

## Wound healing activity of gel extract female coconut flower in mice (*Mus musculus*)

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### Abstract

The purpose of this study to analyzes the wound healing activity of female coconut flower (*Cocos nucifera* L.) gel extract in male mice (*Mus musculus*). Treatment by applying the gel to wound with a concentration of 0% (negative control), 25%, 50%, 75%, 100%, and povidone-iodine 10% as a positive control. The measurement of the wound includes the length, width, and area every 24 hours until the wound is completely absent and the third data calculated become healing percentage and wound healing speed, analyzed by statistic (One Way ANOVA). The results show that the percentage and wound healing speed in male mice were not significantly different between all treatments with the positive control (povidone-iodine 10%). It can be concluded that female coconut flower extract (*Cocos nucifera* L.) is effective against wound healing in male mice (*Mus musculus*) because it has the same ability as povidone-iodine to wound healing. The female coconut flower extract has a high flavonoid content as antioxidant which can inhibit inflammation in the wound. This research can provide great benefits for people around the world that can be developed into effective, inexpensive wound healing products with abundant and easily available ingredients in the surrounding environment and can compete in the world.

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Received 15 April 2021,

Revised 03 Jun 2021,

Accepted 15 Jun 2021

**Keywords:** Coconut, Flower, Healing, Gel, Wound.

## 1. Introduction

According to a scientific publication, in the year 2000 occurred at least 100.000 workplace accidents in a population of 7 to 8 million workers [1]. The simplest thing is the occurrence of a wound. Incisions are very common but are considered normal. However, if the wound deeper body tissues, causing damage to blood vessels, there is a possibility of bleeding, which can cause the sufferer to lack blood and result in death. It shows that blood vessels and muscles are critical for the health of the body [2]. Herbal medicines are safer for consumption, have no effects for the body, and can be easily found in the surrounding environment [3, 4, 5]. Inorganic compounds from herbal medicines have had a major clinical impact since 1978 [6, 7]. One of them is female coconut flower extract (*Cocos nucifera*) used to treat the wound. In this era, herbal medicine is increasingly used by people worldwide as a substitute for chemical drugs [8]. Wound healing with herbs has more advantages because herbs are available in the surrounding environment and safer [3]. Therefore, female coconut flower extract as another alternative to wounds healing is commonly used by specific communities in the Ciamis area, West Java, Indonesia. The novelties of this research were (1) This research is based on local wisdom that only exists in the Ciamis area, West Java, Indonesia. (2) Based on the results of various literature studies, no similar research has been found that using female coconut flower extract for wound healing, although previous studies have used female coconut flower extract to inhibit the growth of *Staphylococcus aureus* bacteria while being an anti-tumor that can inhibit the growth of leukemia cancer cells K-562 [9]. Coconut is a member of the Arecaceae family, originating from the South Asian region [10]. There are male flowers and female flowers, male flowers are smaller with a more significant number of flowers and are above the female flowers [11]. Female coconut flowers have many positive impacts on health, especially for wound healing, because of their active substances [12]. The female flowers on the coconut are known to be antimicrobial, antitumoral, and antioxidants. As an antimicrobial, it inhibits the growth of *Staphylococcus aureus* bacteria, and as an anti-tumor, it can inhibit the growth of leukemia cancer cells [13]. In addition, female coconut flowers have many positive impacts on health because of their secondary metabolites such as flavonoids, saponins, tannins, and capric acid as antibacterial, antifungal, and antiviral [13]. These compounds are primarily phenolic compounds because they have phenol groups. These compounds play a role in various bioactivities such as antioxidants, anti-radicals, allergy, anti-inflammatory, and anti-radiation UV [14]. Phenolic compounds function as inhibitors of histamine release when there is inflammation to prevent atopic dermatitis or inflammation of the skin [15]. Phenolic is a secondary metabolite compound that plants can synthesize in response to various conditions [16]. Previous research explained that these phenolic compounds play a role in antioxidant bioactivity. Furthermore, the correlation between polyphenol content and free radical scavenger bioactivity was explained [17]. One of the phenolic compounds in coconut flowers is flavonoids and saponins. Flavonoids inhibit inflammation in two ways: 1) Inhibiting the release of arachidonic acid from neutrophil and endothelial cells. 2) Inhibits the proliferation phase and exudation phase of the inflammatory process. In addition, flavonoids can shorten the inflammatory phase by eliminating Reactive Oxygen Species (ROS), detoxifying hydrogen peroxide ( $H_2O_2$ ) so it can reduce lipid peroxide levels, increasing levels of antioxidant enzymes in wound tissue, thereby inhibiting the free radical chain effect [18]. Saponins are compounds that initiate the formation of collagen proteins that play a role in the wound healing process. However, saponins interfere with the surface tension of cells so that membrane permeability will be disrupted. As a result, the essential ingredients needed by bacteria are disrupted. Therefore, female coconut flowers are an alternative to wound healing, so this study aims to analyze the effectiveness. Thus, our hypothesis is that female coconut flowers containing a high concentration of phenolic compound, especially flavonoid, can effectively reduce wound healing time. This type of research experiment used six treatments with four replications according to statistical rules [19]. The primary purpose of this

research to analyze the wound healing activity of gel extract female coconut flower (*Cocos nucifera* L.) at certain concentration levels in male mice (*Mus musculus*).

## 2. Materials and methods

This research was conducted for five months in the Research Laboratory and rat rearing house, Department of Biology Education, FPMIPA UPI. This type of research is an experiment using six treatments with four replications [20]. The material used to make the extract is a female coconut flower (*Cocos nucifera*). The animals used were male mice (*Mus musculus*) from the Swiss webster strain, while povidone-iodine is a positive control for the treatment. Alcohol and ether are used for anesthesia. The simplicia maceration was dissolved using ethanol 70%, and the gel ingredients consisted of CMC-Na, glycerin, propylene glycol, aquadest, and coconut flower extract. The tools used for the treatment were animal cages, containers for the mice to eat and drink, and a sterile scalpel to create the wound. The observation and measurement of the wound size using a digital microscope (Micro cam).

### 2.1. Preparation of extracts and gel formulations

This type of research is experimental that is consisting of treatment and replication. This research was done in the laboratory of animal structures Universitas Pendidikan Indonesia from January until May 2019. The subjects of this research are male mice (*Mus musculus*) Swiss webster strains. The sample used 24 mice aged two months and weighing 25 grams. Female coconut flower extraction by maceration method was done by adding 1000 gr simplisia in 7000 ml of 70% ethanol for 1-3 days. The extract was filtered, and then ethanol was evaporated until it formed a thick extract. In this study positive control used povidone-iodine 10%, for treatment used gel concentration 25%, 50%, 75%, 100% and 0% as negative control. The basic gel is following the standard formula CMC-Na gel (Sodium Carboxymethyl cellulose) [9].

Table 1. shows the standard formula of CMC-Na gel containing all components and the measure for each component, including CMC-Na, glycerin, propylene glycol, and aqua dest. The amount of coconut flower extract is adjusted to the amount of aqua dest. If the aqua dest is the same as the amount of extract, then the gel has a concentration of 100%. The prepared material is then weighted according to the predetermined formula. The extract of each concentration was dissolved in part of the water then heated at a suitable temperature so that it did not coagulate and was evenly mixed (about 50 ° C). Then added Na-CMC and stirred until homogeneous. Next, add glycerin, propylene glycol, and partly water with continuous stirring until a gel is formed. The gel formed is then stored in a cold place (10 - 15 ° C) overnight to form a thicker gel preparation.

**Table 1. Standard formula CMC-Na gel**

Component	% w/v
CMC-Na	5 g
Glycerin	10 g
Propylene glycol	5 g
Aquadest	10 ml

### 2.2. Treatment on mice

This research is experiment consisted of 6 treatments and 4 replications according to statistical rules. The number of experimental animals consisted of 24 mice divided into six groups, each group consisting of 4 animals which was the number of replications. The wound positive control was given Povidone Iodine (PI) 10%, a gel concentration of 25%,

50%, 75%, and 100% was applied to the treatment group, and the negative control group was given gel without extract (0%). The parameters measured were the length, width, and area of the wound for two weeks. Observations of the wound were done every 24 hours. After each mouse is grouped, the mouse's posterior dorsal is injured with a size of 1 cm. The parameters measured were the length, width, and area of the wound using a digital microscope. The measurement and gel treatment were done every 24 hours until the wound was healed.

### 2.3. Data Analysis

Data on wound healing percentage and wound healing speed analyzed by One Way ANOVA using the SPSS program.

## 3. Result and Discussion

### 3.1. Wound healing percentage

The initial wound size (length, width, and width) was homogeneous according to statistical analysis from the three data. In addition, it was found that the decrease in the length, width, and area of the wound had a decrease with the same trend. Therefore, the percentage of wound healing was calculated by using the initial wound size to reduce the size of the wound when divided by the length of the initial wound multiplied by one hundred percent [21]. As a result, the reduction size of the wound (length, width, and area) has the same trend, then the third data was calculated to the percentage of wound healing—the data presented in Table 2.

**Table 2. Percentage of wound healing during two week**

Day	NC (Negative Control) (%)	25% (%)	50% (%)	75% (%)	100% (%)	PC (Positive Control) (%)
1	0	0	0	0	0	0
2	3.62	4.05	10.66	5.49	8.99	10.9
3	12.23	35.08	23.75	22.4	35.8	41.02
4	19.02	41.02	38.31	50.3	68.3	55.15
5	44.74	54.51	53.99	71.1	85.9	69.36
6	58.61	66.31	66.93	95.3	100	100
7	69.52	80.25	83.79	100	100	100
8	72.49	86.67	94.58	100	100	100
9	74.09	94.87	100	100	100	100
10	76.77	100	100	100	100	100
11	79.89	100	100	100	100	100
12	88.34	100	100	100	100	100
13	93.19	100	100	100	100	100
14	100	100	100	100	100	100

Table 2. shows that the wound treated with Povidone Iodine 10% as a positive control complete wound healing on day-6, while the negative control complete wound healing on day-14 (two weeks). Higher the concentration of female coconut flower extract gel, the less time it takes to achieve a hundred percent. The highest percentage of wound healing was indicated by the concentration of 100%, while negative controls indicated the lowest percentage of wound

healing. The table also explains that it achieved one hundred percent wound healing after the 14th day when the wound was given negative control treatment. According to statistical analysis, this negative control significantly differs from the positive control, which only takes six days to achieve 100% healed. Meanwhile, the closest treatment to positive control was gel with 100% female coconut flower extract concentration. Although according to statistics, there were no significant differences between all positive controls. This indicates that a 25% female coconut flower extract gel concentration is effective in healing wounds because it has no difference with povidone-iodine. According to the statistic test, positive control has a significant difference from the negative control. The same result also at study used *Euphorbia tirucalli* extract for wound healing [21]. Every wounded body tissue will heal naturally without treatments for the wound, which is called self-healing power [22]. The percentage of wound healing of all treatments did not differ significantly from positive controls. Table 2. also shows that with the higher concentration of the female coconut flower gel extract, wound healing effectivity is better. This is because female coconut flower extract (*Cocos nucifera*) contains secondary metabolites called phenolic compounds as flavonoids as an anti-inflammatory and saponins as antibacterial [14].

### 3.2. Wound healing speed

After the percentage of wound healing was calculated, the data wound healing speed for each concentration and the two controls were also presented. The speed of wound healing is obtained from the size of the initial incision (length/width/area), divided by the number of days of wound healing. So that the unit obtained is cm/day. After that, the wound healing speed data were analyzed based on statistics. Table 3 shows the higher the concentration of female coconut flower extract gel, the higher the speed of wound healing in male mice. The statistical analysis between treatment concentrations did not significantly differ in the speed of wound healing and the results of statistical analysis between treatments and controls. This study tests the effectiveness of the ethanol extract of female coconut flowers in a gel preparation. Female coconut flower extract is made in a gel formulation because the gel can retain water content, is easily evenly distributed, does not leave marks, causes a cool sensation on the skin, and is easy to apply [21]. Female coconut flower extract is an antimicrobial agent [22]. Various solvent concentrations and maceration times vary according to the purpose of which secondary metabolites are to be obtained more. In Christian and Halim's research, it was proven that the highest phenolic and flavonoid content was at 70% ethanol concentration, 24 hours maceration time with a ratio of 1 gram of *Simplicia* : 20 ml of solvent [23]. In the study results, wounds treated with 10% Povidone Iodine as a positive control experienced complete healing on the day 6 (Table 2.). In contrast, the negative control recovered on the day 14 (two weeks). This result is consistent with a similar study by Siti Qomariah in 2014, which used extracts of fractured stems (*Euphorbia tirucalli*) in healing wounds of white rats (*Rattus norvegicus*) with a wound length of 1 cm [24]. Every wounded body tissue will heal naturally on its own (Table 2.), even if no wound medicine is given, and this is called self-healing power [25]. This is in accordance with the data obtained, which shows that the negative control experienced recovery even though it took a relatively long time. Although the 3 variables were separately measured, it is correlated that the overall percentage of wound healing was obtained after calculating the average (Table 3.). For the three variables, the length and width of the wound and the area of the incision had the same healing time in healing 100% each time. This shows that the three variables have the same trend. This is consistent with the research conducted by Pariyana et al. in 2016, which measured the distance of the wound edges when the distance between the edges of the wound was very close, but the length of the wound was still there [26]. From the research results of the percentage of wound healing in terms of the reduction in the length, width, and area of the wounds of male mice (*Mus musculus*), the application of female coconut flower (*Cocos nucifera*) gel extract is effective in healing wounds in male mice, even from a concentration of 25%. According to the

statistical tests, the percentage of wound healing and the speed of wound healing were not different between treatments and for positive control of povidone-iodine. However, 100% concentration has the best effectiveness among other concentrations. The higher the female coconut flower extract gel concentration, the better the effectiveness (Table 3). Female coconut flowers have high phenolic compounds [9]. Phenolic compounds as antioxidant can generally inhibit histamine release when an inflammation of the skin occurs to prevent dermatitis atopic [27]. Histamine in the body causes dilation of blood vessels, irritating the skin, increased capillary permeability, so white blood cells and blood fluids easily enter the site of infection, causing swelling [23]. Flavonoids inhibiting inflammation is preventing the release of arachidonic acid to reduce prostaglandin production causes a reduced histamine effect and Inhibiting exudation phase of the inflammatory and flavonoids can eliminating Reactive Oxygen Species (ROS), detoxifying hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) to reduce lipid peroxide levels, increase levels of antioxidant enzymes [17]. Female flower in coconut (*Cocos nucifera*) as an antimicrobial, antitumoral, and antioxidant agent. Female coconut flowers extract can inhibit the growth of *Staphylococcus aureus* bacteria while being an anti-tumor that can inhibit the growth of leukemia cancer cells K-562 [9].

**Table 3. The average speed of wound healing in male mice**

Gel Concentration	Average speed of wound healing (cm/day)
NC (Gel 0 %)	0,100
PC (Povidone Iodine 10%)	0,190
25 %	0,117
50 %	0,145
75 %	0,186
100 %	0,180

## 4. Conclusion

Female coconut flower extract (*Cocos nucifera*) effectively promotes wound healing in male mice (*Mus musculus*). A suggestion for the future, it would be better to do another test on the gel concentration of female coconut flower extract below 25%, and with a specific concentration ratio and not much different.

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