The Effect Of Giving Salak Skin Extract (*Salacca Zalacca*) On Collagenization And Histopathological Picture Of Skin Tissue In The Healing Process Of Dermapen Wounds In White Wistar Male Rats With Obesity Model

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ABSTRACT

Background This study aims to analyze the effect of snake fruit skin extract (*Salacca zalacca*) in accelerating the healing of dermapen wounds in obese Wistar white rats. The research method used a quantitative experimental design with a post-test only group design. The results of this study are that snake fruit skin extract contains active compounds such as alkaloids, flavonoids, saponins, and tannins which have anti-inflammatory and antioxidant effects. This observation was carried out with the aim of comparing wound healing between the group given the base cream and the group given snake fruit skin extract cream (*Salacca zalacca*) with 3 different concentrations, namely 10%, 15%, and 20%. This observation was carried out every day for 14 days. The treatment group showed an increase in the percentage of wound healing that was faster than the control group. The conclusion of this study is that snake fruit skin extract is effective in accelerating the healing of dermapen wounds in obese rats, with an increase in collagen fiber density at concentrations of 15% and 20%.

Keywords: *Salak Peel Extract, Collagenization, Histopathology*

INTRODUCTION

Salak (*Salacca zalacca*) is one of the medicinal plants. Salak, which belongs to the Arecaceae family, is a native Indonesian plant like aren (enau), coconut, oil palm, fern, and palm, has low branches. The stem of this plant is covered with leaf sheaths that are tightly arranged and thorny, which makes many new shoots emerge from the stem, which are almost invisible. The part of the salak fruit that is very efficacious for traditional medicine is found in the skin of the salak fruit. Salak skin is classified as waste that is difficult to decompose. However, (Nazaruddin and Kristiawati, 2000) argue that salak skin has nutrients such as protein percentage, carbohydrate percentage, water percentage and low fat.

Wounds are partial or partial tissue damage that causes the composition of the organism to be damaged or lost. Wounds can cause loss of almost all or half of the body's impact, bacterial contamination, sympathetic stress response, blood clotting, bleeding, and cell death. Robinson (2008) stated that wound closure is a skin wound that remains and is not connected between the tissue below and the outside environment. This is known as a wound due to fear of blunt objects. Bruises can be called closed wounds. There are two types of bruises: contusions, which are damage to tissue under the skin that only looks like a lump on the outside, and hematomas, which are damage to tissue under the skin followed by bleeding, so that it looks bluish from the outside.

Wounds that experience skin problems or the system below are called open wounds. Open wounds are caused by gunshots, sharp objects, or impacts from hard objects. Types of open wounds include bite wounds (vulnus marsum), abrasions (excoriation), slash wounds (vulnus caesum), gunshot wounds (vulnus sclopetinum), crush injuries (vulnus lacerum), lacerations (vulnus traumaticum), cuts/cuts (vulnus scisum), and burns. Cuts or incisions (vulnus scisum) are caused by slicing with sharp objects including razors, knives, machetes, etc. The resulting injuries are long and straight edged, but the surrounding skin tissue is not injured (Amri, B., et al 2017) argue that wound infections are caused by not being treated.

Phytochemical tests showed that the skin of the snake fruit and its flesh contain flavonoids, alkaloids, and tannins. Meanwhile, Sahputra, FM, (2008) stated that the skin of the snake fruit contains steroids, saponins, and triterpenoids. Kanon, et al. (2012:58) stated that the skin of the snake fruit has the potential to be used as a herbal medicine because the mixture contained a mixture of flavonoids, namely the percentage of blood sugar is lowered. Farida (2009:1) argued that a mixture of saponins, iron, flavonoids, and tannins is consumed as a medicine for diarrhea. The skin of the snake fruit also has the potential to have an impact as a medicine for diarrhea because of the content of the mixture it has. Sukadana, et al. (2009-8:16) argued that flavonoid isolates are thought to be inhibitors of the growth of E. coli bacteria. Melanocytes

absorb some of the ultraviolet (UV) radiation that has a worrying potential from the sun, and mesodermal cells, pigmentation, or melanin are found in the skin.

Samples were taken by administering mouse drops (Combination of ketaminexylazine dose 0.1mg/200gBW), Skin cut samples were taken from healed wounds of each mouse and placed in 10% formalin buffer solution to facilitate histopathological studies. Healed skin samples were cut at a thickness of 5 µm and stained using hematoxylin and eosin (H&E). Samples were placed in a pot with 40% PFA content, kept at room temperature and transferred to the histopathology preparation location, namely the Histopathology laboratory, University of North Sumatra.

Based on previous research conducted by (Girsang, 2020)mentions the many benefits of snake fruit skin for body health which has diverse and potential pharmacological properties due to its high antioxidant content. So the author is interested in further researching the efficacy of snake fruit skin whether there are other contents outside the content that has been studied by previous researchers because Phytochemical investigations in this fruit have revealed the presence of phenolics, flavonoids, glycosides to some volatile and aromatic solutions, including chlorogenic acid, epicatechin, gallic acid, quercetin, proanthocyanidins, lycopene and ß-carotene. Saleh, MS, et al. (2018) in pharmacological studies on the flesh and skin of the fruit have shown extraordinary antioxidant, anti-inflammatory, anticancer, and antidiabetic potential.

LITERATURE REVIEW

The snake fruit tree is dioecious, meaning that male and female flowers grow together on different trees. Male inflorescences have 410 flowers in one cluster and a long stem, while female inflorescences have 420 flowers in one cluster and a shorter stem. Snake fruit plants are usually attacked by wind and insects. To produce fruit, male flowers must be inserted into female flowers slowly. Even in fertile conditions, female snake fruit plants will not produce fruit if they do not do this activity. By planting seeds from fresh fruit, snake fruit can germinate in less than a week in a humid and shady environment. Snake fruit is 312 cm long and 47 cm wide, oval (like an egg) or almost round. Each fruit has thirteen seeds covered in fruit flesh (Kusmana, C., Hikmat, A, 2015). Today's society really likes tropical fruits such as salak. Salak fruit is very good because it has a lot of nutrition. The Directorate of Nutrition, Ministry of Health of the Republic of Indonesia in the Salak Product Technique of the Horticulture Research and Development Center. (1995) stated, the fruit content of every 0.04 milligrams of vitamin B1, 100 grams of 77 calories, 20.9 grams of carbohydrates, 0.5 grams of protein, 28 milligrams of calcium, 18 milligrams of phosphorus, 4.2 milligrams of iron, and 2 milligrams of vitamin C.0020.

The following is the taxonomy of the salak plant:

Kingdom: Plantae

Sub Kingdom: Trachebionta

Super Division: Spermatophyta

Division: Magnoliophyta

Class: Lilopsida

Sub Class: Arecidae

Order: Arecales

Family: Areceae

Genus: Salacca

Species: *Salacca zalacca*

The skin is a complex body part that covers the entire surface of the body. Its function is as a physical barrier between the body and the surrounding environment, it retains water and electrolytes in the skin, and protects it from bacteria and UV radiation. Changes caused by aging problems occur in one of the organs of the body that can be seen directly. Changes in photoaging will encourage the use of cosmetics that improve the skin to get a better appearance.(Yousef H, Alhajj M, Sharma S. Anatomy, 2023). The skin is composed of three layers: epidermis, dermis, and hypodermis. These two layers tend to differ in anatomical and functional conditions. The skin system is composed of a complex relationship that protects the body first from pathogens, ultraviolet sunlight, chemicals, and mechanical injury. In addition, it increases the release of air and the amount of water into the environment.(Yousef H, Alhajj M, Sharma S. Anatomy, 2023). The epidermis has several forms (derivates). There is a stoma, which means hole, where there is a small gap (pore) between the epidermal cells. which is flanked by two uniquely shaped cells that are different from other epidermal cells. The guard cells that are connected to the gap or hole are called stoma.(Trisiswanti & Sugimin, 2020). The epidermis layer consists of several layers, namely(Yousef H, Alhajj M, Sharma S. Anatomy, 2023):

a. **Basal layer** aka stratum germinativum is the deepest layer, starting from the dermis by the basal membrane (basal lamina) and attached to the hemidesmosomes. Cells form cubes to columns and are actively stem cell mitosis

a. **Stratum spinosum** or the arrangement of the spiny cells is a disorderly polyhedron with long, outward-facing processes ("spines") that encounter neighboring cells via desmosomes.

b. **Stratum granulosum** diamond-shaped cells containing keratohyaline powder; aggregates of keratin filaments occur upon cornification of the cells.

c. **Stratum lucidum** is a thin, clear structure composed of eleidin (a transformed product of keratohyalin); generally, appears in thick skin.

d. **Stratum corneum** is the outermost layer, consisting of keratin and horny scales, dead squamous cells (anucleate), the composition varies in thickness, especially thick in callused skin

In general, wounds are divided into 2 types, namely open injuries and open injuries. The following is an explanation of these types of wounds.

Open wound is a wound that causes damage to the skin structure to the point where the skin is in contact with the outside.(Barku, 2019)Some types of open wounds are

1. Abrasion: Wounds caused by skin rubbing against a rough surface. Abrasion wounds often occur in road accidents. An example is falling off a bicycle that causes an elbow or knee injury. Carefully clean the wound from sand or soil debris to avoid wound infection.

2. Laceration: a deep tear that penetrates the tissue. Often the wound involves a sharp object such as a knife that has the potential to cause damage.

3. Avulsion: Avulsion is a type of wound that presents the body tissue with an uneven wound. Wounds caused by wild animal bites or explosions are examples of this type of wound.

4. Puncture wounds: occur due to a small hole piercing the skin. Such as gunshot wounds, knife wounds, or needle injections.

METHODS

The type of research applied is quantitative experimental. Quantitative experimental is the use of true experiment design or laboratory experiment. True experiment is an experimental test that is carried out seriously by controlling all external variables. In this case, namely the administration of snake fruit skin extract (*Salacca zalacca*) and the procedure for dermapen injuries healed the backs of white rats (Rattus norvegicus) Wistar strain suffering from obesity.

A. Variable X (Free): Giving the essence of snake fruit skin (*Salacca zalacca*)

B. Variable Y (Bound): Healing of cuts and imaging Histopathology

Antioxidant Activity Test

- **Wavelength Determination** The maximum wavelength of DPPH was determined by reacting the control solution, which consisted of two milliliters of DPPH solution and two milliliters of methanol. The fiber of the control solution was measured on UV-Vis spectrophotometry, and the maximum wavelength of DPPH was determined by scanning λmax at a wavelength of 400–800 nm.(Molyneux, 2004)]
- **Determining Operating Time** To evaluate the operational time, 2 milliliters of DPPH solution was reacted with 2 milliliters of sample. The solution fiber was then measured on UV-Vis spectrophotometry by calculating the absorbance at the maximum wavelength until a stable absorbance was obtained.(Molyneux, 2004)
- **To determine antioxidant activity** snake fruit skin extract, 25 milligrams of snake fruit skin extract was taken and mixed with 25 milliliters of ethanol per liter in a 25-milliliter measuring flask until a concentration of 1000 ppm was obtained. Then, 20 l, 40 l, 60 l, 80 l, and 100 l of the stock solution were put into a test tube until a concentration of 2 ppm, 4 ppm, 6 ppm, and 10 ppm. After that, 1 ml of 10% TCA solution and 1 ml of 0.2 N phosphate buffer (pH 6.6) and 1 ml of 1% K3Fe (CN)6 were added. Then, centrifuged for approximately 20 minutes at a speed of 3000 rpm. After that, it was put into Furthermore, the maximum wavelength is used to measure the absorbance.

The animal acclimation procedure in this test looks at the modified test and was carried out by Gusbakti et al. Mice are test animals that are kept in groups in experimental animal cages in the laboratory. The plastic mouse cages measure 30 cm x 20 cm x 10 cm and are covered using fine wire mesh. Rice husks 0.5 to 1 cm thick are installed at the bottom of the cage and replaced every day during the study. The lights in the room are set to produce a 12-hour light/12-hour dark cycle. The temperature is set to 25–27 °C, and the humidity is set to normal 35–50%. Mice are given distilled water ad libitum and regular mouse pellets(Rittner D, 2005)

RESULTS

The sample calculation is based on the ferderer formula for 4 groups and the results obtained were 6 per group, so the total sample in this study was 24 mice. The following are the characteristics of the test animals are shown in table 1.

Table 1. Characteristics of test animals

Component	Group			
	Control	**P1**	**P2**	**P3**
Types of Rats	*Rattus norvegicus* white wistar strain			
Gender	Male			
General Condition	White fur color, healthy and active			
Average Body Weight	293gr	245gr	281gr	293gr

This observation was conducted with the aim of comparing wound healing between the group given the base cream with the group given the salak fruit skin extract cream (*Salacca zalacca*) with 3 different concentrations, namely 10%, 15%, and 20%. This observation was conducted every day for 14 days. The results of observations of dermapen wounds on the backs of mice are presented in the following table 2.

Table 2. Average Wound Healing (cm)

Day	Control	P1	P2	P3
1	2	2	2	2
2	1.97	1.96	1.94	1.91
3	1.95	1.91	1.71	1.65
4	1.91	1.85	1.53	1.51
5	1.82	1.71	1.31	1.26
6	1.77	1.65	1.21	0.65
7	1.68	1.32	1.18	0.61
8	1.62	1.21	1.05	0.53
9	1.59	1.11	0.88	0.21
10	1.23	0.85	0.48	0.15
11	1.11	0.51	0.11	0.02
12	0.94	0.25	0	0
13	0.86	0	0	0
14	0.77	0	0	0

Dermapen wound healing consists of several phases, namely the inflammation, proliferation and maturation phases. In the proliferation phase, fibroblasts play an important role in producing proteins for wound healing, one of which is collagen. The

results of histopathological examination using a 400x magnification light microscope on wound healing showed the following differences in collagen density:

Normality Test

The results of the data normality test in this study can be seen in the following table 3.

Table 3. Normality Test Results

Group	df	Sig
Control	14	.097
P1	13	.200
P2	15	.166
P3	14	.154

Based on the results of the normality test that has been carried out using the Kolmogorov-Smirnov Test. The results of significance above 0.05 were obtained for all groups that had been measured in several days of treatment. Data is said to be normally distributed if the p value> 0.05. Therefore, it can be concluded that the data is normally distributed.

Homogeneity Test

Table 4. Homogeneity Test Results

Levene static	**df1**	**df2**	**Sig**
2.209	3	52	0.098

The results of the homogeneity test using the Levene test with 14 days of treatment by 4 groups of mice can be seen in the table 4. The probability value in the significance column is 0.098. 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 come from a population that has the same variance or is homogeneous.

DISCUSSION

This study used test animals in the form of male white rats (Rattus norvegicus) Wistar strain weighing 160-250 grams. The test animals were divided into 4 groups, the control group was only given base cream, treatment group 1 was given salak fruit skin extract cream (*Salacca zalacca*) with different concentrations, namely 10%, 15%, and 20%. The sample calculation

was based on the ferderer formula for 4 groups and the results were 6 per group, so that the total sample in this study was 24 rats. One form of skin damage is wounds. Skin wounds are pathological conditions caused by disease, injury, or physiochemical damage. Based on the origin of the damage and the duration of healing, wounds are classified into acute and chronic wounds. Wounds are divided into acute wounds and chronic wounds based on the duration of healing. Acute wounds are wounds caused by trauma or surgical wounds, and they heal within 8-12 weeks, depending on the size and depth of the wound (Farahani, 2011). The healing process occurs after the wound is formed. To repair tissue damage, wounds usually go through inflammatory, proliferative, and remodeling phases. Collagen, an important part of the extracellular matrix, plays a vital role in regulating the wound healing phase, either in its native form, fibrillar conformation, or in its soluble form in the wound environment (Mathew-Steiner, S.S., Roy, S., & Sen, 2021). In the early stages of wound healing, resident cells, including fibroblasts that secrete collagen, accumulate in the damaged area. Eventually, blood vessels, nerve fibers, and collagen are formed, leaving an immature scar that is red, raised, hard, and painful (Anief, 1997). Dermapen is a motorized microneedle therapeutic system that can be adjusted to skin problems. Dermapen, which is an ergonomic device, can adjust the needle length from 0.25 to 2 mm using disposable needles and guides. Thus, one demapen can be used to treat narrow areas such as the nose, lips, and eyes without damaging adjacent skin (Purbosetyo, 2020). To accelerate wound healing, one way is to bandage the wound with salak fruit skin extract cream (*Salacca zalacca*). The phytochemical content of salak skin contains tannins, phlobathin, carbohydrates, saponins, triterpenoids, flavonoid phenols, flavanol glycosides, proteins, rutin, alkaloids, anthraquinones, anthocyanins, essential oils and steroids. The composition of fatty acids includes palmitic, stearic, linoleic and linoleic oleic acids. Butterfly pea flower antioxidants such as phenolics, flavonoids, anthocyanins, flavonol glycosides, kaempferol glycosides, quercetin glycosides, myristetin glycosides, terpenoids, flavonoids, tannins, and steroids (Valentine et al., 2021). Researchers suspect that there is an effect of giving snake fruit skin extract cream (*Salacca zalacca*) on healing wounds from dermapen in obese male white rats (*Rattus norvegicus*) of the Wistar strain. Researchers conducted a trial on male white rats (*Rattus norvegicus)* of the Wistar strain to prove this suspicion.

The study was conducted by collecting data related to the observation of the treatment procedure. First, the mice were given a preconditioning treatment in the form of a high-fat diet to induce obesity. The high-fat diet given was in the form of quail egg yolks. The parameters used to confirm that the mice were obese were using the Lee Index. The results showed that after a high-fat diet, all test animals had a Lee Index value of 0.33 or greater than 0.3 so it can be concluded that the mice were obese. After a high-fat diet, dermapen wounds were given.

CONCLUSION

1. There is an effect of giving the extractsnake fruit skin (*Salacca Zalacca*) on the collagenization of the skin of mice that were given dermapen treatment and the histopathological picture of skin tissue in the healing process of wounds from dermapen in male white Wistar rats.

2. Secondary metabolite content insnake fruit skin (*Salacca Zalacca)* Through phytochemical tests it is known that the skin of the snake fruit positively contains active compounds such as alkaloids, flavonoids, saponins, and tannins. So that the extract of the snake fruit skin has antioxidant, anti-inflammatory and anti-aging benefits.

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