



## Effect of Variation in Concentration of SCOBY Kombucha Gel Citrus Peel Extract (*Citrus nobilis*) on Wound Incision (*Vulnus Scissum*) in Mice (*Mus musculus*)

Ainina Neihayatus Shofiyya<sup>1</sup>, Eva Agustina<sup>1</sup>, Funsu Andiarna<sup>2</sup>,  
Irul Hidayati<sup>2</sup>, Risa Purnamasari<sup>1\*</sup>

<sup>1</sup>Department of Biology, Faculty of Science and Technology, Sunan Ampel State Islamic University, Surabaya, Indonesia.

<sup>2</sup>Department of Nutritional Science, Faculty of Psychology and Health, Sunan Ampel State Islamic University, Surabaya, Indonesia.

### Abstract

A laceration wound is damage to the skin tissue characterized by a neat outline of the wound edge, due to slices by sharp instruments or incisions during surgery. This study aims to evaluate the effect of variations in the concentration of SCOBY Kombucha gel preparations of orange peel extract (*Citrus nobilis*) on wound healing (*Vulnus Scissum*) in mice (*Mus musculus*). SCOBY Kombucha gel was made with six concentration variations (0%, 2%, 4%, 8%, 10%, and 12%) and tested for its effectiveness in accelerating wound healing. The research method involved making incision wounds on male mice, followed by applying the gel according to the specified concentrations. The results showed that SCOBY Kombucha gel with a concentration of 12% was most effective in accelerating wound healing, approaching the effectiveness of the positive control (bioplacenton). Analysis showed that the increase in gel concentration was directly proportional to the effectiveness of wound healing, where higher concentration gels showed significantly faster wound closure.

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### Keyword

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extract (*Citrus nobilis*);  
incision wound healing;  
mice (*Mus musculus*)

### Introduction

Human activity in various activities can increase the risk of injury. When there is a wound on the surface of the body, blood is released from the body. Following such a wound, blood elements, especially platelets, immediately accumulate around the injured area to initiate coagulation and close the wound. Wounds to tissues can disrupt anatomical continuity. In general, the wound healing process is a complex cellular mechanism that aims to restore the disrupted tissue. Along with epithelial cells, collagen plays an important role in wound healing, with fibroblasts being the main synthesizers of collagen (Muin, 2021).

A wound can be defined as the loss of epithelial integrity of the skin. The skin plays an important role in protection from the body's internal environment and is the largest organ in the human body. So any serious damage to this organ can cause several problems in its continuity. It is therefore very important to restore its integrity as soon as possible (Novyana

& Susanti, 2016). Based on data from RISKESDAS (2018) the prevalence of injury in Indonesia is 9.2%. The highest prevalence was found in Central Sulawesi province (13.8%) and the lowest in Jambi province (5.6%). The highest type of injury experienced by Indonesians is abrasions / bruises as much as 64.1%, then lacerations / cuts as much as 20.1% with the highest rate found in the Papua region at 7.74% while in the Jambi region the incidence of cuts is 4.76%. Indonesia experiences an increase in the number of injury cases every year, this can be seen in 2013 at 8.4% and in 2018 it increased to 9.2%. A study conducted in the US on wound incidence in the world showed that the prevalence of wound patients was 350 per 1000 population. The etiology of wounds varies, one of the data obtained is 110.30 million cases of surgical wounds. The occurrence of wounds requires healing with several appropriate and regular phases so that anatomical integrity and impaired function can be restored, so that there is no delay or failure of healing.

The wound healing process includes three stages: the inflammatory phase, the proliferation phase, and the maturation/remodeling phase. The inflammatory phase consists of the early and late inflammatory phases, which last from day 0 to day 5 post-wounding (Primadina et al., 2019). The main goal of the inflammatory phase is haemostasis or stopping bleeding (Fauziah & Soniya, 2020). Furthermore, the proliferation phase lasts from day 3 to 21 post-wounding and is characterized by fibroblast proliferation and re-epithelialization. Finally, the maturation/remodeling phase focuses on the strengthening of nascent granulation tissue structures, epithelial growth, and scar tissue formation, which occurs from day 21 to 1 year post-injury (Primadina et al., 2019).

Pharmacotherapy is an important determinant in the wound healing process. The incorporation of drugs containing anti-inflammatory or antibiotic properties is essential for the prevention of infection and rapid wound recovery (Fauziah & Soniya, 2020). Plant extracts are the main ingredient in the formulation of gel-based products, which function as antiseptics or wound healers. In addition, SCOBY kombucha emerged as another component that can be used as an anti-inflammatory gel formulation. SCOBY, which stands for Symbiotic Culture of Bacteria and Yeast, is a by-product of kombucha fermentation (Goh et al., 2012). This tea fungus materializes as a pellicle-enveloped gelatinous layer with a thickness of 0.3-1.2 cm, and is shaped according to the container used during fermentation. When cultivated in glucose-rich media, SCOBYs develop into thickened stratified structures (Azizah et al., 2020).

Formulation of gel preparations requires careful consideration in the selection of ingredients. The ingredients used in the preparation of gel preparations include carbomer 940, triethanolamine (TEA), parabens, glycerin, and distilled water. Gel is a semi-solid system consisting of a suspension of small inorganic particles or large organic molecules penetrated by liquid (Sari et al., 2021). Parabens or p-hydroxybenzoic acid esters, known for their antimicrobial properties, are commonly used in food, cosmetics, and pharmaceuticals (Dodge et al., 2015). Parabens that are widely used in the cosmetic and food industry include methylparaben, ethylparaben, propylparaben, and butylparaben (Golden et al., 2005). Parabens, especially methylparaben and propylparaben, are still used in Indonesia, but starting in 2018 the use of parabens as preservatives in traditional medicines has been banned based on a circular letter from the Food and Drug Administration (BPOM). In addition, many studies still question the safety of parabens and the negative impact of long-term use of parabens, especially when certain cancer cells are found in parabens (Fransway et al., 2019). One way to overcome the problem of microbial purity is to use natural compounds (such as herbal extracts) whose preservatives are replaced with raw materials obtained from

plants. Orange peel extract can be combined as a preservative to make various products durable and antimicrobial. One of the disadvantages of natural preservatives compared to parabens is that natural preservatives must be used in high concentrations to achieve maximum effect (Roska et al., 2018). Based on research by Samsudin and Arimurti, (2018) orange peel extract is also beneficial in wound healing because it has anti-inflammatory, antimicrobial, and antioxidant properties.

SCOBY kombucha gel preparations were made with 6 concentration variations, including 0% (negative control), 2%, 4%, 8%, 10% and 12%. SCOBY kombucha gel preparation is expected to be used as a wound medicine as well as an anti-inflammatory drug. Wound infection causes excessive inflammation, resulting in tissue damage that interferes with wound healing. The difference in concentration of SCOBY kombucha gel will certainly affect the wound healing effect. Therefore, this study will examine the effect of variations in the concentration of SCOBY kombucha gel preparation of orange peel extract (*Citrus nobilis*) on wound healing in mice (*Mus musculus*).

## Materials and Methods

### Tools

The tools used in this study include mice cages, mice food and drink containers, analytical scales, pans, spoons, napkins, jars, fur shavers, scalpels (cutters), syringes (1 cc syringes), large vial bottles, plastic wrap, label paper, tissue, erlenmeyer, watch glass, magnetic stirrer, blender, knife, sieve, filter paper, stirring rod, push rod, measuring flask, petri dish, beaker glass, small spatula spoon, ose needle, measuring cup, dropper pipette, measuring pipette, bulb, oven, and rotary evaporator.

### Materials

The materials needed in this study include male mice, husks, mice feed and drink, green tea, sugar, SCOBY, water, bioplacenton, xylazin, ketamine, 70% alcohol, carbomer, triethanolamine (TEA), glycerin, siamese orange peel, ethyl acetate, and distilled water.

### Methods

#### Making SCOBY Kombucha Gel

##### Gel Base

Carbomer 940 as much as 10 grams, sprinkled on 500 mL of distilled water that has been heated in a glass beaker. Carbomer 940 was stirred quickly until a gel was formed, followed by the addition of 10 drops of TEA (Triethanolamine). Paraben (orange peel extract) as much as 1.0 gram was dissolved in 25 mL of distilled water, then poured into a measuring cup and stirred until homogeneous. Next, 5 mL of glycerin was added and stirred until the gel base was homogeneous. Carbomer 940 can disperse in water to create acidic colloids, while TEA acts as a strong alkalizing agent. Therefore, the higher the amount of TEA added, the higher the pH value of the gel preparation. The components of the gel preparation without the incorporation of extracts (negative control), showed a gel-like appearance with a clear color and medicinal aroma because it only contained gel base.

##### SCOBY Kombucha Gel

SCOBY Kombucha gel was made with various variations of SCOBY extract concentrations, namely 0%, 2%, 4%, 8%, 10%, and 12% by weight w/w by weighing 0gr, 2gr,

4gr, 8gr, 10gr, and 12gr of SCOBY extract, SCOBY extract was then added to the gel base until 100gr and homogenized.

## Wound Healing Activity Test

### Animal Preparation

The experimental procedure involved male white mice (*Mus musculus*) with DDY strain, aged between 2 to 3 months and weighing between 20-30 grams. A total of 24 male mice in good health were obtained from the Farma Veterinary Center (PUSVETMA) Surabaya. Prior to treatment, the mice underwent an acclimatization period lasting 1-2 weeks, during which the mice were fed and watered ad libitum, with husk replacement every 3 days. DDY strain mice provide good reproductive performance and superior growth. These mice originated in Germany in 1910-1920 and were developed at the University of Tokyo Institute of Infectious Diseases (now the University of Tokyo Institute of Medical Science), and then established as a strain at the Yoken National Institute of Health (now the National Institute of Infectious Diseases). DDY stands for Deutschland, Denken, and Yoken. This strain has been the model of several studies of diseases such as IgA nephritis, obesity, dyslipidemia and diabetes in mice (Yamazaki et al., 2012).

### Creation of Incision Wounds in Mice

Male mice that will be wounded first undergo an initial acclimatization phase and last for 1-2 weeks. Prior to wounding, the mice were anesthetized using ketamine. The procedure involves epilation of a designated area on the back of the mice, followed by an incision of approximately 0.5 cm using a scalpel (cutter) that has been sterilized with 70% alcohol. This treatment was given to each test animal.

### Wound Care in Mice

After being wounded, mice were treated by applying the gel preparation according to the specified concentration. The application of the gel preparation is done using a small spatula spoon, so that the administration of the gel to each mice has the same dose. Wound incisions in mice were applied twice a day, once in the morning and once in the afternoon thoroughly until the wound incision in the mice was completely closed and the fur grew back.

### Assessment of Wound Healing Time

Assessment of wound healing time in mice (*Mus musculus*) was carried out daily during the healing process. This assessment involved daily direct observation, supplemented by the use of documentation tools that served as evidence of the progress of wound healing in mice.

### Measurement of Incision Wound Diameter

Measurement of wound incisions in mice was carried out to assess differences in wound length at various stages of the healing process. Measurement of wound incisions in mice was carried out using a caliper, and the results of wound measurements obtained were recorded.

## Results and Discussion

Measurement of wound incisions in mice was carried out using a caliper, and the results of wound measurements obtained were recorded. Measurement of wound incisions

in mice was carried out to assess differences in wound length at various stages of the healing process. The results showed that SCOBY kombucha gel can help accelerate the wound healing process. The difference in the wound healing process can be seen in Figure 1.

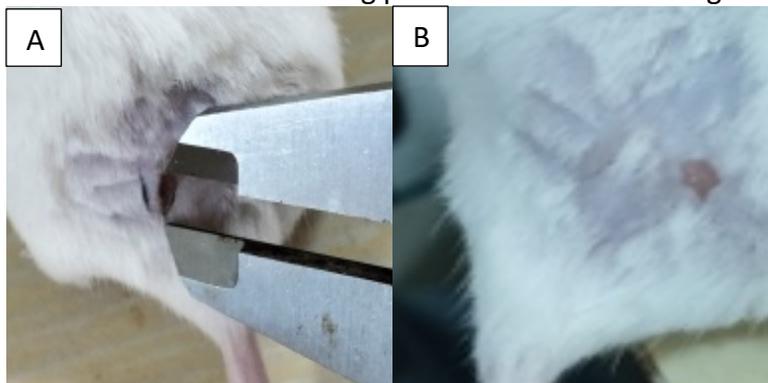


Figure 1. Incision wounds on mice A) New wound; B) Healed wound (arrow) (Personal Documentation, 2024)

The average values for the length of wound healing can be seen in the table below:

Table 1. Mean Value of Incision Length

Concentration	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Without Treatment	0.5	0.44	0.37	0.32	0.18	0.11	0
Bioplacenton	0.5	0.44	0.34	0.24	0.14	0.07	0
0%	0.5	0.47	0.38	0.30	0.18	0.10	0
2%	0.5	0.45	0.37	0.27	0.18	0.09	0
4%	0.5	0.43	0.36	0.27	0.17	0.09	0
8%	0.5	0.42	0.34	0.26	0.17	0.08	0
10%	0.5	0.37	0.32	0.23	0.14	0.07	0
12%	0.5	0.32	0.29	0.21	0.11	0.02	0

Source: (Personal Documentation, 2024)

The incision length graph is shown in the following image:

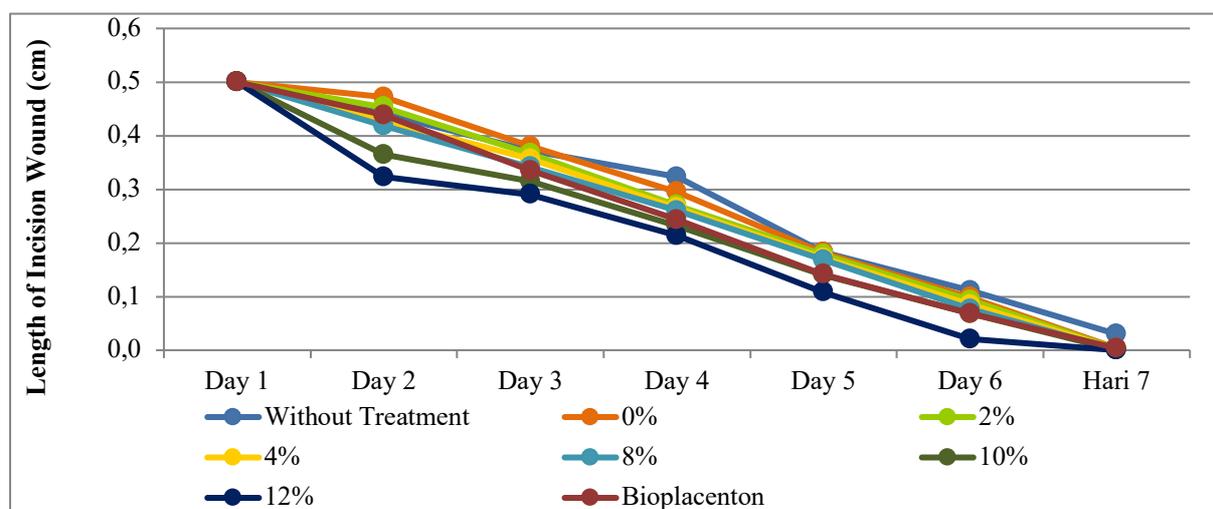


Figure 2. Graph of incision wound length (Personal Documentation, 2024)

Based on the graph of the length of the incision wound in Figure 2, it is known that the 12% SCOBY kombucha gel treatment group has a smaller wound length than the other groups since the second day. SCOBY kombucha gel with a concentration of 12% was closest to the positive control (bioplacenton) and was more effective in healing the incision wound compared to SCOBY kombucha gel 0%, 2%, 4%, 8%, 10%, and the untreated group. SCOBY kombucha gel with 0% concentration had complete wound closure on day 7. At concentrations of 2%, 4%, 8%, 10% and 12%, complete wound closure occurred on day 6, because the incision wounds in mice were completely closed and regrew fur. Complete wound closure in the untreated group and the bioplacenton group also occurred on day 6.

The untreated group showed the slowest wound healing. When compared to the other groups, the untreated group showed the longest healing effectiveness. This is because the untreated group did not receive any intervention that could inhibit the infiltration of pathogenic bacteria into the wound, resulting in a relatively long wound healing process. The negative control still showed a wound healing effect, albeit at a slower rate as it only consisted of a gel base with no active ingredients, and only served as a wound cover without significantly contributing to the healing process.

The 0% kombucha SCOBY gel showed the least effectiveness in wound healing compared to the other concentrations due to the absence of both active ingredients and SCOBY. The length of the wound incision in the positive control group (Bioplacenton) was significantly reduced within a shorter period. This was attributed to Bioplacenton containing placenta extract, thus increasing cell proliferation faster than the other concentrations. Higher concentrations of SCOBY kombucha gel showed the most effective healing for incision wounds. This is evidenced by the fact that the wound healing with the 12% concentration of SCOBY kombucha gel treatment was the closest to the positive control (bioplacenton) and was more effective in healing the wound compared to the 0%, 2%, 4%, 8%, 10%, and untreated groups. SCOBY contains various types of beneficial lactic acid bacteria and yeast. SCOBY gel is often used in skincare products because of its probiotic content and organic acids that are believed to provide benefits to the skin, such as balancing the pH of the skin by helping to keep the skin from being too acidic or alkaline, helping to keep the skin moisturized, relieving inflammation because the compounds in SCOBY have anti-inflammatory properties and repairing damaged skin.

The use of higher extract concentrations in gel formulations has been shown to accelerate wound healing. Several studies have shown that increasing extract concentration not only improves the physical properties of the gel, but also its effectiveness in healing wounds. Based on research conducted by Sukmawati et al. (2024), in the preparation of binahong (*Anredera cordifolia*) leaf extract patches showed that a concentration of 35% was the most effective in healing cut wounds in rabbits. The results showed that wounds treated with this patch closed very quickly and did not leave scars, compared to other formulas that used lower extract concentrations that showed less optimal results. This study confirms that increasing extract concentration can significantly accelerate the wound healing process.

Astriyani (2024) examined the effectiveness of bitter melon (*Momordica charantia L.*) ethanol extract in accelerating wound healing in mice. The results showed that a 50% concentration of this extract gave the best results in wound closure, even exceeding the positive control (povidone iodine). The extract contains bioactive compounds such as flavonoids and saponins that contribute to the wound healing process with antimicrobial and anti-inflammatory mechanisms. In the research of Prasongko et al. (2020), a gel formulated with kedondong (*Spondias dulcis*) leaf extract showed that increasing the concentration from

2.5% to 7.5% increased the effectiveness of burn wound healing. The gel with a concentration of 7.5% showed the highest percentage of healing, reaching 18.45%, compared to the lower formula.

Research by Aoetpah et al. (2019), showed that acacia leaf extract gel (*Acacia auriculiformis*) with a concentration of 11% produced faster wound healing in mice (*Mus musculus*) compared to lower concentrations (7% and 9%). The results showed that the 11% acacia leaf extract gel treatment group experienced significant wound healing in a shorter period of time, suggesting that increased concentration contributes to healing effectiveness. Kaban et al. (2024) evaluated a gel made from ethanol extract of *Jatropha curcas* (L.) leaves in accelerating wound healing in white rats. The gel with 20% concentration showed better results compared to the lower concentrations (10% and 15%), and significantly accelerated the wound healing process within the seven-day observation period. In a study by Sukmawati et al. (2024), lime leaf ethanol extract ointment (*Citrus aurantifolia*) with a concentration of 60% proved to be the most effective in accelerating wound healing in white mice compared to 20% concentration and 40% concentration. The average healing time for this group was 11 days, faster than the positive control group and the group with lower concentrations.

Based on the results of these studies, it can be concluded that the use of higher extract concentrations in gel formulations can result in faster and more effective wound healing. Increasing the concentration of the extract not only improves the physical properties of the gel but also increases the therapeutic effectiveness in accelerating the wound healing process. Further research is still needed to explore the mechanism of action of the active compounds in the extract and their potential application in humans.

Based on the results of this study, it can be concluded that SCOBY gel can help the wound healing process by preventing infection because the acidic environment created by SCOBY can inhibit the growth of bacteria that cause infection, strengthening tissues because some components in SCOBY can help regenerate skin tissue, and reducing inflammation or anti-inflammatory properties of SCOBY can reduce redness and swelling in wounds. SCOBYs produce various biotic compounds such as gluconic acid, chondroitin sulfate, hyaluronic acid, vitamins B1, B6, and B12, and several enzymes that play a role in the human body. The high content of acids, vitamin B, and vitamin C in SCOBY can support SCOBY in alleviating wounds. The moist environment created by the longer drying time of SCOBY can increase the rate of wound healing. The moisture protects the wound and surrounding skin, reduces pain, and maintains an optimal temperature for the healing process. The 12% concentration of SCOBY gel preparation has the highest phenolic content, so there are more phenolic molecules available to neutralize free radicals. This means that the higher the antioxidant potential of the material (Rahmadani et al., 2021).

## Conclusion

Based on the results of the study, it can be concluded that the use of higher concentrations of extracts in gel formulations can produce faster and more effective wound healing. Increasing the concentration of the extract increases the therapeutic effectiveness in accelerating the wound healing process. Further research is still needed to explore the mechanism of action of the active compounds in the extract and their potential application in humans.

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