# The Effect Of Giving Cloves Extract (*Syzygium Aromaticum*) On Hair Growth And Histopathological Picture Of Skin Tissue In The Healing Process Of Dermapene Wounds In Wistar Strain White Rats

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## ABSTRACT

Hair loss is a widespread concern and affects skin quality. Hair loss can occur due to many factors. Based on previous research, clove plants can overcome hair loss problems. This is because cloves can be used as extracts and contain secondary metabolites in the form of flavonoids, saponins, tannins, and steroids.

This study is a true experimental study, using a post-test only control group design. The sample in this study was 24 animals.

The results of the study concluded that administration of clove extract cream (Syzygium aromaticum) affected hair growth. Based on the difference in average hair length in the groups after testing. The conclusion of the research results stated that the greatest hair growth was in test group 3 which was given clove extract with a concentration of 12%. The group with the least hair growth was in the control group which was only given base cream. Administration of clove extract cream (Syzygium aromaticum) in the group after testing with concentrations of 3%, 9%, and 12% extra affected the healing process of dermapen wounds in white rats (Rattus norvegicus) Wistar strain. Based on phytochemical tests, it was shown that clove extract (Syzygium aromaticum) contains secondary metabolites in the form of flavonoids, saponins, tannins, and steroids. These compounds, especially flavonoids, help heal dermapen wounds and stimulate hair growth.

***Keywords:*** *Clove extract, Hair growth, Wounds.*

## INTRODUCTION

Socially and mentally, those with good skin benefit (Huang, 2022). Skin problems and psychology have been shown to be clearly correlated, with those with skin issues more likely to suffer social isolation, loneliness, despair, and a lower quality of life (Krasuska, 2018). Skin issues have been addressed using a range of methods, such as chemical peels, topical drug formulations, and ablative and non-ablative laser rejuvenation. These techniques have been used extensively to address wrinkles, acne scars, hyperpigmentation, and skin aging. Additionally, more complex skin conditions like hair loss can now be treated because to advancements in cosmetic applications (Lawson, 2017). Hair is a specialized skin appendage that is essential for several functions, including thermoregulation, immunological surveillance, and sebum production. A certain number of hair follicles that develop during the embryonic stage are present in mammals at birth. These hair follicles undergo cycles of regression and regenerative regeneration after birth, which replicate many embryonic signaling pathways. Moreover, the hair cycle has a direct influence on skin wound healing, disease conditions, and skin regeneration in homeostasis (A, 2020). Because hair constantly enters the resting period and subsequently falls out, humans continue to lose hair. A healthy adult's head may lose between 70 and 100 hairs per day. However, because new hair continually grows to replace it, this natural hair loss is undetectable. Although about 100 hairs fall out every day, the number is much higher in the early stages of hair loss disorders because of disturbances in the normal cycle of hair formation, which is referred to as hair loss (N. Sadgrove, 2023). The frequent problem of hair loss affects the quality of life for both men and women. Hair loss can be caused by a variety of factors, such as hormone problems, poor nutrition, skin conditions, anomalies in hair formation, vitamin and mineral deficiencies, genetic predispositions, and more (Gokce N, 2022). Hair loss is also known as alopecia areata (AA). The common condition known as alopecia areata (AA), which is characterized by non-scarring hair loss, is caused by the immune system. The lifetime incidence of AA varies from 1.7 to 2.1% and is higher in younger patients (ages 21 to 40), but there is no appreciable difference between men and women. Like other skin disorders like atopic dermatitis and psoriasis, AA can have a major negative impact on a patient's quality of life. The pathogenesis of AA is now understood to entail an autoimmune process that includes T cell infiltration, immune system breakdown in the hair follicle, and melanogenesis-related peptides as autoantigens (Thompson, 2017). A wound is any injury to the body, including a break in the epidermis of the skin and a disruption of its natural structure and function. Wound healing is a complicated process that necessitates the coordinated action of several cell types and tissues. Angiogenesis, inflammation, matrix deposition, cell migration, and proliferation must all be strictly regulated. It may take weeks for larger wounds from trauma, serious illness, or major

surgery to heal, leaving a fibrotic scar that might affect tissue function. On the other hand, minor skin injuries might recover in a few days. Since ancient times, the importance of wound healing has been recognized, and several attempts have been made to develop innovative wound dressings that use the best materials to facilitate rapid and effective wound healing. Herbal remedies greatly assist in the healing process of wounds. One type of medicinal plant that may be used to cure wounds is cloves. Cloves (*Syzygium aromaticum*) are one of the herbs that have been used for antiquity because of their well-known antibacterial and antioxidant properties. Considering this, scientists are interested in learning if clove extract influences the formation of hair and the healing of cutaneous wounds.

## LITERATURE REVIEW

Hair is part of the pilosebaceous unit, which also consists of sebaceous glands and arrector pili muscles (Lim, 2018). Hair not only protects against radiation, heat, cold, and other environmental elements, but it also improves a person's appearance and personality. Hair loss contributes to psychological, social, and behavioral problems and has a cosmetic and societal impact on our society. Hair includes water, lipids, minerals, and melanin pigment in addition to keratin, which has a high quantity of the amino acid cysteine. The main protein that gives hair fibers their structure is keratin. Hair passes through three distinct growth stages: anagen, catagen, and telogen. Wounds are caused by damage to biological tissues, such as the skin, mucous membranes, and organs. Numerous injuries can cause wounds, which need to be appropriately cleansed and treated to prevent infection (Herman TF, 2024). The skin's surface is where wounds occur. The skin is the organ that covers the human body's inside. Its origin is ectodermal. The skin protects internal organs, bones, muscles, and other soft tissues from poisons, infections, and other harmful environmental elements. The skin plays an important role in sensation, vitamin D, and body temperature regulation. Furthermore, the body's first line of defense against infections is the skin. Additionally, systemic variables (congenital pathology linked to collagen deficit or synthesis, pathology that raises the likelihood of bacterial infection, and nutrition) and local factors (wound wetness, wound ischemia, and bacterial infection) affect wound healing (Thanh Tuan, 2023). Tissue repair is the outcome of an ordered series of overlapping stages that characterize wound healing. Hemostasis, inflammation, proliferation, and the development of mature scar tissue are all steps in this process (H. Grubbs, 2024). Cloves are among the plants that may be used as a natural remedy for wound healing. Known as spices, cloves (*Syzygium aromaticum*) are indigenous to Indonesia, particularly the Maluku Islands. The primary portion of the plant that is now used is dried clove buds (Alfian et al., 2019). Strong antibacterial, antifungal, analgesic, antiparasitic, antioxidant, anti-inflammatory, and anticarcinogenic qualities are all displayed

by cloves. Eugenol, caryophyllene, hydroxycinnamic acid, eugenyl acetate 3-allyl-6-methoxyphenol, tyranton, flavonoids, and hydroxyphenyl propens are among the primary components (60–90%) of the clove extract. Meanwhile, vitamins K, A, B1, B6, C, and β-carotene are small components (5–10%) (Uddin MA, 2017). The following is the conceptual framework used in this study:

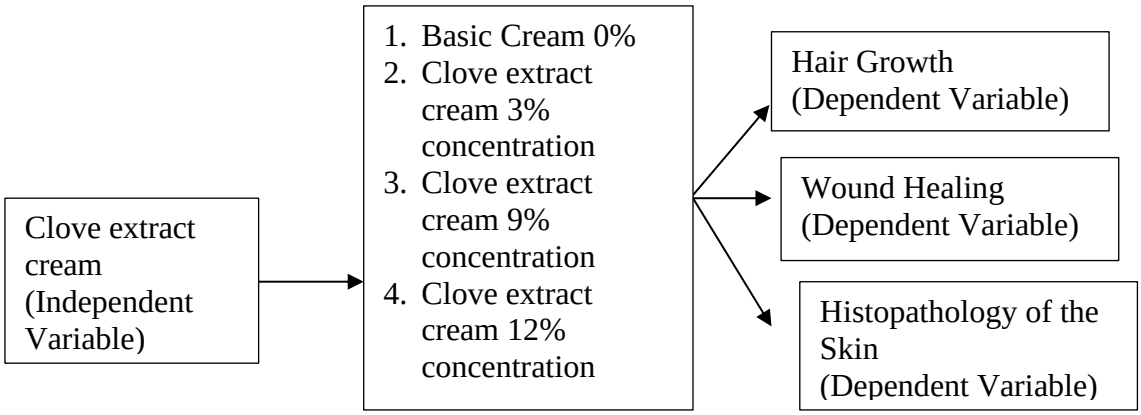



**Figure 1. Conceptual Framework**

## METHODS

This research is true experimental research, with the research design used being the post-test only control group design, which is a type of research that only makes observations on the control and treatment groups after being given a test. Acclimatization is the process of adjusting to a new environment, climate, condition, or atmosphere. Before giving treatment, all male Wistar rats went through a seven-day acclimatization process in the Laboratory of the Department of Pharmacology and Therapeutics, Faculty of Medicine, University of North Sumatra. The rats were given time to adapt to the new environment, as well as their food and drink. The provision of feed and drink to the rats was carried out according to their standard needs (ad libitum). Ethical Clearance will be submitted to the Health Research Ethics Commission (KPEK) of Prima Indonesia University and is still in process. All procedures comply with animal welfare guidelines to address any ethical issues related to animal handling and care. A male Wistar strain white rat (*Rattus norvegicus*) weighing 150–200 grams and two to three months of age served as the study's research sample. According to the sample calculation above, there were a minimum of six test animals each group, for a total of twenty-four test animals in this study. Rats were randomly assigned to one of four test groups: the control group (K-0), which received a 0% base cream smear; test group 1 received a 3% concentration of clove extract cream; test group 2 received a 9% concentration; and test group 3 received a 12% concentration every day for 14 days. The first step in getting the test animals ready was to shave the mice's backs. A hair clipper with a 4x4 cm area was used to shave each mouse's back. To get rid of all the hair on the surface, the remaining hair was coated with

depilatory cream (Veet Cream) for three to five minutes. Rinse with water until all hair has been removed. Using a marker, a box measuring 2 by 2 cm was created for each test region on the mice's shaved backs. Before the clove extract ointment could be administered, the test animals were left for a full day. Dermapen activity was used to create the second wound in Wistar strain white rats. To prevent discomfort and excessive movement caused by the dermapen action, the rats were first rendered unconscious using a mixture of ketamine (80 ml/kg BW) and xylasin (5 ml/kg BW). Additionally, dermapen activity was applied to the white rat's skin in the targeted region, which measured 2 by 2 cm, up to one time. The needle moved perpendicular to the skin that was stretched. The bleeding point served as the termination point after pressure was applied point by point to each scar (needle depth: 2.5 mm). Sterile gauze was used to clean up any blood. and the skin was held with sterile saline. The mice who had undergone the testing process had their back skin biopsied. On a 400x magnification object, histopathological scoring values for the density of collagen tissue distribution were calculated using one field of view. Collagen density received a score of 0 for extremely low (less than 25%) collagen growth, 1 for low (less than 25%) collagen growth, 2 for moderate (50%) collagen development, and 3 for dense (75–100%) collagen growth (Sembiring et al., 2021).

**Preparation of Clove Extract Cream**

Samples of cloves in the shape of flowers are termed simplicia once the flower stems are crushed to a size of 60–70 mesh. The first step in the extraction process is to soak the simplicia in a 90% ethanol solvent at a 1:10 ratio while stirring the sample. Additionally, the simplicia is soaked in ethanol again after the solution is filtered every three days, and so on, until the filtering procedure is completed three times. A rotary evaporator is used to evaporate the resultant filtrate until all the solvent is gone and the mixture has solidified into a paste. All materials for making the shipment, each weighed according to the desired formula as presented in the following table 1.

**Table 1 Clove Extract Cream Preparation Formula (O/W)**

| Material Name | Cream Formula (grams) | | | |
|---|---|---|---|---|
| | **F0** | **F1** | **F2** | **F3** |
| **Clove Extract** | **0** | **3** | **9** | **12** |
| Cetyl alcohol | 4 | 4 | 4 | 4 |
| Glycerin | 15 | 15 | 15 | 15 |
| TEA (triethanolamine) | 3 | 3 | 3 | 3 |
| Stearic acid | 12 | 12 | 12 | 12 |

| | | | | |
|---|---|---|---|---|
| Methyl paraben | 0.2 | 0.2 | 0.2 | 0.2 |
| Propyl paraben | 0.02 | 0.02 | 0.02 | 0.02 |
| Aquades until.. | 100 | 100 | 100 | 100 |

Following microscopic analysis, data from histopathological observations were gathered and then scored. Following the tabulation of the study data, the modifications discovered were examined and given in a descriptive manner. We used SPSS (Statistics of Package for Social Science) 25.0 for Windows to evaluate data from observations of wound healing and hair growth. The Kolmogorov-Smirnov test method was used to examine the data normalcy test ($p>0.05$). One-way analysis of variance, or One Way ANOVA, was employed at a 95% confidence level ($p <0.05$) to determine whether there was a significant difference between the trial groups. The Post Hoc Test with the LSD approach was used for additional analysis or testing.

## RESULTS

For 14 days, 24 experimental animals were weighed both before and after testing. According to the test animals' features, the mice were generally in good health both before and after treatment throughout this investigation. Twenty-four test animals in all were able to complete this trial without dropping out. Clove extract (*Syzygium aromaticum*) includes secondary metabolites in the form of flavonoids, saponins, tannins, and steroids, according to the findings of the phytochemical tests that were conducted. The following are the screening results obtained in table 2.

**Table 2. Phytochemical Tests**

| **Secondary Metabolites** | **Testing** | **Color** | **Results** |
|---|---|---|---|
| Flavonoid | Wilstater | Yellow | + |
| Saponins | Forth | Yellow and foamy | + |
| Tannin | FeCl3 | Blackish blue | + |
| Alkaloid | Wagner | Yellow | + |

| | | | |
|---|---|---|---|
| Steroid | Lieberman – Burchard | Green | + |

**Hair Growth Observation Results**

Observation of mouse hair growth was carried out on days 7 and 14 by plucking 6 strands of the longest mouse hair, then straightening it and attaching it to adhesive tape. The length of the hair was measured using a caliper. The following are the results of observations of mouse hair growth are shown in table 3.

**Table 3. Rat Hair Length (cm)**

| **Rat hair length (cm)** | | | |
|---|---|---|---|
| **Group** | **Repetition** | **Day 7** | **Day 14** |
| Control | 1 | 0.21 | 0.48 |
| | 2 | 0.33 | 0.41 |
| | 3 | 0.12 | 0.23 |
| | 4 | 0.24 | 0.37 |
| | 5 | 0.18 | 0.28 |
| | 6 | 0 | 0.19 |
| Test 1 (3%) | 1 | 0.55 | 0.78 |
| | 2 | 0.71 | 1.12 |
| | 3 | 0.56 | 1.09 |
| | 4 | 0.42 | 0.82 |
| | 5 | 0.73 | 1.12 |
| | 6 | 0.33 | 0.95 |

| | | | |
|---|---|---|---|
| Test 2 (9%) | 1 | 0.78 | 1.18 |
| | 2 | 0.69 | 1.12 |
| | 3 | 0.81 | 1.31 |
| | 4 | 0.71 | 1.03 |
| | 5 | 0.59 | 1.01 |
| | 6 | 0.72 | 1.14 |
| Test 3 (12%) | 1 | 0.85 | 1.34 |
| | 2 | 0.91 | 1.43 |
| | 3 | 0.82 | 1.56 |
| | 4 | 0.93 | 1.61 |
| | 5 | 0.79 | 1.45 |
| | 6 | 0.87 | 1.57 |

Based on the results of the observations made, it shows that there is a hair growth process in all control groups and test groups. Researchers calculated the comparison of the average hair length per group to see the post-test group with the fastest hair growth, and the following results were obtained in table 4.

**Table 4. Average Length of Rat Hair (cm)**

| **Average Rat Hair Length (cm)** | | |
|---|---|---|
| Group | Day 7 | Day 14 |
| Control | 0.18 | 0.32 |
| Test 1 | 0.55 | 0.98 |
| Test 2 | 0.71 | 1.13 |

| | | | |
|---|---|---|---|
| Test 3 | 0.86 | 1.49 | |

Based on the average value of the length of the rat's hair, it can be seen that there was a development in hair growth in each group for 14 days. On the last day, the hair length in the control group was 0.32 cm. The hair length of the group after testing 1 was 0.98 cm, the test group 2 was 1.13 cm and the test group 3 was 1.49 cm. Based on this average difference, the researcher concluded that the test group with the greatest hair growth was in the group after testing 3 with treatment given clove cream extract (*Syzygium aromaticum*) 12%. While the group with the least hair growth was in the control group compared to test groups 2 and 1 which were given clove cream extract (*Syzygium aromaticum*) 3% and 9%

**Wound Healing Observation Results**

The results of observations of dermapen wounds on the backs of mice are presented in the following table 5:

**Table 5. Average Wound Healing (cm)**

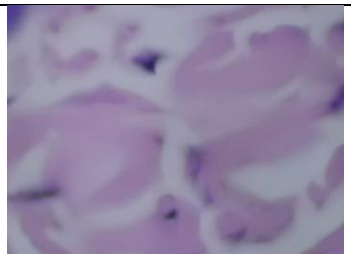
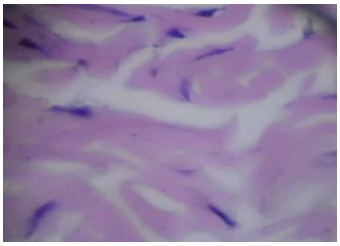
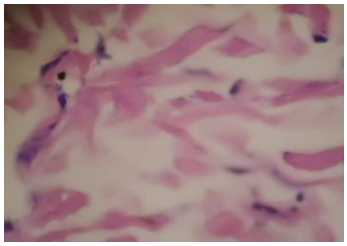
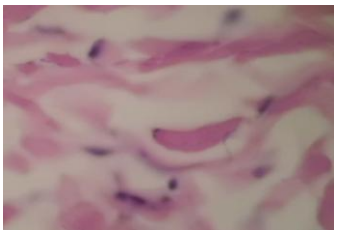
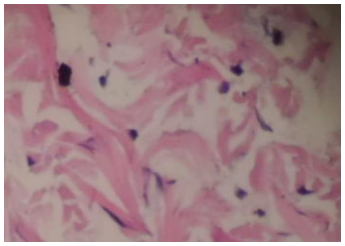
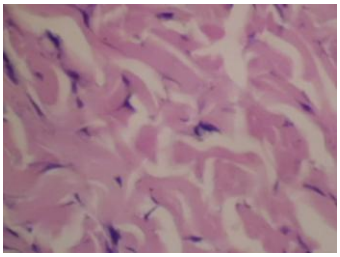
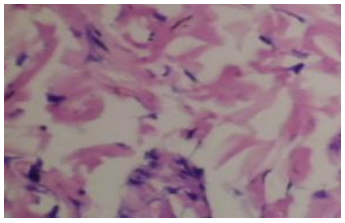
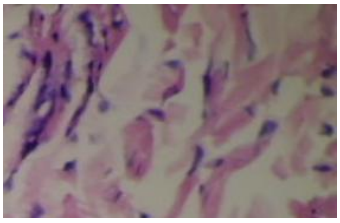
| Day | Average Wound Healing (cm) | | | |
|---|---|---|---|---|
| | **Control** | **Test 1** | **Test 2** | **Test 3** |
| 1 | 2 | 2 | 2 | 2 |
| 2 | 1.97 | 1.94 | 1.93 | 1.92 |
| 3 | 1.91 | 1.82 | 1.80 | 1.79 |
| 4 | 1.86 | 1.76 | 1.70 | 1.62 |
| 5 | 1.65 | 1.60 | 1.52 | 1.31 |
| 6 | 1.49 | 1.40 | 1.32 | 1.03 |
| 7 | 1.36 | 1.12 | 1.06 | 0.88 |
| 8 | 1.27 | 1.01 | 0.93 | 0.73 |
| 9 | 1.06 | 0.85 | 0.74 | 0.53 |
| 10 | 0.95 | 0.76 | 0.57 | 0.34 |

| | | | | |
|---|---|---|---|---|
| 11 | 0.89 | 0.63 | 0.44 | 0.15 |
| 12 | 0.80 | 0.45 | 0.26 | 0 |
| 13 | 0.65 | 0.28 | 0 | 0 |
| 14 | 0.53 | 0 | 0 | 0 |

The following are the average results of the comparison of the length of the rat wounds in each group. From the data, those who experienced perfect wound closure were in test groups 1, 2, and 3, namely 0 cm, on the 14th day and the group with the largest wound length in the control group, namely 0.58cm. To compare the percentage of healing of wounds from dermapen between treatments, the length of the wound was calculated every day in each test group. The initial wound was considered 0.00%, thus it can be said that the percentage of wound healing before treatment in all research groups was the same. Based on the results of observations made on all groups, it showed that there was a process of healing wounds from dermapen in white rats (*Rattus norvegicus*) Wistar strain. There was a difference in the average percentage of healing from the control group and the test group. The average percentage of healing of wounds from dermapen on the last day of the control group was 73.5%, group after test 1 100% on the 14th day, group after test 2 100% on the 13th day and group after test 3 100% on the 12th day. So, it can be concluded that the control group did not experience total healing and the group after test 3 required the fastest time to experience total healing than the groups after test 1 and test 2. Histopathological observations were carried out using a light microscope with 400x magnification. The purpose of this observation was to see the structure and morphology of wound healing cells in the control group using the base cream and the group after testing which was given clove extract cream (*Syzygium aromaticum*) with concentrations of 3%, 9%, and 12%. Clove extract cream (*Syzygium aromaticum*) was given every day in the morning. The following is a picture of the collagen density of each group after testing:

**Table 6 Histopathological Description of Skin Tissue**

| No | Group | Histopathological Image of Skin Tissue |
|---|---|---|

| | | | |
|---|---|---|---|
| 1 | Control (Base Cream) |  |  |
| 2 | Test 1 (3%) |  |  |
| 3 | Test 2 (9%) |  |  |
| 4 | Test 3 (12%) |  |  |

The study's findings suggest that giving male white rats (*Rattus norvegicus*) of the Wistar strain clove extract cream (*Syzygium aromaticum*) may have an impact on collagen development in wound healing from dermapen. The treatment with clove extract (*Syzygium aromaticum*) showed thick and dense growth in microscopic pictures. Collagen grew quickly in groups 1, 2, and 3 following testing.and thick (75–100%). Compared to the groups after testing 1 and 2, which were smeared with 3% and 9% clove extract cream, the histopathological image of the group after testing 3, which was smeared with 12% clove extract cream, had the densest collagen structure. The chemicals in clove extract (*Syzygium aromaticum*) cannot be distinguished from the density of collagen generated in the histopathology of the skin of male white rats (*Rattus norvegicus*) of the Wistar strain.

## DISCUSSION

As free radical transporters that arise from dermal wounds, the secondary metabolites in clove extract—tannins, triterpenoids, flavonoids, and saponins—promote hair growth and hasten wound healing. Phytochemicals have shown promising results in the prevention and treatment

of wounds and microbiological infections. Antimicrobial, antioxidant, and wound-healing phytochemicals aid in blood coagulation, infection prevention, and wound healing. Phenolics enhance wound healing due to their astringent, antimicrobial, and free radical scavenging properties. By inhibiting lipid peroxidation, flavonoids and other polyphenolic components may have antibacterial and antioxidative properties that aid in the successful healing of wounds. This reduces cell damage and promotes the survival of collagen fibrils (Vitale et al., 2022). According to Banerjee et al. (2020), clove has potent antibacterial, antifungal, antiseptic, analgesic, antiparasitic, antioxidant, anti-inflammatory, and anticarcinogenic properties. The main components (60–90%) of the clove extract are eugenol, caryophyllene, hydroxycinnamic acid, eugenyl acetate 3-allyl-6-methoxyphenol, tyranton, flavonoids, and hydroxyphenyl propens. Vitamins K, A, B1, B6, C, and β-carotene, on the other hand, are minor ingredients (5–10%). White rats (*Rattus norvegicus*) Wistar strain is administered clove extract cream (*Syzygium aromaticum*), which is believed to affect hair development and cutaneous wound healing. Researchers employed Wistar strain white rats (*Rattus norvegicus*) to test this notion. Cloves are one of the many medicinal herbs that are used to cure and prevent a wide range of illnesses. According to Rasouli et al. (2017), the presence of recognized bioactive chemicals in plants indicates a variety of possible biological activities that may be crucial for hair development and wound healing. Clove extract (*Syzygium aromaticum*) included secondary metabolites in the form of flavonoids, saponins, tannins, alkaloids, and steroids, according to the findings of phytochemical testing. According to earlier studies by El Faqer et al. (2022), clove extract includes alkaloids, steroids, flavonoids, tannins, and saponins. Following 14 days of the wounds being smeared with base cream in the control group and clove extract cream in the treatment group, many data analyses, including normality, homogeneity, and significance tests, were required to process and test the data. Using SPSS, data for the normality test was collected using the Kolmogorov-Smirnov test. At a significance level of 0.200, the results of the hair growth and wound healing tests for each test group were normally distributed. As a result, the data might be considered normally distributed or representative of the population. The Levene test checks for homogeneity to see whether normally distributed data comes from a population with the same variance. Next, the homogeneity test and the One-Way Anova test were performed. The results of the first observation, namely on the growth of hair on the back following a dermapen wound. Based on the average length of the mouse hair, the study concluded that treatment group 3, which was given a 12% concentration of clove extract, had the fastest rate of hair development. The control group, on the other hand, experienced the least quantity of hair growth. The control group received only a base cream. The second observation pertained to the process of wound healing. The results of the observation showed that the average percentage of healing differed

between the control and treatment groups. The average dermapen wound healing percentage for the control group was 73.5% on the last day; on the 14th and 12th days following tests 1 and 2, the groups experienced 100%, 13 and 12, respectively, and 100%. Considering this, it can be said that the control group did not undergo complete healing, and that the group after test 3 needed the least amount of time to do so compared to the groups following tests 1 and 2.

## CONCLUSION

1. Phytochemical test results show that clove extract (*Syzygium aromaticum*) contains secondary metabolites in the form of flavonoids, saponins, tannins, and steroids. These compounds, especially flavonoids, help heal wounds from dermapen and stimulate hair growth.
2. Clove extract cream (*Syzygium aromaticum)* affects hair growth. Based on the difference in average hair length, the researcher concluded that the group with the greatest hair growth was in the group after test 3 which was given clove flower extract with a concentration of 12%. The group with the least hair growth was in the control group which was only given the base cream.
3. Administration of clove extract cream (*Syzygium aromaticum*) with concentrations of 3%, 9%, and 12% affected the healing process of wounds from dermapen in obese white rats (*Rattus norvegicus*) Wistar strain. The treatment group experienced total healing, while the control group given the base cream did not.
4. The results of histopathological observations showed that the control group produced very thin collagen growth <25%, while in the group after testing which was given clove extract cream (*Syzygium aromaticum*) with concentrations of 3%, 9%, and 12% the collagen was fully filled and dense (75-100%).

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