflammation

P = Treatment (*) = Sign of significant difference in Mann-Whitney test (Sig.p<0.05), P1 = Mediclin 1%, P2 = SCOBY 0% Citrus Peel Extract, P3 = SCOBY 2% Citrus Peel Extract, P4 = SCOBY 4% Citrus Peel Extract, P5 = SCOBY 8% Citrus Peel Extract, P6 = SCOBY 10% Citrus Peel Extract, P7 = SCOBY 12% Citrus Peel Extract, P8 = No Gel Application.

Based on Figure 1, it can be seen that the highest healing activity is in P7 (12% SCOBY Gel) with a value of 26. The higher the value in Figure 1, the greater the effectiveness of the treatment group. P7. has the best healing activity compared to other treatment groups. This is by Table 4.12 which shows that the diameter of acne

inflammation in P7 has healed faster than other groups. P7 has shown an inflammatory diameter of 0.00 at the 90th hour observation marked by the fading of redness on the ears of mice. This was influenced by the amount of SCOBY added to the gel. In P7, the content of SCOBY added is more than the other concentration variations. The slowest treatment group in the healing process of inflammatory diameter is P8 (untreated group). Judging from Figure 1 which shows the mean rank value in P7 is the lowest value compared to other treatment groups. The mean rank value of P8 is 2, which means that this group has a slow effectiveness in healing inflammation caused by *S. aureus*. This can occur because in P8 the inflammation is not treated so there is nothing that plays a role in accelerating the healing process of acne by *S. aureus*. The treatment group without gel application means that the inflammation will be allowed to heal itself without encouragement by active substances so that the healing process will take longer. This is consistent with Table 1, where group P8 still experienced a reduction in inflammatory diameter after 48 hours. However, the reduction in inflammatory diameter at each observation tends to be slower so that at the last observation (114th hour) P8 still showed an inflammatory diameter of 0.19 cm. This happens because of the body's defence system that can fight against pathogenic bacteria that cause acne.

Discussion

Based on Figure 1 and the description above, the higher the concentration of SCOBY kombucha, the faster the healing process on acne caused by *S. aureus*. It can be concluded that the fastest healing process of the right ear injected by *S. aureus* is P7 (12% SCOBY gel). This is shown in the diameter of acne inflammation in 12% SCOBY gel at the 90th hour which has successfully cured acne inflammation up to 0.00 cm. Based on Rezaldi *et al.* (2022), 40% kombucha concentration can inhibit *S. aureus* up to 15.7 mm which is classified as a strong category. Whereas the lower concentration of kombucha (30%) could only inhibit *S. aureus* up to 7.9 mm which is classified as a medium inhibition zone category. This is due to SCOBY kombucha which is the result of kombucha fermentation. The higher the content of kombucha compounds, the more compounds are absorbed by SCOBY kombucha. Therefore, the higher the concentration of SCOBY added, the greater the antibacterial ability.

The antibacterial ability of SCOBY kombucha comes from the basic ingredient, green tea. Based on the research of Andaryekti *et al*. (2015) it was showed that green tea leaf extract gel (*Camelia nobilis*) with a concentration of 10% already has antibacterial activity with a clear zone of 17 mm around *S. aureus* bacteria. This shows that green tea can inhibit *S. aureus* with moderate inhibition. This is because green tea contains flavonoids which can be an antibacterial agent. In addition, SCOBY kombucha contains yeast and *Acetobacter xylinum* bacteria which are *lactic acid bacteria* (LAB). *A. xylinum* will convert fructose and glucose in yeast into ethanol and will produce acetic acid at the end of fermentation. Not only acetic acid, green tea kombucha fermentation will also produce other types of acids that can reduce pH and will damage the bacterial cell wall. The fermented acids will be absorbed by the new SCOBY kombucha layer to form a cellulose layer that floats above the fermentation solution. Therefore, SCOBY has antibacterial potential by damaging the lipid bilayer structure of bacteria by introducing protons from the acid into the bacterial cytoplasm. This will make the cytoplasm acidic conditions and cause protein denaturation until cell death. The higher the organic acid, the higher the ability to inhibit the growth of pathogenic bacteria (Abdillah *et al.*, 2022).

Fermentation of green tea kombucha produces secondary metabolites that will be absorbed by SCOBY and can damage peptidoglycan in the bacterial cell wall secondary metabolites in SCOBY are easier to have antibacterial potential on gram-positive bacteria because their cell walls contain higher peptidoglycan. In the research of Abdillah *et al*. (2022) stated that the highest antibacterial activity of 20% bay flower kombucha was on *S. aureus* with an average inhibition zone of 17.13 mm (strong category) while on *S. epididymis, P. aeruginosa*, and *E. coli* had an inhibition zone diameter of 13-15 mm. This is due to the higher permeability of the cell wall so that active compounds easily damaged it. *S. aureus* has a thin bacterial cell wall so that components that are antibacterial can more easily penetrate the bacterial cell wall to destroy the peptidoglycan contained in the cell wall of pathogenic bacteria (Rezaldi *et al*., 2022).

The addition of siamese orange peel extract certainly strengthens the antibacterial activity of SCOBY kombucha gel against *S. aureus*. Citrus peel extract contains antioxidant compounds and flavonoids that can counteract free radicals so that it plays a role in antibacterial. According to Dewi (2019), orange peel extract (*Citrus nobilis*) can be used as a preservative because at a concentration of 10 mg/mL, total phenolic content of 55.46 mg*GAE and antioxidant activity of 66.41% were obtained. This shows that orange peel extract has good antibacterial properties. In Dewi's research (2019) it was also mentioned that orange peel extract was able to inhibit *S. aureus* as evidenced by the appearance of a clear zone of 16 mm around the bacteria (medium category). Phenol compounds can inhibit cell wall formation and essential oils can inhibit bacteria by disrupting the process of forming cell membranes and walls (Mehmood *et al.,* 2015). Therefore, the combination of orange peel extract and SCOBY kombucha will certainly be better in reducing inflammation caused by *S. aureus*.

Based on the results obtained in this study, SCOBY kombucha gel combined with orange peel extract tested in vivo in mice can reduce the diameter of inflammation caused by *S. aureus*. The use of orange peel extract in SCOBY gel is known to have high antioxidant activity. In addition, this addition is done to utilise orange peel which is considered less valuable waste. SCOBY gel combined with orange peel extract has good healing effectiveness by reducing the diameter of inflammation in the ears of mice caused by *S. aureus*. From this study, a new alternative is produced as a solution in healing acne using natural ingredients that have less side effects. This study needs further research to test the shelf life and stability of the product to ensure product quality in long-term storage.

CONCLUSIONS

The difference in the addition of SCOBY kombucha to the gel can affect the inflammatory healing activity characterised by a decrease in the diameter of acne inflammation in the ears of mice infected with *S. aureus*. The most effective SCOBY kombucha gel in reducing the diameter of inflammation caused by *S. aureus* is 12% SCOBY gel combined with orange peel extract. The higher the concentration of SCOBY added, the faster the healing activity. Meanwhile, the lowest concentration of SCOBY (2% SCOBY) had a slower healing activity compared to the gel with a higher concentration of SCOBY.

Competing Interests: The authors declare that there are no competing interests.

REFERENCES

Abdilah, N. A., Rezaldi, F., Kusumiyati, K., Sasmita, H., & Somantri, U. W. (2022). Aktivitas Antibakteri Kombucha Bunga Telang (*Clitoria Ternatea L*) Yang Difermentasi Dengan Gula Aren Pada Konsentrasi Berbeda. *Tirtayasa Medical Journal*, 1(2), 29-39. Doi: https://doi.org/10.52742/tmj.v1i2.15139

Ardana, M., Aeyni, V., & Ibrahim, A. (2015). Formulasi Dan Optimasi Basis Gel HPMC (Hidroxy Propyl Methyl Cellulose) Dengan Berbagai Variasi Konsentrasi. *Journal of Tropical Pharmacy and Chemistry*, 3(2), 101-108. Doi: https://doi.org/10.25026/jtpc.v3i2.95

Dewi, A. D. R. (2019). Aktivitas Antioksidan dan Antibakteri Ekstrak Kulit Jeruk Manis dan Aplikasinya Sebagai Pengawet Pangan. *Jurnal Teknologi & Industri Pangan*, 30(1), 83-90. Doi: https://doi.org/10.6066/jtip.2019.30.1.83

Dhillon, K. S., & Varshney, K. R. (2013). Study Of Microbiological Spectrum In Acne Vulgaris: An In Vitro Study. *Journal App. Med. Sci,* 1(6), 724-727. Doi: 10.36347/sjams.2013.v01i06.0017

Fajriyah, Y. D. N. (2015). Pengaruh Kombucha Sari Buah Belimbing Wuluh (*Averrhoa bilimbi L*.) Terhadap Pertumbuhan Bakteri *Escherichia coli* Serta Pemanfaatannya Sebagai Buku. *Skripsi.* Universitas Mataram.

Falahhudin, I., Apriani, I., & Nurfadilah. (2017). Pengaruh Proses Fermentasi Kombucha Daun Sirsak (Annona Muricata L.) Terhadap Kadar Vitamin C. Jurnal Biota, 3(2), 90-95. Doi: https://doi.org/10.19109/biota.v3i2.1323

Fitriani, I., Lubis, M. S., Yuniarti, R., & Rahayu, Y. P. (2022). Perbandingan Efektivitas Produk Topikal Anti Jerawat Terhadap Tikus Putih Jantan (*Rattus novergicus*) Secara *In Vivo*. *Farmasainkes: Jurnal Farmasi, Sains, Dan Kesehatan,* 2(1), 67-76. 60. Doi: https://doi.org/10.32696/fjfsk.v2i1.1375

Friatna, E. R., Rizqi, A., & Hidayah, T. (2011). Uji Aktivitas Antioksidan Pada Kulit Jeruk Manis (*Citrus nobilis*) Sebagai Alternatif Bahan Pembuatan Masker Wajah. *Pelita-Jurnal Penelitian Mahasiswa* UNY, 1(2), 59.

Imasari, T., & Emasari, F. (2021). T Deteksi Bakteri *Staphylococcus sp.* Penyebab Jerawat Dengan Tingkat Pengetahuan Perawatan Wajah Pada Siswa Kelas Xi Di Smk Negeri 1 Pagerwojo. *Jurnal Sintesis: Penelitian Sains, Terapan Dan Analisisnya*, 2(2), 58-65. Doi: https://doi.org/10.56399/jst.v2i2.20

Malik, W. A., & Javed, S. (2021). Biochemical Characterization Of Cellulase From *Bacillus Subtilis* Strain And Its Effect On Digestibility And Structural Modifications Of Lignocellulose Rich Biomass. *Frontiers In Bioengineering And Biotechnology,* 9. Doi: https://doi.org/10.3389/fbioe.2021.800265

Mehmood, B., Dar, K. K., Ali, S., Awan, U. A., Nayyer, A. Q., Ghous, T., & Andleeb, S. (2015). In Vitro Assessment Of Antioxidant, Antibacterial And Phytochemical Analysis Of Peel Of *Citrus Nobilis*. *Pakistan Journal Of Pharmaceutical Sciences*, 28(1).

Miratunnisa, Hajar, S., & Mulqie, L.. (2015). Uji Aktivitas Antibakteri Ekstrak Etanol Kulit Kentang (*Solanum Tuberosum L*) Terhadap *Propionibacterium*. *Prosiding Penelitian SPESIA Unisba*. Universitas Islam Bandung.

Nina, H. F. (2014). Isolasi Dan Karakterisasi Senyawa Metabolit Sekunder Dari Ekstrak Etil Asetat Albedo Buah Pamelo (*Citrus maxima (Burm.) Merr.). Skripsi*. Universitas Andalas.

Nurikasari, M., Puspitasari, Y., & Siwi, R. P. Y. (2017). Characterization And Analysis Kombucha Tea Antioxidant Activity Based On Long Fermentation As A 64 Beverage Functional. *Journal Of Global Research In Public Health*, 2(2), 90-96. Doi: Prefix 10.30994

Pratami, H. A., Apriliana, E., & Rukmono, P. (2013). Identifikasi Mikroorganisme Pada Tangan Tenaga Medis Dan Paramedis Di Unit Perinatologi Rumah Sakit Abdul Moeloek Bandar Lampung. *Medical Journal of Lampung University*, 2(5), 85-94.

Putri, H. A. (2017). Pengaruh Konsentrasi Pelarut Etanol Terhadap Hasil Rendemen Ekstrak Daun Kemangi (*Ocimum Sanctum*) Sebagai Zat Antiseptik Pada Pembuatan Gel Hansanitizer. *Skripsi*. Universitas Muhammadiyah

Rinawati, R., Tirta, I., Budiarti, B., Putri, D. A. E., & Kurniaty, I. (2022). Pengaruh Konsentrasi Ekstrak Kental Daun Kanyere (*Bridelia Monoica (L). Merr)* Sebagai Antiinflamasi Dalam Sediaan Gel Luka Bakar. *Jurnal Teknologi*, 14(1), 79-90. Doi: https://dx.doi.org/10.24853/jurtek.14.1.79-90

Rezaldi, F., Junaedi, C., Ningtias, R. Y., Pertiwi, F. D., Sasmita, H., Somantri, U. W., & Fathurrohim, M. F. (2022). Antibakteri *Staphylococcus Aureus* dari Sediaan Sabun Mandi Probiotik Kombucha Bunga Telang (*Clitoria Ternatea* L) Sebagai

Produk Bioteknologi. *Jurnal Biotek*, 10(1), 36-51. Doi: https://doi.org/10.24252/jb.v10i1.27027

Rommana & Hanna, (2016). Pengaruh Pemberian Teh Kombucha Terhadap Pertumbuhan *Salmonella Typhi. Jurnal Majori Ty*. 5(5), 48–54.

Rosalina, Z. (2018). Deteksi Gen Coa Pada Methicillin Resistant *Staphylococcus aureus* (MRSA). *Skripsi.* Universitas Muhammadiyah Semarang.

Rosmania, R., & Yanti, F. (2020). Perhitungan Jumlah Bakteri Di Laboratorium Mikrobiologi Menggunakan Pengembangan Metode Spektrofotometri. *Jurnal Penelitian Sains,* 22(2), 76-86. Doi: https://doi.org/10.56064/jps.v22i2.564

Rezaldi, F., Setiadi, T., Nuris, F. F., Jhoni, K. A., Ma'ruf, A., & Rustini, R. (2022). Pelatihan Pembuatan Deodoran Berbahan Aktif Fermentasi Kombucha Bunga Telang Kepada Mahasiswa Farmasi Universitas Mathla'ul Anwar Banten. SEWAGATI: *Jurnal Pengabdian Masyarakat Indonesia,* 1(3), 11-23. https://doi.org/10.56910/sewagati.v1i3.138

Sasanti, T. J., Wibowo, M. S., Fidrianny, I., & Caroline, S. (2012). Formulasi Gel Ekstrak Air Teh Hijau Dan Penentuan Aktivitas Antibakterinya Terhadap *Propionibacterium Acne*. School Of Pharmacy ITB, Bandung.

Yanti, Y. N., & Mitika, S. (2017). Uji Efektivitas Antibakteri Ekstrak Etanol Daun Sambiloto (*Andrographis Paniculata Nees)* Terhadap Bakteri *Staphylococus Aureus. Jurnal Ilmiah Ibnu Sina*, 2(1), 158-168. Doi: https://doi.org/10.36387/jiis.v2i1.93