

# Effectiveness Of Ethanol Extract Gel Of Leaf Leaves Of *Cymbopogon Nardus L*) Against Grade Ii A Burn-In White Rats (*Rattus Norvegicus*)

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## Abstract

The writing of the results of this study was carried out to assess the comparison of the effectiveness of the ethanolic extract gel of citronella leaves with concentrations of 5%, 10%, and 15% of each extract on the diameter and thickness of the granulation tissue produced in second-degree burns induced in white rats. This study uses an experimental method with a post-test-only design and the sample is taken using purposive sampling. The effectiveness test was carried out by making IIA degree burns with an iron plate measuring  $\pm 3$ cm which had been heated and then attached to the skin of rats that had been anesthetized and shaved, after that they were given treatment in the form of applying 5%, 10%, and 10% citronella ethanol extract gel. 15% for each treatment group. The results of the data obtained from this study were continued with the Oneway-Anova test which was continued with the Post-Hoc test, wherein the test results there were significant differences from each treatment given. The results of the measurement of the average diameter of burns resulting from the administration of ethanolic extract gel of citronella leaves given for 21 days with a concentration of 15% showed total healing of burns, while the concentration of 10% showed results of 1.12 cm and 5% of 1.59. cm. From the results of the calculation of the thickness of the granulation tissue, it was found that the administration of ethanol extract gel of citronella leaves with a concentration of 15% was 1074.067 m, a concentration of 10% was 1016,787 m and a 5% concentration was 478.38 m.

**Keywords:** Gel, Ethanol Extract, Citronella Leaves, Grade IIA Burns.

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## I. INTRODUCTION

One of the organs that serve as a protector of the body is the skin. The skin itself is one of the largest and heaviest organs of the human body, accounting for 16% of the total weight of the human body. In terms of area, the skin is about 1.5-1.9 square meters (Abeng, Kalangi, and Wangko, 2016). There are 3 layers of human skin structure, the epidermis is the outermost layer of skin, then after that is the dermis layer and the last is the subcutis layer. The stratum corneum, lucidum, granulosum, spinosum, and basal are the constituents of the epidermis layer of human skin. The epidermis layer itself has a thickness of about 75-150  $\mu$ m, while the dermis is 1-4 mm, and the subcutis is the deepest layer located under the dermis (Sari, 2015). A wound is a condition that causes damage or loss of some tissue in the body. Trauma from sharp and blunt objects, extreme temperature changes, chemicals, explosions, electric shocks, and wounds from animal bites are the most common causes. Wounds themselves have several classifications, namely: open and closed wounds. Burns, lacerations, penetrating wounds, gunshot wounds, incisions, and abrasions are examples of open wounds (Nofriyanti, Sinata, and Mistawati, 2020). Burns or what is known as Combustion is a type of open wound that can occur due to exposure to extreme temperatures, chemicals, or electric currents (Ardhiansyah et al., 2020). The gel is a topical preparation as the most common treatment used for burns.

The reason for choosing the gel as a treatment for burns is because of its transparent, elastic characteristics and most importantly, the gel is very easy to penetrate the skin, this is because the basis for making the gel is water (Halim et al., 2019). Another reason is that the gel is cool, moisturizes the skin, and is not sticky and not greasy, this makes gel preparations convenient to use (Prasongko, Lailiyah, and Muzayidin, 2020). One of the herbal plants that can be used in herbal medicine is the citronella plant (*Cymbopogon nardus L.*). Lemongrass is a type of green grass from the Poaceae family that grows in tropical and sub-tropical areas such as Asia, Africa, and America (Arpiwi, Muksin, and Kartini, 2020). If it is related to the chemical content of citronella (*Cymbopogon nards L.*) and burns, it is possible that citronella can be an alternative in the treatment of burns, this is due to the content of citronella, namely tannins whose activity is as astringent, where its performance can shrink the size of pores in the skin, increases skin thickness, and reduces light bleeding (Rinaldi Fauziah; Musfira, Yuni, 2019). Research on the effectiveness of the ethanol

extract of citronella (*Cymbopogon nardus L.*) leaf extract on burns has never been done before. This is what prompted researchers to conduct research on the effectiveness of citronella leaves as an alternative treatment for burns.

## II. LITERATURE REVIEW

### 2.1. Fragrant Lemongrass Leaves

Lemongrass grows in tropical and sub-tropical areas such as Asia, Africa, and America (Arpiwi, Muksin, and Kartini, 2020). In Indonesia, lemongrass can be found in West Sumatra, South Sumatra, West Java, Central Java, and South Sulawesi. Citronella plants grow from the lowlands to an altitude of 1,200 m above sea level (asl). However, the optimum production will be at 250 masl so that it is easy to cultivate (Sulaswatty et al., 2019). Some of the active chemical constituents found in citronella plants are alkaloids, saponins, tannins, flavonoids, phenol steroids, and essential oils. And the content of citronella essential oil is  $\alpha$ -citral,  $\beta$ -citral, geraniol, myrcene, nerol, citronellal, terpinolene, geranyl acetate, linalool, terpinol, methilheptenon, borneol, linalyl acetate, limonene, and linalool isobutyrate (Afrina, Nasution, and Rahmania, 2018). The citronella plant is generally used as aromatherapy which is commonly used for massage as well as a stress reliever and mosquito repellent. Some of the benefits in the pharmaceutical field are anti-bacterial, and anti-fungal (Anwar, Ningtiyas, and Simanjuntak, 2020).

### 2.2. Skin Anatomy

The skin is the outermost and largest organ in the human body, this organ has the function to protect internal organs from trauma from outside the body. Another function of the skin is not only as a barrier but also as a sensory receptor device that can capture external stimuli and plays an important role in the homeostatic system. The skin has several layers, the outermost layer is called the epidermis, the second layer is the dermis and the deepest layer is called the sub-dermis.

### 2.3. Burns

Burns or what is known as Combustion is a type of open wound that can occur due to exposure to extreme temperatures, chemicals, or electric currents (Ardhiansyah et al., 2020). The degree of burns is classified into 3, namely (Anonymous, 2012):

1. First-degree burns: First-degree burns are usually caused by sunburn, characterized by redness (erythema), pain, and no bullae. The depth of the wound is usually only on the surface of the skin in the epidermis.
2. Second-degree burns: For second-degree burns, are usually caused by hot liquids or explosions. It is characterized by a reddish or mixed color accompanied by swelling and bullae. The surface is wet, watery, and painful even though only exposed to the wind. The depth of the wound has reached the dermis of the skin. Second-degree burns are also divided into 2 types, namely II A degree burns, where the wound is still superficial and only reaches the superficial dermis, while II B degree burns are deep enough and reach the deepest part of the dermis.
3. Third-degree burns: Third-degree burns, are usually caused by hot objects, electric shocks, hot liquids, and chemicals. It is characterized by the presence of black and stiff sores. The skin color is white like wax, red to black, painless, and dry. And the depth of the third-degree burn has reached the subcutaneous and the nerve endings.

### 2.4. Wound healing

The wound healing stage will take place immediately after the occurrence of a wound process, where there are several stages in wound healing, namely (Comino-Sanz et al., 2021):

1. Coagulation Stage: The first stage in the wound healing process that takes place quickly after an injury occurs where blood will clot to close and heal the wound as well as the body's physiological response to prevent excessive blood loss.
2. Inflammation Stage: The next stage is when the blood vessels begin to dilate and flow fresh blood to the injured area of the body, causing symptoms of warmth, swelling, and redness in the injured area. At this stage, leukocytes will also act as a destroyer of germs in the wound area.

3. Proliferation Stage: At this stage, the formation of scar tissue based on cells begins to occur.  
Remodeling Stage: At this stage, the damaged tissue will be completely healed, and the skin will also be strong as before being injured.

### III. METHODS

This type of research is experimental laboratory research. Where this research includes sample collection and processing, extract making, phytochemical testing, gel preparation, gel preparation testing for burns, and measuring burn diameter. The research location was carried out at the Bio-Molecular Laboratory of Prima Indonesia University and North Sumatra University on October 19, 2020. The population used in this study were white rats (*Rattus norvegicus*) which were obtained from the Eldwin Cipta Competence Laboratory. White rats with Wistar strain, male sex with 2-3 months of age, and body weight ranging from 160-200 grams. The extracted sample used in this study was citronella leaves obtained on November 9, 2020, from Cikampak City, Torgamba sub-district, South Labuhan Batu, North Sumatra, Indonesia. The number of samples used was 25 male white rats, which were calculated using the Federers formula:

$$(t-1)(n-1) > 15$$

Information:

$$t = \text{Number of groups} = 5$$

$$n = \text{Number of subjects per group}$$

$$= (5-1)(n-1) > 15$$

$$= 4(n-1) > 15$$

$$= 4n - 4 > 15$$

$$= 4n > 19$$

$$= n > 4,75 = 5 \text{ subjects per group. Where the 5 groups are:}$$

1. Control Group (+)
2. Control Group (-)
3. Treatment Group 1
4. Treatment Group 2
5. Treatment Group 3

#### 3.1. Tools and Materials

The tools used are a knife, cutting board, blender, filter paper, razor, beaker glass, object glass, label paper, stationery, 200 mesh sieve, hotplate magnetic stirrer, Rotary evaporator, dropper pipette, measuring cup, ruler, analytical balance, measuring flask, micropipette, jerry can, syrup bottle, aluminum foil, plastic wrap, coin plate, microscope, cage. The materials used were 1 kg of citronella leaf powder (*Cymbopogon nardus L.*), male white rat (*Rattus norvegicus*), 70% ethanol, 70% alcohol swab, Na-CMC, glycerin, propylene glycol, Aquadest.

#### 3.2. Extraction

The leaves of citronella are separated from the stems and cleaned using a cloth, then air-dried at room temperature so that the metabolite content is not lost or damaged by direct sunlight. After drying, the citronella leaves are cut using a knife, then in a blender, until it becomes a powder, sieved using a sieve (Rita and Retna, 2012). The amount of citronella leaf powder that is obtained is 500 grams. The citronella leaf powder that has been obtained is then measured as much as 100 grams and then mixed with 400 ml of 70% ethanol in a glass bottle, which is then covered with aluminum foil for 3 days, then filtered every 3 days to obtain 1 liter of Solvent. . After that, the solvent that has been collected is evaporated using a rotary evaporator, and followed by a hotplate magnetic stirrer to thicken it.

#### 3.3. Gel Preparation

In this study, gel preparations were made with 3 concentration variations, 5%, 10%, 15%. The standard formulation of carboxyl methylcellulose (CMC) gel base can be seen in the table below:

**Table 1.** CMC Gel Base Standard Formulation

| Component        | %b/ b |
|------------------|-------|
| CMC              | 5%    |
| Glycerin         | 10%   |
| Propylene glycol | 5%    |
| Aquabidest       | 100   |

In this study, gel preparations were made with 3 concentration variations, 5%, 10%, 15%. The standard formulation of carboxyl methylcellulose (CMC) gel base can be seen in the table below:

**Table 2.** Gel Concentration Formulation

| Ingredients      | Concentration |        |        |
|------------------|---------------|--------|--------|
|                  | 5%            | 10%    | 15%    |
| Extract          | 2,5 g         | 5 g    | 7,5 g  |
| CMC              | 2,5 g         | 2,5 g  | 2,5 g  |
| Glycerin         | 0,25 g        | 0,25 g | 0,25 g |
| Propylene glycol | 0,41 g        | 0,41 g | 0,41 g |
| Aquades ad       | 50 g          | 50 g   | 50 g   |

The method of manufacture is that all the materials used are weighed in advance according to the formulation. CMC is dissolved in Baker Glass which has been partially filled with water and has been heated at 50oC. The extract was added according to the concentration (5%, 10%, 15%) and stirred using a magnetic stirrer until homogeneous. Glycerin, propylene glycol, and water were added with continuous stirring until a gel was formed, after being stored at room temperature for 1 night.

### 3.4. Manufacture of Grade II A Burns

Prior to the treatment on the sample, the back of the white rat should be shaved  $\pm$  3 cm which was carried out the day before the treatment. Before making burns, white rats should be given an anesthetic injection of Ketamine-Xylazine in a ratio of 2:1 as much as 0.03 ml intramuscularly, then the backs of the rats were cleaned using 70% ethanol and then treated with an iron plate measuring  $\pm$  3 cm (Indriani, Almasyhuri, and Pratama, 2020), heated over a blue fire for 10 seconds and directly attached to the skin of a white rat that had been shaved for 5 seconds so that second-degree burns formed A. (Wardani, 2020).

The way to measure burns is to measure 4 diameter sizes using a ruler and then calculate using the formula:

$$dx = \frac{d1 + d2 + d3 + d4}{4}$$

Information: dx = x day wound diameter

d1 = diameter 1 (cm)

d2 = diameter 2 (cm)

d3 = diameter 3 (cm)

d4 = diameter 4 (cm)

### 3.6. Data Analysis

The results of data from the effectiveness of the ethanol extract of citronella leaves on changes in the average diameter of burns will be processed and analyzed using SPSS (Statistical Program Service Solution) software with ONEWAY-ANOVA as the type of test with a 95% confidence level and followed by Post Hoc Test Tukey.

H0: There is no effect of giving ethanol extract gel of citronella leaves against second-degree burns in white rats.

Ha: There is the effectiveness of giving ethanol extract gel of citronella leaves against second-degree burns in white rats.

#### IV. ANALYZE AND RESULT

##### 4.1. Phytochemical Test Results

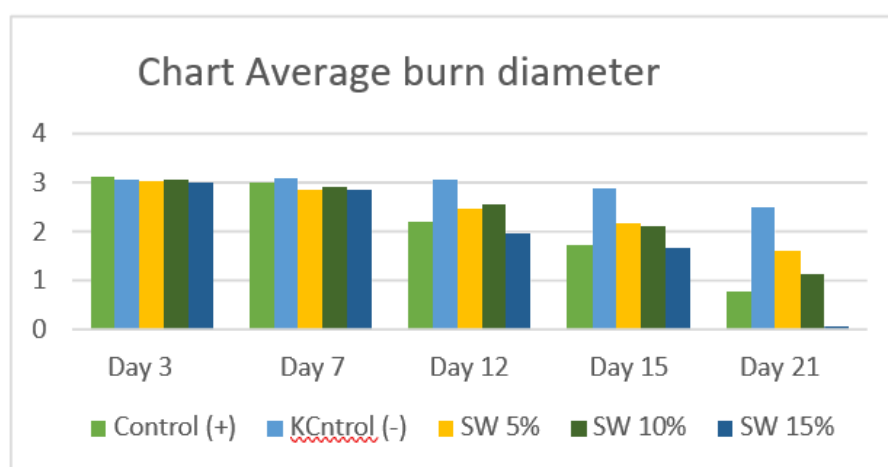
The active chemical constituents of citronella leaves (*Cymbopogon nardus L.*) that have been tested are flavonoids, alkaloids, saponins, and tannins (Rita and Retna, 2012; Afrina, Nasution and Rahmania, 2018). From observations, the results of the Alkaloids test with Bouchardart and Wagner reagents were the formation of a brick red color which indicated a positive result, while with Maeyer's reagent there was a color change to cloudy yellow which meant a positive result, and with Dragendorf's reagent, there was a color change to white which meant positive. The results of the Saponin test with the Aquadest reagent showed a color change to clear yellow indicating a positive result, the results of the Flavonoid test with 5% FeCl<sub>3</sub> reagent showed a change in color to dark green which indicated a positive meaning, and the results of the Tannin test with 1% FeCl<sub>3</sub> reagent showed a change dark green color shows positive meaning.

##### 4.2. Diameter of the Burn that has been given a Gel of Ethanol Extract of Fragrant Lemongrass Leaves (*Cymbopogon nardus L.*)

The speed of healing produced by the Ethanol Extract Gel of Serai Wangi Leaves for second-degree burns can be seen from the change in the diameter of the burn. Measurement of the diameter of the burn using a ruler by adding up the measurement results from the four latitudes, namely d1, d2, d3, and d4 which is then divided by four, this is done for each treatment so that the average diameter is obtained as shown in the table below. In this treatment, Bioplacenton was used as a control (+) and CMC Gel as a control (-).

**Table 1.** Average Burn Diameter with Ethanol Extract Gel Application of Fragrant Lemongrass Leaves

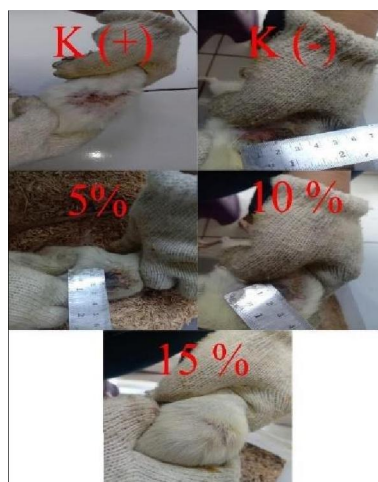
| No. | Day      | Treatment |         |         |         |         |         |
|-----|----------|-----------|---------|---------|---------|---------|---------|
|     |          | K (+)     | K (-)   | 5%      | 10%     | 15      | K (+)   |
| 1.  | 3rd day  | 3,10 cm   | 3,06 cm | 3,01 cm | 3,05 cm | 3,00 cm | 3,10 cm |
| 2.  | 7th day  | 2,99 cm   | 3,07 cm | 2,85 cm | 2,89 cm | 2,83 cm | 2,99 cm |
| 3.  | 12th day | 2,20 cm   | 3,06 cm | 2,45 cm | 2,54 cm | 1,95 cm | 2,20 cm |
| 4.  | 15th day | 1,70 cm   | 2,86 cm | 2,17 cm | 2,11 cm | 1,66 cm | 1,70 cm |
| 5.  | 21th day | 0,76 cm   | 2,49 cm | 1,59 cm | 1,12 cm | 0,06 cm | 0,76 cm |



**Fig 1.** Diagram of the average diameter of burns with the administration of citronella leaf extract gel

Based on the results obtained from Table 5.1 and Figure 5.1 above, the Ethanol Extract Gel of Serai Wangi Leaves (*Cymbopogon nardus L.*) has good effectiveness in healing second-degree burns. has shown complete healing after being given for 21 days, while a concentration of 10% produces a wound diameter of

1.12 cm and a concentration of 5% produces a wound diameter of 1.59 cm. This is because the content, namely Tannins has activity as an astringent, where its performance can shrink the size of pores on the skin, increase skin thickness, and reduce light bleeding, several other chemical ingredients such as alkaloids that work antimicrobial to minimize infection and the content of flavonoids. has a role as an antioxidant (Rinaldi Fauziah, Musfira, and Yuni, 2019).



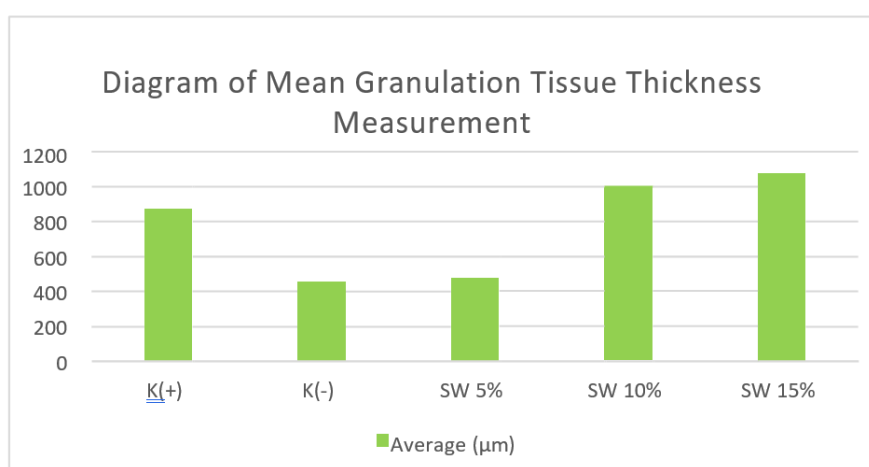
**Fig 2.**Picture of the diameter of the burn on the 21st day after being given treatment

#### 4.3. Burns Granulation Tissue Thickness that has been given Gel Ethanol Extract of Fragrant Lemongrass Leaves (*Cymbopogon nardus L.*)

One of the other parameters to assess the speed of healing of a burn wound is through the histopathological appearance of the thickness of granulation tissue formed during the healing process. In this study, the average thickness of granulation tissue formed during the healing process of second-degree burns that had been induced with Ethanol Extract Gel from Lemongrass Leaves can be seen in the following table and diagram.

**Table 2.** Histopathology means granulation of tissue thickness in rat skin (*Rattus Norvegicus*)

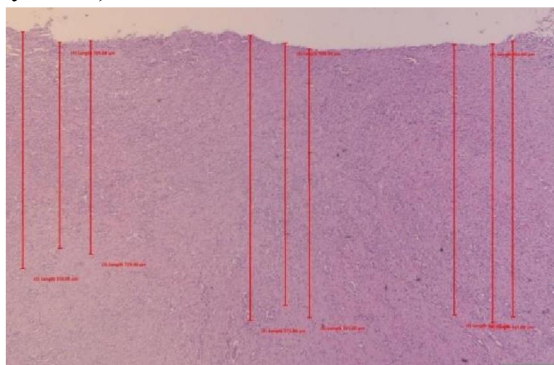
| No. | Group    | Measurement |        |        |       |        |        |        |        |        | Ave<br>rage<br>( $\mu\text{m}$ ) |
|-----|----------|-------------|--------|--------|-------|--------|--------|--------|--------|--------|----------------------------------|
|     |          | Sinistr     |        |        | Medi  |        |        | Dextr  |        |        |                                  |
|     |          | I           | II     | III    | IV    | V      | VI     | VII    | VIII   | IX     |                                  |
| 1   | K        | 705,00      | 810,00 | 729,60 | 898,8 | 975,00 | 921,00 | 954,00 | 945,00 | 927,00 | 873,93                           |
| 2   | K (-     | 489,00      | 492,00 | 468,00 | 505,2 | 497,40 | 483,00 | 424,20 | 378,00 | 366,00 | 455,86                           |
| 3   | SW (5%)  | 458,68      | 431,05 | 459,85 | 429,1 | 437,21 | 415,93 | 551,98 | 573,62 | 548,02 | 478,38                           |
| 4   | SW (10%) | 1076,3      | 1068,1 | 1085,3 | 982,2 | 1008,9 | 976,64 | 1011,4 | 964,69 | 977,22 | 1016,78                          |
| 5   | SW (15%) | 967,2       | 972    | 1005   | 1066, | 1026   | 1087,2 | 1176,6 | 1141,2 | 1225,2 | 1074,06                          |



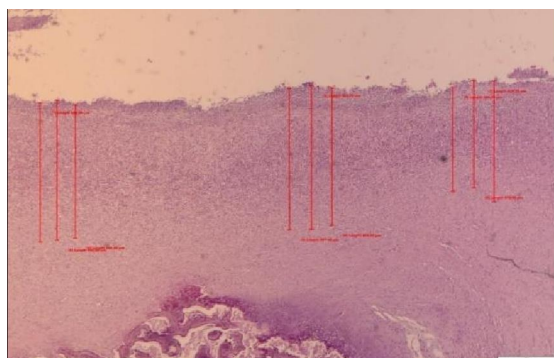
**Fig 3.**Histopathological diagram of the mean thickness of granulation tissue in rat skin



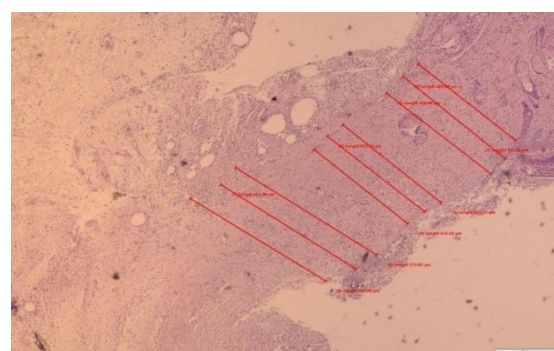
Referring to table 2 and Figure 3 above, the results of the measurement of granulation tissue thickness from the histopathological description that has been treated using Lemongrass Leaf Ethanol Extract Gel, where the granulation tissue formed at the proliferation stage will increase which indicates the wound healing process. From the results obtained, it was found that the best granulation tissue thickness was when using Lemongrass Leaf Ethanol Extract Gel with a concentration of 15% where the average thickness of granulation tissue was 1074.067 m, while at a concentration of 10% it produced 1016.787 m, and the lowest was at a concentration of 5%, which is equal to 478.38 m. This is because the chemicals contained in citronella leaves are tannins and flavonoids as polyphenolic substances that can help the wound healing process, where these two substances play a role in the fibrogenesis stage which causes fibroblast cells to proliferate so that if the granulation tissue gets thicker, the healing process will occur. the wound will get better. (Sari, Rashid, and Liberty, 2020).



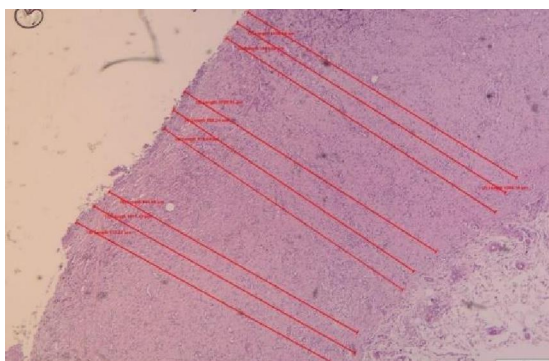
**Fig 4.** Histopathology of granulation tissue thickness using a positive control



**Fig 5.** Histopathology of granulation tissue thickness using a negative control



**Fig 6.** Histopathology of granulation tissue thickness using ethanol extract gel of citronella leaves with a concentration of 5%



**Fig 7.** Histopathology of granulation tissue thickness using ethanol extract gel of citronella leaves with a concentration of 10%



**Fig 8.** Histopathology of granulation tissue thickness using ethanol extract gel of lemongrass leaves with a concentration of 15%

#### 4.4. Results Data with SPSS

From the data on the results of the wound healing effectiveness test, it was analyzed statistically using SPSS software with the One Way Anova Test followed by the Tuckey Post Hoc Test. Before testing using the One Way Anova Test, the Normality Test was carried out first and the results were  $> 0.05$ , which means the study had a normal data distribution. The homogeneity test was then carried out with the results  $> 0.05$ , which means that the data is homogeneous. Then the One Way Anova test was carried out and the sig.  $< 0.05$  where  $H_1$  is accepted and  $H_0$  is rejected, which means that there is an Effectiveness of Ethanol Extract Gel of Fragrant Lemongrass Leaves (*Cymbopogon nardus L*) on the Healing of Grade IIA Burns.

**Table 3.** One Way Anova Test with SPSS Version 24

|                | Sum of Squares | df | Mean Square | F     | Sig. |
|----------------|----------------|----|-------------|-------|------|
| Between Groups | 2.789          | 4  | .697        | 1.058 | .043 |
| Within Groups  | 13.184         | 20 | .659        |       |      |
| Total          | 15.973         | 24 |             |       |      |

## V. CONCLUSION

Based on the results of the Phytochemical Tests carried out, it was found that the ethanolic extract of citronella leaves in the form of, Alkaloids, Flavonoids, Saponins, and Tannins.

1. Based on the results of the measurement of the diameter of the burn, it was found that the Ethanol Extract Gel of Serai Wangi Leaves (*Cymbopogon nardus L*) had good effectiveness in healing second-degree burns, whereas from the three variations of concentration, the ethanolic extract gel of lemongrass leaves with a concentration of 15% had showed complete healing after being given for 21 days, while for a concentration of 10% it produced a wound diameter of 1.12 cm and a concentration of 5% produced a wound diameter of 1.59 cm.
2. From the histopathology results obtained, it was found that the best granulation tissue thickness was when using Lemongrass Leaf Ethanol Extract Gel with a concentration of 15% where the average thickness of granulation tissue was 1074.067 m, while at a concentration of 10% it



produced 1016.787 m, and the average thickness of granulation tissue was 1074.067 m. the lowest was at a concentration of 5%, which was 478.38 m.

3. 3. And from the results of the analysis using the One Way Annova test with the results of sig. <0.05 where H1 is accepted and H0 is rejected, meaning that there is an Effectiveness of Ethanol Extract Gel of Fragrant Lemongrass (*Cymbopogon nardus L*) Leaves for Healing Grade IIA Burns.

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