The Effect Of Giving Cloves Extract (*Syzygium Aromaticum*) On Collagenization And Histopathological Picture Of Skin Tissue In The Healing Process Of Dermapenese Wounds In White Wistar Male Rats

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ABSTRACT

Clove is a native plant of Indonesia but is currently cultivated in several parts of the world including Brazil in the state of Bahia. This plant represents one of the richest sources of phenolic compounds such as eugenol, eugenol acetate and gallic acid and has great potential for pharmaceutical, cosmetic, food and agricultural applications. The antioxidant and antimicrobial activities of cloves are higher than those of fruits. This study was conducted to test and analyze the effectiveness of giving clove extract cream (*Syzygium aromaticum*) on collagenization and wound healing from dermapen in male white rats (*Rattus norvegicus*) of the Wistar strain. The sample in this study was male white rats (*Rattus norvegicus*) of the Wistar strain weighing 160 – 200 grams and aged 2 – 3 months. Determination of the sample using the Ferderer formula for 4 groups and the overall results were 24 rats which would be divided into 4 different groups. Phytochemical test results showed that clove extract (*Syzygium aromaticum*) contains secondary metabolite compounds, namely flavonoids, saponins, tannins, alkaloids, and steroids which have antioxidant, antimicrobial, and anti-inflammatory effects and play a role in the healing process of dermapen wounds and collagenization. Clove extract cream (*Syzygium aromaticum*) with a concentration of 12% is most effective in accelerating wound healing, namely in 12 days. The results of observations of histopathological images of skin tissue showed that treatment groups 1, 2, and 3 with the administration of clove extract cream (*Syzygium aromaticum*) with concentrations of 4%, 8%, and 12% had a collagen density of 75 – 100 compared to the control group <25%.

Keywords: *Wounds, Collagen, Dermapen, Skin, Cloves*

INTRODUCTION

The skin, as a general defense system, is the largest organ in the human body, accounting for 15% of body weight. It plays an important role in protective, thermoregulatory, sensory and immunological functions, as well as body fluid balance (Liang et al., 2022). The skin has four main functions: protection against the external environment, including ultraviolet light and infection, temperature regulation through blood vessels and sweat glands, sensation of different nerve endings, and endocrine function by producing vitamin D (Labib & Winter, 2023). The skin is made up of three main layers: the epidermis, dermis, and subcutis. The epidermis is the outermost layer, and is made up of five sublayers. The basal layer or stratum germinativum is made up of columnar cells that continually divide to regenerate the skin. It also contains melanin-producing cells (melanocytes) and neuroendocrine cells (Merkel cells). The stratum spinosum is made up of polyhedral cells, and forms the bulk of the epidermis. It contains antigen-presenting cells, called Langerhans cells. The next sublayers are the stratum granulosum and stratum lucidum, which are made up of large, flat cells. These cells eventually die to form the outermost layer of the epidermis, the stratum corneum. The dermis is the next layer of skin, accounting for 90% of the skin's thickness. The dermis is made up primarily of collagen and elastin and contains blood vessels, sweat glands, sebaceous glands, and hair follicles. It can be further divided into the papillary and reticular dermis. The deepest layer is the subcutaneous or hypodermis, which is mostly composed of fat and collagen. This layer acts as an insulator and shock absorber (Labib & Winter, 2023). A wound is a disruption of the cellular, anatomical, and functional integrity of living tissue caused by physical, chemical, electrical, or microbial threats to the tissue (Sharma et al., 2021). Wounds tend to disrupt the integrity of the skin epithelium and can occur with or without microbial infection. Wounds can occur multiple times throughout a person's life due to the chemical, physical, and microbial factors that influence them. The individual's physiological response to an injury is referred to as wound healing, which involves a concerted biochemical and cellular event that leads to the restoration of functional and structural integrity at the site of injury (Soni et al., 2023).

LITERATURE REVIEW

The skin is the largest and most important protective organ of the body, covering its entire outer surface and serving as a first-order physical barrier to the environment. Its functions include temperature regulation and protection against ultraviolet (UV) light, trauma, pathogens, microorganisms, and toxins. The skin also plays a role in immunological surveillance, sensory perception, control of insensible fluid loss, and general homeostasis. The skin is also highly adaptive with varying thicknesses and specialized functions in different

parts of the body (Lopez-Ojeda et al., 2022). The skin is the largest organ in the human body, consisting of various cells, layers, such as hair follicles, sebaceous and sweat glands, and with nerve endings that sense pain, pressure, vibration and temperature. All these entities act together to sense and protect humans from the external environment, such as physical, chemical and biological influences (temperature, radiation, drying, trauma, chemicals, microbes, etc.). The skin is also very important for humans to understand our environment and to communicate through receptors present in the skin (Mirastschijski et al., 2020).The skin is basically composed of three layers. The top layer is the epidermis, the layer beneath the epidermis is the dermis, and the third and deepest layer is the subcutaneous tissue (Lopez-Ojeda et al., 2022). Cloves (*Syzygium aromaticum*) are a spice plant in the form of aromatic flower buds from a tree species in the *Myrtaceae* family. This plant was first discovered in the Maluku Islands, Indonesia (Sundari et al., 2019). The following is the classification of clove plants:

Kingdom: Plants
Division: Spermatophyta
Class: Dicotyledoneae
Order: Myrtales
Family: Myrtaceae
Genus: Syzygium
Species: *Syzygium aromaticum*

Cloves contain bioactive compounds, namely 10-13% tannin, terpenoid, glycoside, and 14-21% essential oil. Essential oil compounds contain eugenol, caryophyllene, furfural, vanillin, methyl salicylate, pyrocatechol, methyl ketone, & valerate aldehyde, eugenin, isoeugenitol, isoeugenitin, eugenitin, tannin, mucilage, sitosterol, estigmaterol, resin, oleanolic acid, resin, cellulose, and fixed oil. Cloves have a strategic role in various industries including the pharmaceutical, cosmetics, food and beverage, cigarettes, botanical pesticides, fisheries, active packaging and other chemical industries (CortésRojas et al., 2014). The medicinal use of clove oil can be observed in many cultures throughout the world, clove oil has been used as a natural antiseptic and as a remedy for various symptoms either in topical or inhalant form (Primandaru et al., 2023). It’s antiseptic properties, as well as its anti-inflammatory potential, are due to its high eugenol content (Uddin et al., 2017).

METHODS

The type of research used in this study is quantitative experimental using a true experiment or laboratory experimental design. Experimental research is carried out by controlling all external variables that can affect experimental activities. This study uses a post-test only control group design to determine and analyze the effect of giving clove extract in accelerating the healing of wounds from dermapen in white rats (*Rattus norvegicus*) Wistar strain and how the histopathology of their skin is. The sample of this study was a rat (*Rattus norvegicus*) Wistar strain weighing 160-200gr and aged 2-3 months. The researcher chose male Wistar rats as the test subjects because this animal has characteristics and physiology that are almost the same as humans and is also one of the most widely used animals in biomedical research.

The operational definition in this study can be seen in table 1

Table 1 Operational Definitions

Variables	Operational Definition	Methods and Measuring Tools
Clove extract cream	Cream containing clove extract with a semi-solid emulsion, which is used for external use.	Measuring with the help of scales
Collagenization of mouse wound healing	Dermapen wound healing as seen from the length of healing time, measuring the length of the wound,	Vernier calipers,
Histopathology of the Skin	To assess the level of healing of the backs of mice that have been given clove extract through histopathological images of skin tissue.	Identified using a microscope with 400x magnification

Data from histopathological observations through microscopic examination were collected in the form of then scored. The research data were tabulated, then the changes found were analyzed and presented descriptively. Wound healing observation data were analyzed using SPSS (Statistic of Package for Social Science) 25.0. for windows. Data normality tests were analyzed using the Kolmogorov-Smirnov test approach ($p > 0.05$). To test the significance between test groups, one-way variance analysis technique or One Way ANOVA was used at a 95% confidence level ($p < 0.05$). Further analysis or testing was carried out using the Post Hoc Test with the LSD technique. Acclimatization is the process of adjusting to a new environment, climate, condition, or atmosphere. Test animals undergo a seven-day

acclimatization process in the Laboratory of the Department of Pharmacology and Therapeutics, Faculty of Medicine, University of North Sumatra. Test animals are given time to adapt to the new environment, as well as their food and drink (ad libitum).

RESULTS

The The subjects used in this study were male white rats (*Rattus norvegicus*) Wistar strain weighing 160 – 200 g. The sample calculation used the Ferderer formula and the results obtained were 6 per group, so that the total sample in this study was 24 rats. The test animals were randomly divided into 4 groups, the control group which was only given base cream, treatment group 1 was given clove extract cream (*Syzygium aromaticum*) topically with a concentration of 4%, treatment group 2, given clove extract cream (*Syzygium aromaticum*) topically with a concentration of 8%, and treatment group 3 was given clove extract cream (*Syzygium aromaticum*) topically with a concentration of 12%.

The characteristics of the test animals used in this table can be seen in Table 2.

Table 2 Characteristics of test animals

	Group			
Component	**Control**	**P1**	**P2**	**P3**
Types of Rats	*Rattus norvegicus*white wistar strain			
Gender	Male			
General Condition	Healthy and active			
Average Initial Body Weight	189gr	182gr	181gr	200gr
Average Final Weight	188gr	181gr	180gr	198gr

Characteristics of the test animals, in general the mice were in a healthy and active condition during this study, namely before and after treatment. A total of 24 test animals were able to participate in this study until the end without any drop outs. The average initial body weight of mice in the control group was 189 grams, after treatment it became 188 grams. The average initial body weight of treatment group 1 was 182 grams, after treatment it became 181 grams. The average initial body weight of treatment group 2 was 181 grams, after treatment it became 180 grams. Finally, the average initial body weight of treatment group 3 was 200 grams, after treatment it became 198 grams. Based on the average body weight that has been described, there was a decrease in body weight in mice. The average weight loss in all groups of mice was 1.25 grams. There was no significant weight loss in all groups. The results of the phytochemical screening test of this test sample can be seen in the Table 3.

Tabel 3. Phytochemical screening test

Secondary Metabolites	Color	Results
Flavonoid	Yellow	+
Saponins	Yellow and foamy	+
Tannin	Blackish blue	+
Alkaloid	Yellow	+
Steroid	Green	+

Phytochemical testing is carried out to examine the content of secondary metabolite compounds contained in clove extract (*Syzygium aromaticum*). Phytochemical tests include tests for flavonoids, saponins, tannins, alkaloids, and steroids/triterpenoids. First, a flavonoid test is carried out. 1 gram of clove extract (*Syzygium aromaticum*) is put into a test tube, then concentrated HCl is added and heated for 15 minutes in a water bath. If a red or yellow color is formed, it means that flavonoids (flavones, chalcones and aurones) are positive. In testing flavonoids, a yellow liquid is formed, which means that it is positive for flavonoids.

DISCUSSION

This study was conducted to test and analyze the effectiveness of clove extract cream (*Syzygium aromaticum*) on collagenization and wound healing from dermapen in male white rats (*Rattus norvegicus*) of the Wistar strain. The sample in this study was male white rats (*Rattus norvegicus*) of the Wistar strain weighing 160-200 grams and aged 2-3 months. The sample determination used the Ferderer formula for 4 groups and the overall results were 24 rats which would be divided into 4 different groups. The first group as the control group, in this group the rats were only given base cream (0%). The treatment group was given clove extract cream (*Syzygium aromaticum*) with concentration variations of 4%, 8%, and 12%. The procedure begins by eliminating the test animal's consciousness by using a combination of ketamine (80 ml/kg BW) and xylasin (5 ml/kg BW) so that the rat does not feel pain and avoids excessive movements made by the rat due to the dermapen action. The treatment uses a dermapen tool with the direction of needle movement perpendicular to the stretched skin. Pressure is initiated point by point on all scars (needle depth 2.5 mm) and the end point is the bleeding point. The wounds formed were then smeared with base cream in the control group and clove extract cream (*Syzygium aromaticum*) with concentrations of 4%, 8%, and 12% in the treatment group for 14 days and produced data that needed to be processed and tested first, so it was necessary to carry out several data analyses in the form of normality, homogeneity, and significance tests. Normality test data were obtained with the help of SPSS using the Kolmogorov-Smirnov test. The results showed that wound healing data in each group were

normally distributed with a significance value of 0.200 in all test groups. So it can be concluded that the data is normally distributed, or can represent the population. Normally distributed data are then tested for homogeneity using the Levene test to determine whether the data comes from a population with the same variance. The results of the homogeneity test of wound healing data obtained showed a significance value of 0.751. The probability value of significance obtained is greater than 0.05, so it can be concluded that the control group, treatment group 1, treatment group 2, and treatment group 3 are homogeneous or come from the same population. The normally distributed and homogeneous data were then tested for effectiveness and significance using the One-Way Anova test. The results of the One-Way Anova test on wound healing showed a significance value of 0.000 or greater than 0.05. The results of the analysis showed that there was a significant difference between the control group and treatment groups 1 ($p = 0.000$), 2 ($p = 0.000$) and 3 ($p = 0.000$). Based on the observation results, there is a difference in the average percentage of healing from the control and treatment groups. The average percentage of healing of wounds from dermapen on the last day of the control group was 72.5%, treatment group 1 100% on the 13th day, treatment groups 2 and 3 100% on the 12th day. So it can be concluded that the control group did not experience total healing and treatment groups 2 and 3 required the fastest time to experience total healing than treatment 1. Wound healing occurs within the first 24 hours after injury and can take up to 2 weeks in normal wounds and much longer in chronic non-healing wounds. Mast cells release granules containing enzymes, histamine, and other active amines, which are responsible for the characteristic signs of inflammation, redness, heat, swelling, and pain around the wound site. Neutrophils, monocytes, and macrophages are key cells during the inflammatory phase. These cells clear the wound of infection and debris and release soluble mediators such as proinflammatory cytokines (including IL-1, IL-6, IL-8, and TNF-α), and growth factors (such as PDGF, TGF-β, TGF-α, IGF-1, and FGF) that are involved in the recruitment and activation of fibroblasts and epithelial cells in preparation for the next phase of healing (Shcultz et al., 2011). The results of phytochemical tests on clove extract (*Syzygium aromaticum*) showed that there were secondary metabolite contents in the form of flavonoids, saponins, tannins, alkaloids, and steroids. Previous research conducted by Lone & Jain (2022) showed that clove extract contains secondary metabolite compounds in the form of alkaloids, flavonoids, tannins, steroids, and triterpenes, all of which were found, according to phytochemical examination. Cloves are also the most important and valuable spices. Flavonoid compounds can help the body overcome wounds and stimulate collagen growth. Flavonoid compounds can bind free radicals formed due to dermapen wounds. High amounts of free radicals cause fibroblasts to change into adhesive types, which promote scar tissue formation by increasing collagen synthesis and deposition. The presence of flavonoids breaks

down free radicals and reduces ROS, and increasing their levels can provide adequate conditions for wound healing (Piera-Velazquez et al., 2021). Flavonoid compounds help promote regular cell proliferation and migration (Zhang et al., 2022).

CONCLUSION

1. The results of phytochemical tests show that clove extract (*Syzygium aromaticum*) contains secondary metabolite compounds, namely flavonoids, saponins, tannins, alkaloids, and steroids which have antioxidant, antimicrobial, and anti-inflammatory effects and play a role in the wound healing process of dermapen and collagenization.
2. Administration of clove extract cream (*Syzygium aromaticum*) with concentrations of 4%, 8%, and 12% can accelerate wound healing and increase collagen density in white Wistar rats that have dermapen wounds.
3. Clove extract cream (*Syzygium aromaticum*) with a concentration of 12% was most effective in accelerating wound healing, namely within 12 days.
4. The results of observations of histopathological images of skin tissue showed that treatment groups 1, 2, and 3 with the administration of clove extract cream (*Syzygium aromaticum*) with concentrations of 4%, 8%, and 12% had a collagen density of 75-100% compared to the control group <25%.

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