Biota: Jurnal Ilmiah Ilmu-Ilmu Hayati, Vol. 10(3): 331-344, Oktober 2025
p-ISSN 2527-3221, e-ISSN 2527-323X, https://ojs.uajy.ac.id/index.php/biota/issue/view/497
DOI: 10.24002/biota.v10i3.11282

Effectiveness and Role of Symbiotic Culture of Bacteria and Yeast (SCOBY) Kombucha Gel on Incisional Wound Healing in Mice (*Mus musculus*)

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Abstract

Incision wounds are common occurrences in daily life, and appropriate wound management is essential to promote and accelerate the healing process. Wound care can be performed using topical preparations such as gels containing bioactive compounds. SCOBY kombucha is believed to contain bioactive compounds, such as compounds from green tea (especially polyphenols) and metabolites produced by microbes (organic acids, alcohols, vitamins, etc.) The aim of this research is to determine the effectiveness of SCOBY kombucha gel on the healing of incision wounds in mice (*Mus musculus*). The method used in this research is a Completely Randomized Design (CRD) with 8 treatment groups, including 2 control groups and 6 test groups. The concentrations of SCOBY kombucha gel used are 0%, 2%, 4%, 8%, 10%, and 12%. The SCOBY kombucha gel is applied twice daily until the wound closes. Observation of the incision wound is carried out by measuring the length of the wound that has not yet closed. Based on the conducted research, it was found that SCOBY kombucha gel is effective in healing incision wounds as evidenced by the reduction in wound size due to the bioactive compounds in SCOBY. The most significant wound healing effect was observed with the 12% SCOBY kombucha gel, as there was a significant difference compared to the negative control group, 0%, 2%, 4%, and 8% SCOBY kombucha gels.

Keywords: Incision wound, gel, SCOBY, wound length, wound healing

Submitted: 27 March 2025 ; Revised : 24 July 2025; Accepted : 14 November 2025

Introduction

Skin is the body's largest and outermost organ, functioning as a sensory receptor, secretion organ, and protector of the body. It protects the body from wind, water, sunlight, bacteria, chemical elements, and so forth. Additionally, the skin functions as a sensory organ, controls body temperature, and protects the body from fluid loss (Devi et al., 2023). External disturbances can make the skin prone to damage. Damage to the skin's structure is referred to as a wound. Wounds can be caused by various factors, including injury, surgery, pressure or friction, or even diabetes (Mustamu et al., 2020). Based on their causes, wounds are classified into several types, including bruises (contusions) caused by blunt trauma, abrasions, lacerations (vulnus laceratum), incisions or cuts (vulnus scissum), puncture wounds (vulnus punctum), and chop wounds (vulnus caesum) (Simanungkalit et al., 2019).

One common type of wound encountered in daily life is an incision wound, which is considered a minor wound. Incision wounds result from cutting movements with sharp objects such as knives or razors. These wounds are characterized by smooth edges, shallow depth, and elongated shape (Shoviantari et al., 2021). According to data from the Indonesian BPS (Badan Pusat Statistik), the number of minor wound victims

How to Cite : Nafisah, R. F., Agustina, E., Purnamasari, R., Andiarna, F., & Hidayanti, I. (2025). Effectiveness and Role of Symbiotic Culture of Bacteria and Yeast (SCOBY) Kombucha Gel on Incisional Wound Healing in Mice (Mus musculus). Jurnal Ilmiah Ilmu-Ilmu Hayati 10(3): 331-344.

in Indonesia has increased from 107,743 in 2015 to 130,571 in 2018, and 160,449 in 2022. Generally, incision wounds are require proper care to prevent infection and promote optimal healing. Proper wound care will accelerate the healing process. The use of such medications is one factor that determines the final outcome of the wound healing process. The necessary medications for wound healing are those containing anti-inflammatory or antibiotic properties that can prevent infection and enhance healing (Fauziah & Soniya, 2020). Generally, the form of medication used for wounds is a topical preparation. Topical medications consist of two main components: the active ingredient and the carrier or inactive part. The active ingredient is the component that provides the healing effect, while the carrier is the inactive part that brings the active ingredient into contact with the skin (Yanhendri & Yenny, 2012).

Topical preparations can take the form of gels, creams, pastes, ointments, and lotions. Generally, the topical preparation used as a wound healing agent is a gel. Gel is a semi-solid preparation consisting of a suspension of organic and inorganic particles (Yanhendri & Yenny, 2012). The advantages of gels are their ease of use, non-sticky nature, and easy removal (Kaban et al., 2022). The active ingredients in gels are typically derived from materials with antiseptic and anti-inflammatory properties that can enhance wound healing, such as plant extracts containing secondary metabolites. Secondary metabolites such as tannins, phenolics, and their derivatives are believed to expedite the wound healing process. This is supported by research by Gufron et al., (2023) on incision wound healing with Saliara leaves and research by Milasanti et al., (2023) on Aloe vera extract for incision wound healing. These studies showed that wound healing required 9 days for Saliara leaves and 7 days for Aloe Vera extract. These substances play a role in wound healing due to their secondary metabolites, specifically polyphenols (flavonoids and tannins).

Based on the aforementioned studies, it is known that secondary metabolites like polyphenols affect wound healing. These secondary metabolites can be found in plants that are the base ingredients of kombucha, specifically green tea. Kombucha is a traditional Chinese beverage produced from the fermentation of tea and sugar solutions with the aid of bacteria and yeast (Cardoso et al., 2020). Kombucha contains phenolic compounds and exhibits high antioxidant activity. The fermentation of kombucha produces a thin biofilm layer known as SCOBY (Symbiotic Culture of Bacteria and Yeast). SCOBY results from the symbiosis between bacteria and yeast in kombucha. SCOBY is a thick, dense cellulose layer that can be transferred from one batch of mature kombucha to a new batch of tea. The formation of SCOBY is influenced by the presence of acetic acid bacteria that produce cellulose (Harrison & Curtin, 2021). SCOBY has almost the same content as kombucha.

Kombucha has been known primarily as a health drink, and the resulting ferment, known as SCOBY, is used as a starter for kombucha production. After its usage period, SCOBY becomes a production waste (Chagas et al., 2024). According to Laavanya et al., 2021, the application of SCOBY in new products remains very limited. Previous research has focused on the physical characteristics of SCOBY used in textiles and packaging (Zulaikha Sudin & Azila, 2024). Aditiawati et al., (2021) showed that SCOBY could be transformed into nanocellulose fibers used as raw materials in the textile industry. Thus, this research aims to examine the effectiveness of SCOBY kombucha gel preparations at varying concentrations of 0%, 2%, 4%, 8%, 10%, and 12% on incision wound healing in mice.

Materials and Methods

This study employed an experimental design with a Completely Randomized Design (CRD) consisting of 8 treatment groups, including 2 control groups (a negative control without treatment and a positive control with Bioplacenton gel) and 6 test groups (SCOBY kombucha gel 0%, 2%, 4%, 8%, 10%, and 12%). The test subjects used in this study were male white mice (*Mus musculus*) of the DDY strain, aged 3-5 months, with three repetitions in each treatment group. The number of repetitions is calculated using Federer's formula.

Materials

Kombucha starter, green tea, sugar, water, SCOBY (Symbiotic Culture of Bacteria and Yeast), Carbomer 940, triethanolamine (TEA), glycerin, methylparaben, distilled water, Bioplacenton gel, ketamine, xylazine, male white mice (*Mus musculus*) of the DDY strain, food and water for mice.

SCOBY Kombucha Preparation

2000 ml of water was boiled, and 200 grams of sugar (10% w/v) and 10 grams of dried green tea (0.5% w/v) were added. After straining, the solution was covered and cooled to room temperature. When the tea reached around 28-37°C, 200 ml of kombucha starter culture (10% w/v) was added to the tea solution. The glass container was tightly covered with cloth and rubber bands, then left for 30 days (Nafisah et al., 2023). By the 7th day of fermentation, SCOBY growth was visible, but to achieve optimal physical characteristics, fermentation was proceeded forward to the 60th day. SCOBY, a colony of bacteria and yeast, remained functional even when sliced, cut, or blended. The equipment used had to be sterile and not exposed to extreme temperatures (cold or hot). The refined SCOBY, that has been pureed, was used as a base material for gel preparation.

SCOBY Gel Preparation

10 grams of Carbomer 940 was weighed and sprinkled into 500 ml of preheated distilled water. Carbomer 940 was stirred quickly in a beaker glass to form a gel mass, then 10 drops of TEA (triethanolamine) were added. Next, 1 gram of methylparaben was weighed, dissolved in 25 ml of distilled water, and added to a smaller beaker glass. The mixture was stirred until homogeneous, then poured into the beaker glass containing Carbomer 940 and distilled water. 5 ml of glycerin was added and stirred until the gel base became homogeneous (Rinawati et al., 2022). SCOBY extract concentrations of 0%, 2%, 4%, 8%, 10%, and 12% were prepared by weighing 0 grams, 2 grams, 4 grams, 8 grams, 10 grams, and 12 grams of SCOBY, then added to the gel base to reach a total weight of 100 grams and homogenized.

Incision Wound Preparation on Mice

The test subjects were male white mice of the DDY (Deutschland Denken Yoken) strain, aged 3-5 months and weighing approximately 20-40 grams. The mice were healthy and obtained from the Pusvetma (BBVFP) Surabaya. Before wounding, the mice were acclimatized for 1 week. The mice were anesthetized with ketamine and xylazine at a ketamine : xylazine ratio of 2:1, with a dose of 0.03 ml administered intramuscularly before the incision. The dorsal area of the mice, where the incision would be made, was shaved, and a 0.5 cm long incision was made to a depth reaching the dermis layer, indicated by blood. The incision was made slowly using a sterilized scalpel.

Incision Wound Care

The mice were treated by applying the formulated gel preparation according to the specified concentration. The gel was applied twice daily, uniformly, with each experiment repeated three times over seven days.

Macroscopic Observation

Macroscopic observation was performed by measuring the length of the incision wound in the mice. The length of the unhealed wound was measured using vernier calipers for each treatment.

Data Analysis

Data collection aimed to test the effectiveness of SCOBY kombucha gel in healing incision wounds in mice. The data were statistically analyzed using SPSS software. The data were tested for normality and homogeneity. Data were considered normally distributed and homogeneous if $p > 0.05$. Normally distributed and homogeneous data were further analyzed using One-Way ANOVA (Analysis of Variance) with a 95% confidence level, followed by Duncan's Post Hoc test to identify significantly different treatment groups. Data that were not normally distributed or normally distributed but not homogeneous were tested with the Kruskal-Wallis test. If significant differences were found, the Mann-Whitney test was conducted with a significance level of $p < 0.05$ to determine differences between treatments.

Results and Discussion

Figure 1. SCOBY Produced During 60 Days of Fermentation

Antibiotics are commonly used in wound healing treatments, especially for open wounds or those infected by bacteria, due to their ability to rapidly eliminate microorganisms. However, continuous use of antibiotics can lead to bacterial resistance (Cahyadi et al., 2019). This resistance raises concerns about relying on antibiotics as a solution for wound healing. As an alternative, natural substances can be utilized to replace antibiotics in wound treatment. One such natural substance is SCOBY kombucha, which is believed to have antimicrobial and anti-inflammatory properties that support the wound healing process.

Kombucha is a traditional beverage made from sweet tea fermented by SCOBY (Symbiotic Culture of Bacteria and Yeast), comprising complex microbes such as acetic acid bacteria, lactic acid bacteria, and yeast (Hamed et al., 2023). During kombucha fermentation, the main components are SCOBY and the medium. The ingredients used to make kombucha include green tea, kombucha starter, water, sugar, and SCOBY. The fermentation period used in this study exceeded 30 days to obtain an optimal amount of SCOBY. After 60 days of fermentation, SCOBY kombucha was obtained with a diameter of 9 cm and a height of 3.5 cm (Figure 1).

Subsequently, the SCOBY fermented for 60 days was used as the base material for making SCOBY kombucha gel by blending it into a fine consistency. The SCOBY kombucha gel was prepared in concentrations of 0%, 2%, 4%, 8%, 10%, and 12%. The various concentrations of the SCOBY kombucha gel can be seen in Figure 2 below.

Based on the research conducted, it was observed that each concentration of SCOBY kombucha exhibited different physical properties, such as aroma, texture, and color of the gel formulation at each concentration. The gel's color changed from clear to a deep yellowish-brown. The texture of the gel became more fluid, and the aroma became increasingly acidic. These changes in aroma, texture, and color were caused by the active ingredient, SCOBY, added to the gel. The physical appearance of SCOBY is influenced by the fermentation duration. The longer the fermentation period, the thicker the SCOBY becomes and its color changes from brown to dark brown. This is due to the presence of tannin compounds in green tea, which can bind with SCOBY cellulose (Khaerah & Akbar, 2019). Therefore, the more SCOBY added to the gel formulation, the more intense its color. The accumulation of organic acid compounds in SCOBY results in a more acidic aroma. The higher the SCOBY concentration in the gel formulation, the more acidic the aroma of the gel.

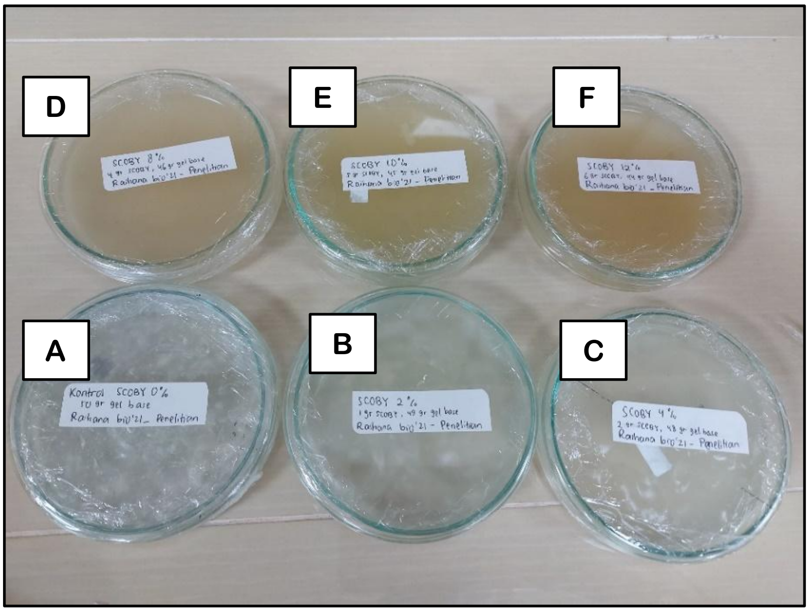

Figure 2. SCOBY Kombucha Gel with Variation of Concentrations: A) 0%; B) 2%; C) 4%; D) 8%; E) 10%; F) 12%

Healing of Incision Wounds in Mice (*Mus musculus*)

Treatment of incision wounds with SCOBY kombucha gel was performed twice daily until the wounds healed. Signs of wound healing included skin closure and wound drying. Observation of incision wound healing was carried forward by measuring the wound length. The chart of the incision wound length measurements in mice (*Mus musculus*) can be seen in Figure 3.

Based on the figure above, the data reveals that the positive control group (Bioplacenton gel) and the 12% SCOBY kombucha gel group showed complete wound closure by the 5th day. The treatment groups with SCOBY kombucha gel concentrations of 0%, 2%, 4%, 8%, and 10% achieved complete wound closure by the 6th day. The negative control group experienced complete wound closure by the 7th day. According to the graph, the 12% SCOBY kombucha gel treatment group had smaller wound sizes from the second day onward compared to the other groups. The chart in Figure 3 demonstrates that the 12% SCOBY kombucha gel is more effective in healing incision wounds compared to the 0%, 2%, 4%, 8%, and 10% SCOBY kombucha gels and the untreated group, as the 12% gel results closely approached those of the positive control (Bioplacenton) from the second day. All lines on the graph show a reduction in incision wound length from the first to the seventh day.

The data obtained were statistically analyzed using IBM SPSS Statistics 26. The first step was to test the data normality using the Shapiro-Wilk test, which showed that the data were not normally distributed (Sig. < 0.05). Therefore, the analysis continued with a nonparametric test, the Kruskal-Wallis test, to determine significant differences between groups. The Kruskal-Wallis test results showed a significance value of 0.006 (< 0.05), indicating that H0 was rejected and H1 was accepted, signifying a significant difference. After rejecting H0, the data were further tested using the Mann-Whitney test to identify significant differences between two independent groups.

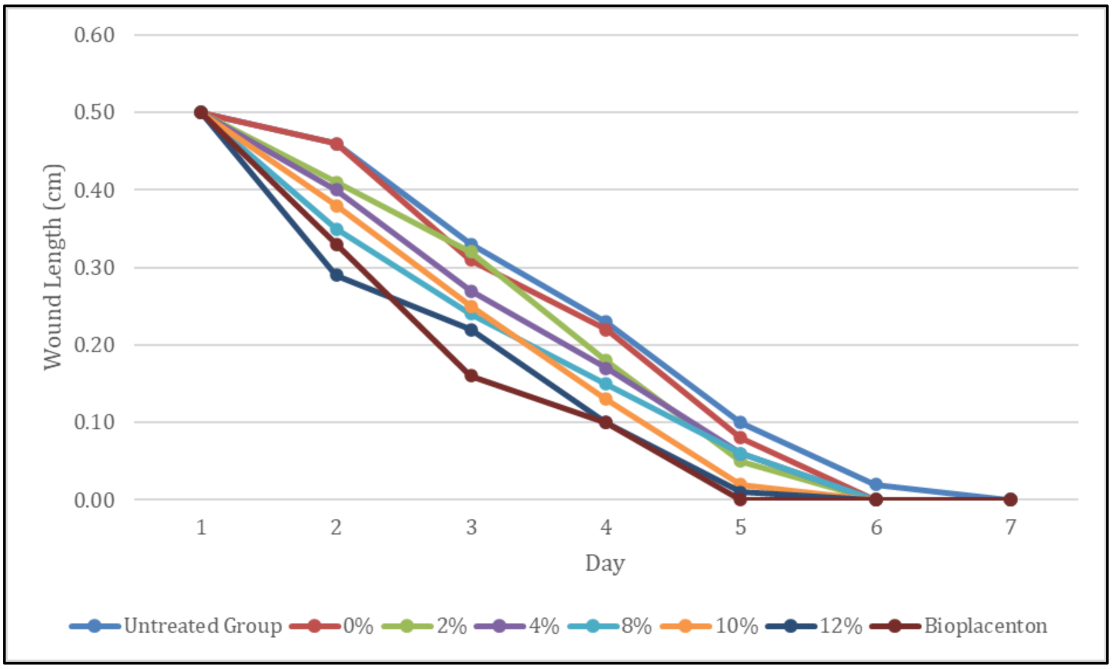

Figure 3. Incision Wound Length Size Chart

Table 1. Mann-Whitney Test Results

	K (-)	0%	2%	4%	8%	10%	12%
0%	0.827		0.127	0.127	0.05	0.05	0.046*
2%	0.275	0.127		0.827	0.184	0.127	0.046*
4%	0.05	0.127	0.827		0.184	0.127	0.046*
8%	0.05	0.05	0.184	0.184		0.275	0.046*
10%	0.05	0.05	0.127	0.127	0.275		0.072
12%	0.046*	0.046*	0.046*	0.046*	0.046*	0.072	
K (+)	0.05	0.05	0.05	0.05	0.05	0.05	0.105

Note: *) H0 is rejected. There are significant differences.

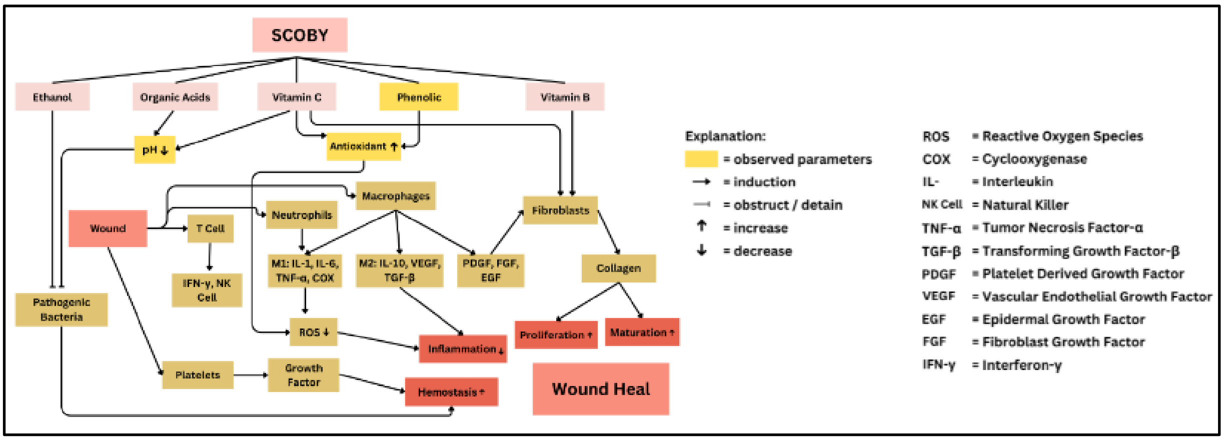

Figure 4. Mechanism of Action of SCOBY Ingredients in Healing Incisional Wounds

Figure 5. First Day Wound

Symbiotic Culture of Bacteria and Yeast (SCOBY) is a pellicle or cellulose composed of acetic acid bacteria, lactic acid bacteria, and yeast. These microorganisms can form various bioactive compounds that may play a role in wound healing (see Figure 4). The yeast in SCOBY can convert sucrose into glucose and fructose. The glucose is then converted into ethanol by Saccharomyces cerevisiae through the glycolysis pathway. According to Suparman et al., (2021), ethanol is effective as an antimicrobial agent. The antimicrobial properties of ethanol can influence the wound healing process, particularly during the hemostasis and inflammatory phases. The hemostasis phase occurs on the first day of injury (see Figure 5). When the body experiences a wound, the damaged or ruptured blood vessels cause bleeding. Naturally, the body initiates the hemostasis process to manage the wound. Hemostasis occurs when the blood exiting the wound comes into contact with collagen and the extracellular matrix, triggering platelets to aggregate and form a clot. The clot then fills the wound cavity and forms a provisional matrix, which serves as a framework for the migration of inflammatory cells during the inflammatory phase (Primadina et al., 2019).

Wound healing can be hindered by infections, which typically cause redness, swelling, and pus. Infections are caused by pathogenic microorganisms that enter and proliferate, such as *Pseudomonas aeruginosa* (Percival et al., 2012) and *Staphylococcus aureus* (Putri et al., 2024). After the hemostasis phase, the wound enters the inflammatory phase (see Figure 6). During this phase, the body sends immune cells to the wound area to combat infection and initiate healing. Ethanol helps reduce the growth of pathogenic bacteria that can disrupt this process, allowing immune cells to work more efficiently in cleaning the wound and facilitating healing. Ethanol can also reduce inflammation caused by pathogenic bacteria, enabling the inflammatory phase to proceed effectively. According to Wandler et al., (2008), ethanol has significant anti-inflammatory effects by reducing IL-6, IL-8, and TNF-α, which can decrease wound infection. These cytokines are pro-inflammatory cytokines (Primadina et al., 2019)

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Figure 6. Second Day Wound

During fermentation, acetic acid bacteria can convert the ethanol produced during fermentation into acetic acid. Acetic acid can then be further converted into gluconic acid and glucuronic acid, which can subsequently be converted into L-ascorbic acid or vitamin C (Antolak et al., 2021). Ethanol will be oxidized to acetaldehyde and then further converted into acetic acid by *Acetobacter* sp. (Simanjutak & Hanna, 2016). The bacteria *Acetobacter* sp. use the enzymes alcohol dehydrogenase and aldehyde dehydrogenase to convert ethanol into acetic acid (Hafsari et al., 2021). Additionally, *Acetobacter* sp. can oxidize glucose into gluconic acid (Simanjutak & Hanna, 2016). Acetic acid bacteria, such as *Gluconobacter oxydans* and *Komagataeibacter xylinus*, oxidize D-glucose into gluconic acid, which is then converted by *Gluconobacter* into glucuronic acid, a precursor to vitamin C. Acetic acid bacteria from the genus *Gluconobacter* also influence the production of DSL (D-saccharic acid-1,4 lactone) via the glucuronic acid pathway, which is believed to have strong antioxidant properties and reduce oxidative damage (Antolak et al., 2021).

Bacterial cellulose in SCOBY can provide rejuvenating effects due to its low pH, which inhibits bacterial growth. This condition can stimulate cell regeneration, induce capillaries under the skin to increase blood flow to the surface, and create new cells (Soares et al., 2021). Acidic substances formed accumulate in the medium, resulting in a pH decrease. Acid-tolerant yeast will survive in a low pH medium. Bacteria in kombucha are highly tolerant of acidic conditions, whereas other bacteria cannot survive in such conditions (Hafsari et al., 2021). Bacteria entering the wound area can multiply and form colonies. This leads to tissue hypoxia in the surrounding area. Moreover, bacterial colonization can impede cell migration and prevent antibiotic and antibody penetration, thereby hindering the wound healing process (Hidayat et al., 2024).

Apart from ethanol, organic acids produced by microorganisms in SCOBY also play a role in the wound healing process, particularly in the hemostasis and inflammatory phases. These organic acids exhibit antimicrobial properties due to their proton and anion content, which can disrupt and damage protein formation in bacteria upon entering bacterial cells. Organic acids can also damage DNA by disrupting the cell membrane and lowering the intracellular pH. Microbes attempt to neutralize the acidity by moving protons, but this reduces cellular energy (Simanjutak & Hanna, 2016). In this study, the inflammatory phase could be observed on the second day post-injury (see Figure 7). This phase is characterized by redness and warmth at the wound site due to increased blood flow to the area. During the inflammatory phase, arterioles supplying blood to the wound dilate, allowing more blood to flow into the local microcirculation. This causes capillaries to dilate and fill rapidly with blood, clinically known as congestion or hyperemia. Hyperemia results from vascular changes in response to inflammation (Laut et al., 2019). In this study, the inflammatory phase appeared to occur from the second to the fourth day post-injury. On the fourth day, a thin red layer covering the wound indicated the process of capillarization (Zauharoh et al., 2020).

During the inflammatory phase, neutrophils and macrophages arrive at the wound site and begin producing large amounts of Reactive Oxygen Species (ROS), along with pro-inflammatory cytokines and proteolytic enzymes such as matrix metalloproteinases (MMPs). ROS aid in attacking and killing pathogens for phagocytosis. However, excessive ROS production can damage surrounding tissues (Kurahashi & Fujii, 2015). High ROS production in this inflammatory environment, known as oxidative burst, is a primary defense mechanism against environmental pathogens (Susilawati, 2021). Small amounts of ROS function as cellular signals in response to stimuli. Moderate levels of H2O2 help regulate the production of vascular endothelial growth factor (VEGF), a key factor in angiogenesis, accelerating new blood vessel formation. ROS also play a role in re-epithelialization by activating epidermal growth factor (EGF) and keratinocyte growth factor (KGF) receptors and promoting TGF-α production in fibroblasts (Kurahashi & Fujii, 2015).

Neutrophils release free oxygen radicals that cause high oxidative stress, which, if uncontrolled, can trigger chronic inflammation (Snyder et al., 2016). Besides neutrophils, ROS is also produced by proliferating cells. Antioxidants play a role in accelerating the inflammatory process by suppressing ROS. The human body can naturally produce several types of antioxidants such as glutathione peroxidase, catalase, glutathione transferase, and superoxide dismutase. However, when the number of free radicals in the body exceeds the capacity of natural antioxidants to neutralize them, the body needs additional antioxidants from external sources (Fadhilah et al., 2021). Antioxidants are found in SCOBY because SCOBY contains phenolic compounds and organic acids such as vitamin C. According to Chagas et al., (2024), research on the concentration of bioactive compounds in SCOBY is still limited. However, researchers argue that the phenolic compounds in SCOBY are similar to those in kombucha, differing only in concentration. Based on this hypothesis, SCOBY may contain the similar phenolic compounds as kombucha.

SCOBY serves as a matrix for microbial growth and the production of phenolic compounds. These compounds tend to accumulate in SCOBY, so the phenolic content in SCOBY may be higher than in kombucha. The phenolic compounds in SCOBY can accelerate the wound healing process. This is supported by previous studies have shown that plant extracts containing phenolic compounds have positive effects on accelerating wound healing. Research by Zauharoh et al., (2020) on the effectiveness of green tea ethanol extract for incision wound healing in mice found that wounds treated with 60% and 70% green tea extract closed on days 4 to 7, whereas 80% and 90% green tea extracts closed wounds on day 6. Green tea contains antioxidant and anti-inflammatory compounds, such as epicatechin gallate, which can accumulate collagen. Additionally, epicatechin gallate can accelerate blood vessel formation in the wound area, speeding up the wound healing process (Zauharoh et al., 2020).

Green tea is the base material for making kombucha and SCOBY. Dried tea leaves contain approximately 42% polyphenolic compounds in the form of catechins. Epigallocatechin gallate (EGCG) is a catechin derivative with the strongest antioxidant activity (Fadhilah et al., 2021). EGCG has a structure that functions as an antioxidant by donating hydrogen atoms or electrons to counteract free radicals (Luo et al., 2024). The strength of antioxidant activity depends on the number of OH groups in flavonoid or phenolic molecules. The more hydroxyl groups substituted in flavonoids, the higher their antiradical activity (Puspitasari et al., 2016). Antioxidants can react with free radicals through a mechanism involving hydroxyl groups, which is a hydrogen atom transfer reaction, where free radicals bind one hydrogen atom from the antioxidant, and the antioxidant itself becomes a radical (Mokra et al., 2022). The released hydrogen atom can bind with free radicals.

In addition to phenolic compounds, SCOBY also contains vitamin C with antioxidant properties. Vitamin C is produced by acetic acid bacteria in SCOBY. *Acetobacter xylinum* produces enzymes that can reduce glucose to D-sorbitol, which is then converted into L-sorbose and oxidized into 2-keto-L-gulonic acid. During fermentation, this compound is converted into 2-L-ascorbic acid (vitamin C) (Yuningtyas et al., 2021). Vitamin C is known as an electron donor, so it can be considered an antioxidant. Vitamin C has strong acidic and reducing properties (Asih et al., 2022). Moreover, the antioxidant properties of vitamin C can enhance the wound healing process by increasing collagen synthesis and reducing ROS and the damage caused by ROS (Dasopang et al., 2021).

SCOBY also contains lactic acid bacteria, in addition to yeast and acetic acid bacteria. Some lactic acid bacteria, such as *Lactobacillus fermentum, Lactobacillus plantarum, Lactococcus lactis*, and *Lactobacillus brevis*, can produce vitamin B. Besides lactic acid bacteria, yeasts like

Zygosaccharomyces bailii and *Saccharomyces cerevisiae* can also generate B complex vitamins (B1, B2, B5, B6, B7, B9, B12) (Antolak et al., 2021). Vitamin B1 acts as an antioxidant by suppressing oxidative stress and regulating NF-kB. Vitamin B6 is crucial for the maintenance of lymphoid tissue and immunological function. Vitamin B9 can inhibit NF-kB activation and is essential for T cell survival. Vitamin B12 is crucial for cellular immunity, particularly concerning CD8+ and NK cells (Dasopang et al., 2021).

Lactic acid bacteria in SCOBY also produce bacteriocins, which are toxic proteins or peptides produced by bacteria that are active against other bacteria without harming their own cells (Antolak et al., 2021). Bacteriocins can combat and prevent the growth of pathogenic microorganisms. Bacteriocins are non-toxic to humans and only active against specific groups of microorganisms. Lactic acid bacteria known as major bacteriocin producers include *Lactobacillus* spp., *Lactococcus* spp., and *Leuconostoc* spp. (Antolak et al., 2021). Bacteriocins are typically produced in liquid cultures and synthesized through the ribosomal pathway during the exponential growth phase of cells (Hafsan, 2014). According to Arqués et al., (2015), bacteriocins are effective against pathogens like *Staphylococcus aureus*. Additionally, bacteriocins produced by *Lactobacillus* spp. can inhibit the growth of bacteria such as *Escherichia coli*, *Klebsiella oxytoca*, *Enterobacter cloacae*, and *Pseudomonas aeruginosa* (Shavira et al., 2022).

After the inflammatory phase, the wound enters the proliferation or re-epithelialization phase. In this study, the proliferation phase occurred on the fifth to the sixth day post-injury (see Figure 7). Antioxidant compounds such as flavonoids can promote new epithelial formation by enhancing keratinocyte migration and epithelial attachment to the wound. Flavonoids can also stimulate the release of growth factors that increase epithelial mitosis and hyperplasia. In this review, the studied flavonoids were found to activate pathways that promote keratinocyte proliferation, differentiation, and secretion, and increase MMP-9 expression, which is crucial for re-epithelialization and migration through internal fibroblast signaling pathways (Carvalho et al., 2021).

Figure 7. Fifth Day Wound

Figure 8. Seventh Day Wound

The final phase in the wound healing process is the maturation phase. During this phase, the wound undergoes contraction, new tissue formation, and tissue maturation. This phase is considered complete when all signs of inflammation and proliferation have disappeared (Mara, 2022). Wound healing is deemed to have reached the maturation phase when the epidermal layer and collagen fiber network have formed (Suwiti, 2010). In this study, incision wound healing entered the maturation phase on the seventh day post-injury (see Figure 8). This phase was marked by complete wound closure and the absence of redness in the wound area. During the healing stage, the wound area had consolidated, collagen fibers had formed, and fibroblasts and blood vessels were present, similar to normal skin (Suwiti, 2010). On the seventh day, the consolidated collagen caused the scar to appear flat, thin, and form a white line. The active compounds in SCOBY that influence this phase are phenolic compounds. Phenolic compounds like epicatechin gallate can accumulate collagen, thus compressing blood vessels more effectively and accelerating wound healing (Zauharoh et al., 2020).

Based on observations on the seventh day, hair growth was observed in the wound area in some mice. This hair growth occurred when the collagen tissue in the wound area had become dense and mature. During the maturation phase, the collagen formed during the proliferation phase undergoes strengthening and densification, creating an environment conducive to new hair follicle growth. Hair growth requires FGF-10 (Fibroblast Growth Factor-10). FGF-10 can stimulate hair growth by activating FGF-2 receptors (Li et al., 2017). Hair follicles consist of epithelial and mesenchymal cells that work together to form the hair shaft (Wang et al., 2015). The cells around the dermal sheath can help repair the dermal papilla, allowing the hair follicle growth cycle to continue, even though the resulting hair shaft may be smaller (Wang et al., 2015).

Conclusion

Based on the research conducted, SCOBY kombucha gel with concentrations of 0%, 2%, 4%, 8%, 10%, and 12% has shown effectiveness in healing incision wounds, as indicated by the reduction in wound size. The 12% SCOBY kombucha gel has proven to be the most effective in wound healing. This is evidenced by statistical analysis, which shows a significant difference ($p < 0.05$) between the 12% SCOBY gel and the negative control, as well as the 0%, 2%, 4%, and 8% SCOBY gels. The effectiveness of SCOBY kombucha gel in wound healing is attributed to the bioactive compounds in SCOBY, which possess antibacterial, anti-inflammatory, and antioxidant properties that accelerate the wound healing process.

Acknowledgement

We would like to express our deepest gratitude to LPPM Universitas Islam Negeri Sunan Ampel Surabaya for providing the funding for this research.

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