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TEST OF BURN HEALING ACTIVITY OF TIBARAU ROOT FRACTION (*Saccaharum spontaneum* L.) IN MALE WHITE MICE

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ABSTRACT

Burns have a major impact both psychologically and physically, including severe trauma. So far, burn treatment has used chemicals such as bacitracin, mafenide acetate, silver sulfadiazine, silver nitrate, povidone-iodine, and mupirocine. However, the use of chemicals can cause side effects such as rashes, allergies, and hemolysis. Therefore, other alternatives are needed, one of which is using natural ingredients that are believed to have fewer side effects. One of them is using tibarau (Saccharum spontaneum). The purpose of this study was to determine the burn healing activity of the tibarau fraction, so that new candidates were found as burn medicine. The method in this study was an experiment with 5 groups of mice, namely the 15% tibarau extract group, 15% n-hexane fraction, 15% ethyl acetate fraction, and 15% n-butanol fraction, control and comparison. The parameters of wound healing were the percentage of wound healing, epithelialization time, and histopathology. From the results of the study it is known that the n-Butanol fraction has the best effect in healing burns. With a wound healing percentage of 99.8% on the 21st day, epithelialization time of approximately 14 days, and the formation of collagen, fibroblast cells, and epithelialization cells that are better than other groups. Calculations using the One-Way ANOVA statistical test for n-Butanol stated (P <0.05)

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significantly different. From these results it is concluded that the 15% n-Butanol fraction has the best burn healing activity.

INTRODUCTION

One of the causes of inflammation in the skin can be caused by burns. Burns that are deep enough can damage the submucosa, muscular lining, and significant mucosal loss. It then causes an active inflammatory response in the burn area followed by esophageal dysmotility and granulated tissue with fibroblasts that carry the collagen fiber matrix to the newly formed connective tissue. The formation of a burn describes the local response of a tissue, with or without a systemic response to energy transfer originating from physical (mechanical, thermal, radiation, electrical) or chemical sources (Ananta, 2020). Burns can have a very detrimental impact on humans both psychologically and physically, because burns include trauma in severe forms that have made humans suffer since ancient times (Ricca, 2023). Therefore, it is necessary to find alternatives to help speed up the healing process of the burns.

So far, burn treatment uses chemicals that can kill bacteria or inhibit bacterial growth. Some of the chemicals used as burn remedies are *bacitracin*, *mafenide acetate*, *silver sulfadiazine*, *silver nitrate*, *povidone-iodine*, and *mupirocine*. These medications can kill and reduce bacteria, thus preventing the risk of spreading bacteria to surrounding skin tissue and helping to prevent infection. On the other hand, the use of these chemical drugs has disadvantages such as causing side effects if used for a long time such as allergies, itchy rashes, hemolysis, *methamoglobinemia*, and so on (Frederick, 2022). Therefore, another solution is needed in healing burns. One of them is by utilizing natural materials. Because it is believed that the use of natural ingredients has fewer side effects than the use of synthetic chemical obak (Mellova, 2022).

One of the plants that has the potential to be used as a burn remedy is tibarau (*Saccharum spontaneum* L). *Saccharum spontaneum* is an economically helpful plant, widely grown in tropical and subtropical areas. This plant is considered a low-cost biomass because it belongs to weeds. Phytochemical tests of plant extract *Saccharum spontaneum* L. showed the presence of carbohydrates, glycosides, alkaloids, tannins, and flavonoids (Selvaraj, 2020). Research conducted by (Nada, 2020) obtained results on phytochemical screening of ethanol extract of tibarau root containing tannins, phenolates, flavonoids, alkaloids and terpenoids. Where each of these substances has a role in the process of healing burns. Flavonoids from the flavone group, flavonols and isoflavones have anti-inflammatory activity. Tannins are polyphenol compounds from the flavonoid group that have a function as a powerful antioxidant and also anti-inflammatory (Ananta, 2020).

Ethanol extract *Saccharum spontaneum*. is believed to contain quite high flavonoids. Based on various research studies that have been conducted, it is believed that flavonoids are one of the groups of phenolic compounds that have antioxidant properties and play a role in preventing damage to cells and their cellular components by reactive free radicals. Flavonoids have anti-inflammatory, antioxidant effects and also flavonoid content is believed to have benefits in the wound healing process (Hassan, 2024), Research conducted (Lapuz, 2016)

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showed that the cream of the 2% root extract of *the previously formulated Saccharum spontaneum* had anti-inflammatory activity rather than antibacterial activity. In this study, the researcher developed *Saccharum spontaneum extract* in the form of another preparation, namely ointment.

Based on the above description, the researcher chose the root of tibarau (*Saccharum spontaneum* L.) as a burn remedy because it has strong antioxidant levels that act as an antidote to free radicals and has anti-inflammatory activity. So the researcher is interested in conducting a study with the title Burn Healing Activity Test of Ointment Fraction of Tibarau Plant (*Saccharum spontaneum* L.).

RESEARCH METHODS

The research method describes the approach, activity design, scope or object, main materials and tools, place, data collection technique, operational definition of research variables, and analysis techniques.

This research is an experimental research. extraction and fractionation methods. A total of 18 male white mice were formed with burns on their backs.

Instrument used in this study are

Instrument

Standard Laboratory glassware includes: a set *of rotary evaporators*, maceration containers, droppers, stirring rods, tweezers, spatels, porcelain crucifixes, cruising pliers, watch glasses, vapor cups, spray bottles, mortars and stempers, calipers, funnels, parchment paper, digital scales, filter paper, test tubes, beaker glass, measuring cups, test tube racks, erlenmeyer, hot solder, stainless plates, blenders, surgical scissors, shaver and split funnel.

Material

The materials used for this study include: tibarau root (*Saccharum Spontaneum*), ethanol 96%, formalin 10%, Bioplacenton®, vaseline flavum, n-hexane solution, ethyl acetate solution, n-butanol solution, pure liqudium paraffin, HE staining, acetone, chloroform, albumin-glycerin, ether, aquadest and standard food.

Research Procedure

1. Sample Processing

The roots that have been collected are washed with running water to remove dirt and microbes that stick to the sample, then drained and then dried until dry. Drying aims to prevent simplicia quality degradation, is not easily damaged and is resistant to long storage. Dried simplisia is weighed as much as 2 kg, then mashed until it forms a powder, stored in a tightly closed container and protected from sunlight.

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2. Preparation of Tibarau Root Extract and Fraction

The extraction process is carried out by the maceration method where this method is a very simple cold extraction method. Simplisia is weighed as much as 2 kg. Simplicia powder is put into the maser, then 96% ethanol is added until all the powder is submerged. Let stand for 3 x 24 hours while stirring frequently. The maserat obtained is filtered into the container. Then the maserat is concentrated with *a low-pressure rotary evaporator* at a temperature of 35-40°C until a thick extract is obtained..

Weighed as much as 5 g of thick extract dissolved in 50 mL of aquades. The solution is then partitioned by adding 50 mL of n-hexane, shaken in a separate funnel and left until there are two layers (aquades in the lower layer and nheksan in the upper layer). Take the n-hexane layer. The aquades layer is then re-fractioned in the same way using the solvents ethyl acetate, and n-butanol. The fractionation results of n-hexane, ethyl acetate, and n-butanol are vaporized with the solvent using a *rotary evaporator* until a viscous fraction is obtained.

3. Burn Ointment Formulation

Table 1. Formulation of Burn Ointment from the plant suddenly (Saccharum Spontaneum)

Ingredient Name	F1	F2	F3	F4
Ethanol extract	3gram	-	-	-
n-Heksan Tibarau Faction		3gram	-	-
Ethyl Acetate Faction	-	-	3gram	-
n-Butanol faction tibaru				3gram
Vaselin Vlum and Ad.20 g	17gram	17gram	17gram	17gram

4. Treatment Procedure on Mice

a. Test Animal Setup

A total of 18 mice weighing 25-30 grams aged 2-3 months were used in this study. Where before conducting the study, the test animals received acclimatization treatment for 7 days which aimed to make the mice adapt to the new environment. The animal is declared healthy if during acclimatization it does not show weight deviations of more than 10% and visually no symptoms of the disease occur.

b. Burn Making

The creation of burns is carried out by determining the location of the burn, namely on the back of the mouse. The burns are shaped in the same way and the same method to be able to see which group is most effective in the healing process of the burn. Previously, a shave was carried out with an area of 2-4 cm on the back of the mouse. Anesthesia is

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performed with ether solution, then a 1 cm burn is made using an iron plate that has been heated to a temperature of 100oC and exposed to the skin of mice for 5 seconds without pressure. Then the wound is given a preparation according to the group.

c. Classification of Test Animals

The test animals were divided into 6 groups, each group consisting of 3 mice, namely: group I (15% ethanol extract), group II (15% n-hexane fraction), group III (15% ethyl acetate fraction), group IV (15% n-butanol fraction), group V (positive control), group VI (comparator).

d. Test Animal Treatment

The wounds that occurred were smeared with extracts and fractions as well as comparators according to the group, until evenly distributed 1 x a day for 21 days and then the results were observed.

5. Wound Healing Parameters

a. Wound Healing Percentage

Observations were made by measuring the area of the wound or the percentage of wound healing. The percentage of burn wound healing was recorded on the 7th day, 14th day and 21st day. The percentage of burn wound healing is calculated using the formula

$$\% \text{ penyembuhan luka} = \frac{\text{luas luka awal} - \text{luas luka akhir}}{\text{luas luka awal}} \times 100\%$$

b. Epithelial Time

Epithelialization time is the time it takes for the formation of a new epithelium that is perfectly formed covering the burn. In this case, the day of exfoliation of scab tissue from the wound is recorded without leaving any residual wound in the burn area.

c. Preparation of Histopathology Preparations

Skin collection is done on day 21. Previously, euthanasia was carried out using an excess dose of ether solution perinhalation on mice. The back area where the skin will be taken is cleaned of hair that has begun to grow back, the skin is cut with a thickness of ± 3 mm to subcutaneous and 2.5 cm long. The excised skin tissue is inserted into a 10% formalin solution. Network fixation time 18- 24 hours. Fixation is complete, then the tissues are dehydrated in 2x acetone solution for 1 hour each. Next, the tissue is cleared in 2x chloroform solution for 1 hour each. Then the tissue was infiltrated in a solution of chloroform paraffin for 1.5 hours and paraffin infiltration for 1.5 hours. The tissue is planted in paraffin blocks. The already dense tissue is cut to 5

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microns thick with microtomes. Pieces of tissue are attached to the glass of an object that has previously been smeared with albumin-glycerin as an adhesive. The tissue on the glass object is placed on the hot plate until it dries. It is then stained with eosin hematoxylin (HE) staining for microscopic examination. Furthermore, observe under a microscope with criteria based on the density of collagen fibers, epithelial cells, and fibroblast cells by assessment using a score system.

6. Analysis Data

The results of the research observations are reported as data from the results of the extract test *Saccharum spontaneum*, n-hexane fraction of the root of the sudrau (*Saccharum spontaneum*), ethyl acetate fraction of the root of the sudrau (*Saccharum spontaneum*), n-Butanol fraction of the root of the sudrau (*Saccharum* Etanol akar *spontaneum*), control (+) and comparison against burn healing by measuring the parameters of wound healing percentage, epithelialization time, and histopathology to see the density of collagen fibers, epithelialized cells and fibroblast cells in male white mice were statistically treated with one-way ANOVA using SPSS 16.0 and continued with the Duncan test.

RESULTS AND DISCUSSION

This study was conducted to see the effectiveness of healing burns from the root of the tree to take advantage of the availability of nature as one of the alternative treatments because of the process of increasing the "*back to nature*" style in other words, people believe that active compounds from natural ingredients are Safe with synthetic chemical compounds (Mellova, 2022).

As much as 2 kg of dried roots are then crushed and extracted by the maceration method, maceration is the process of extracting or extracting compounds from symphylicia using solvents with several times of shaking or stirring. The maceration process is carried out using 96% ethanol solvent because it has properties that can dissolve almost all substances, be it non-polar, semi-polar and polar (Arifin, *etal*. 2006). Hasil maserat dikentalkan menggunakan *rotary Evaporator* until thick and obtained a viscous extract of 70.95 grams with a yield of 3.547%, drying loss of 1.55% and ash content of 7.67%.

Some viscous ethanol extracts are subjected to a cascade fractionation process with a separation funnel that will separate compounds based on their degree of polarity using 3 solvents, namely n-Hexane (non-polar), ethyl acetate (semi-polar), and n-Butanol (polar) to adjust the physical properties and chemical properties contained in the material (Wikarta *et al*. 2012). The use of non-polar, semi-polar, and polar solvents applies the *rule of like dissolved like* which states that non-polar solvents will be dissolved in non-polar solvents, semi-polar solvents will be dissolved in semi-polar solvents and polar solvents will be dissolved in polar solvents.

The result of multi-level fractionation with 3 solvents, namely non-polar, semi-polar, and polar, is then thickened using *a rotary evaporator*. The result of the viscous n-Hexane

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fraction weighed 7.81 grams with a yield of 0.390%, a drying loss of 0.42% and an ash content of 0.48%. The yield of viscous ethyl acetate fraction was 9.56 grams, yield 0.478%, drying shrinkage of 1.05% and ash content of 6.37% and the yield of n- Butanol fraction was 11.83 grams, yield of 0.591%, drying shrinkage of 1.82% and ash content of 9.20%.

In the phytochemical examination, the results were obtained that the ethanol extract contains flavonoids, phenolics, terpenoids, and tannins. The n-hexane fraction has chemical content of flavonoids, steroids, and tannins. The ethyl acetate fraction and the n-butanol fraction have the same chemical content, namely flavonoids, phenolics, steroids, and tannins.

The results obtained in the examination of the drying shrinkage of the extract and fraction have met the standard with a moisture content of no more than 11% and in accordance with the provisions of SNI, which is a maximum of 5%. In the ash content examination, the results obtained from extracts and fractions have met the applicable standards, which are no more than 16.6% (Depkes RI, 2008).

Organoleptic examination of extracts and fractions includes: shape, odor, and color. The results of the examination on the ethanol extract were thick, distinctive smell, and dark brown color. The results of the examination on the n-hexane fraction are thick, n-hexane's distinctive odor, and dark green color; the ethyl acetate fraction is thick, with a distinctive ethyl acetate odor, and a reddish-brown color; and the n-butanol fraction is thick, with a distinctive n-butanol smell, and a dark brown color.

The formation of burns on the back of mice with a diameter of 1 cm using an iron plate heated to a temperature of 100oC is then exposed to the back of the mouse for 5 seconds until it forms a 1st degree burn. Mice that have been injured are given treatment 1x a day according to their respective groups for 21 days. The following is an example of treatment on mice on the 7th, 14th, and 21st days given a 15% n-butanol fraction can be seen in Figure 1.

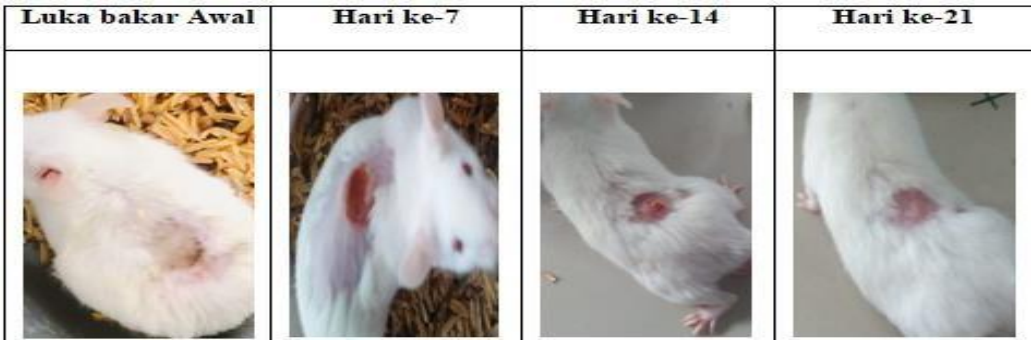

Figure 1. Observation of Burns in Experimental Animals II with 15% n-butanol fraction treatment.

The results of this study can be obtained visually by looking at the diameter of the wound to calculate the percentage of wound healing, and epithelialization time as well as histopathologically based on the value or suspension by looking at the density of collagen fibers, epithelialized cells and fibroblast cells which are the parameters of wound healing.

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From the results of the calculation of the wound healing percentage, if the longer the time required in the wound healing process, the higher the wound healing percentage value, which can be seen in Figure 2.

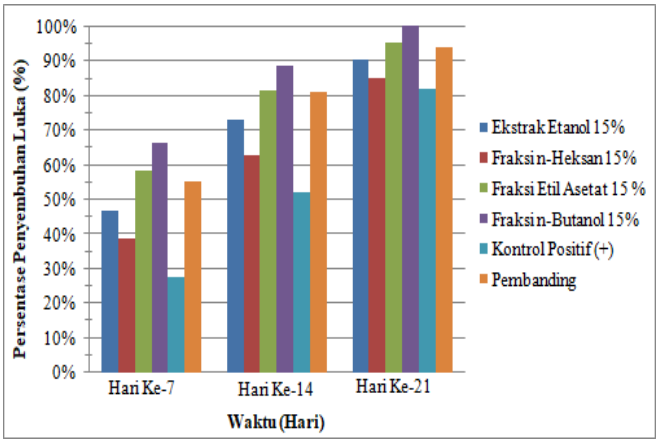

Figure 2. Wound Healing Percentage Chart

In group I (15% ethanol extract), the results of observation of wounds on day 7: 46.85%; 14th: 72.89%; 21st: 90.33%. In group II (n-Hexane fraction 15%), the results of observation of wounds on day 7: 38.85%; 14th: 62.88%; 21st: 85.09%. In group III (ethyl acetate fraction 15%), the results of observation of wounds on day 7: 58.34%; 14th: 81.49%; 21st: 95.40%. In group IV (n-Butanol fraction 15%), the results of observation of wounds on day 7: 66.24%; 14th: 88.48%; 21st: 99.98%. In group V (positive control) the results of observation of wounds on day 7: 27.69%; 14th: 51.91%; 21st: 81.88%. In group VI (comparator) the results of observation of wounds on day 7: 55.09%; 14th: 81.03%; 21st: 93.77%.

From the data, it is explained that the longer the day, the area of the wound that heals has a high percentage of wound healing. The diagram above shows that group IV had higher wound healing activity than the comparison group, while group III and group I had almost the same burn healing activity as the comparison group, and group II and group V had much lower burn activity than the comparator. This is due to the difference in the type of solvent used, where group IV goes through a fractionation process with a polar solvent, namely n-Butanol. Polar solvents are able to attract more polar compounds in them, as the results obtained from chemical content tests state that the n-Butanol fraction has a chemical content of flavonoids, phenolics, tannins, and steroids that have the ability to heal burns.

Epithelialization time is the time needed for the formation of new epithelial cells to completely cover the burn which can be seen from the exfoliation of the burn scab without leaving a residual wound in the burn area. The results of the epithelialization time in each group can be seen in Figure 3.

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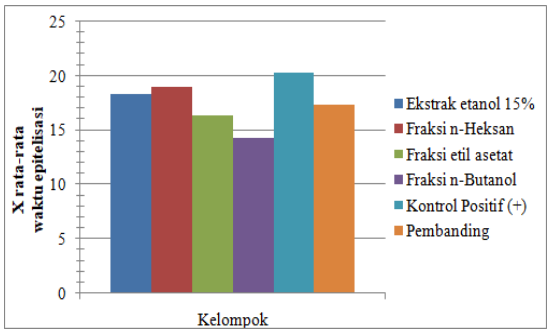

Figure 3. Epithelial Time Diagram

The chart data above shows that the longer the day of scab peeling, the higher the diagram shown, on the contrary, the faster the scab peeling process, the lower the diagram shown. From the diagram above, the positive control group had the longest epithelialization time, which is indicated by the height of the chart among the other groups. Where the time required in exfoliating scabs by extracts and fractions had an average value of ethanol extract 15% (group I) 18.33, n-hexane fraction 15% (group II) 19, ethyl acetate fraction 15% (group III) 16.33, n-Butanol fraction 15% (group IV) 14.33, positive control (group V) 20.33, comparator (group VI) 17.33.

Collagen plays a very important role in every stage of wound healing. Collagen can perform homeostasis, increase fluid exudation, interaction with fibronectin, interaction with platelets, increase cellular components, as well as promote the process of fibroplasia and sometimes in the proliferation of the epidermis. The results of histopathology of collagen fiber density after 21 days from the respective groups can be seen in Figure 4.

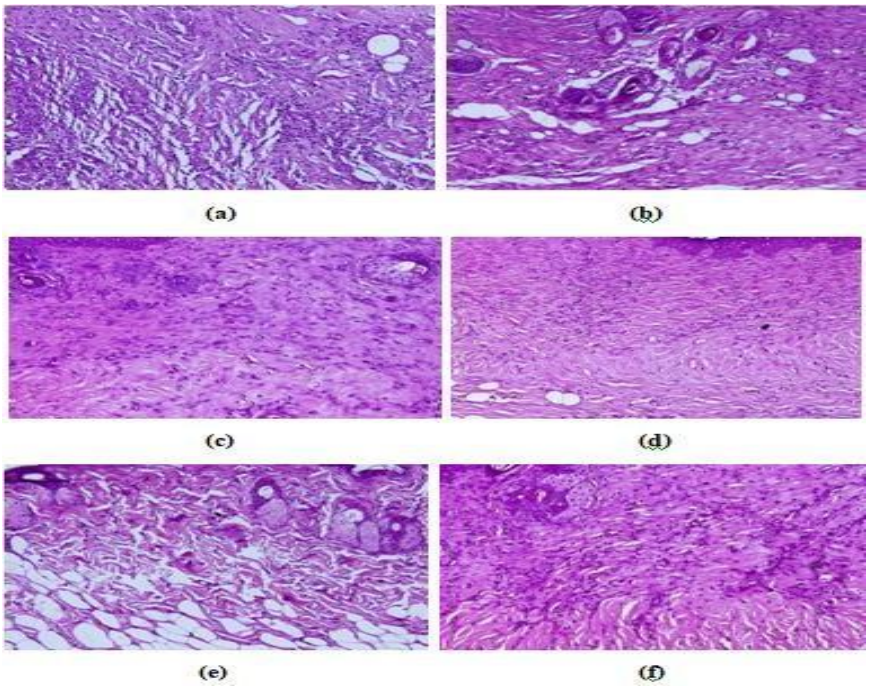

Figure 4. Histopathology of collagen fiber density, where: (a) ethanol extract 15%, (b) n-

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hexane fraction 15%, (c) ethyl acetate fraction 15%, (d) n-Butanol fraction 15%, (e) positive control, (f) comparator.

The above data shows the results of collagen fiber density seen from the objective 40x lens on the 21st day that the 15% n-Butanol fraction group and the 15% ethyl acetate fraction group have collagen fibers that have completely covered the wounded skin, followed by the comparison group and ethyl acetate 15% while for the ethanol extract group 15%, the positive control and the 15% n-hexane fraction show that the collagen fibers have not been fully formed which contain granulation tissue with collagen that Loose to moderate so that the skin after the burn still shows many cavities that have not been completely closed. In each treatment, the average score of ethanol extract was 15% (group I) 2, n-hexane fraction (group II) 1.6, ethyl acetate fraction 15% (group III) 3, n-Butanol fraction 15% (group IV) 3, positive control (group V) 1.33, comparator (group VI) 2.67.

The observation of epithelialized cells aims to see the process of forming new epithelial cells in the burn tissue. Epithelial cells are one of the parameters of burn healing. The longer the wound healing period, the more perfect the epithelialization process is with the formation of new epithelial cells to their normal shape. The histopathological results of epithelial formation are seen on a 10x objective lens, which can be seen in Figure 5.

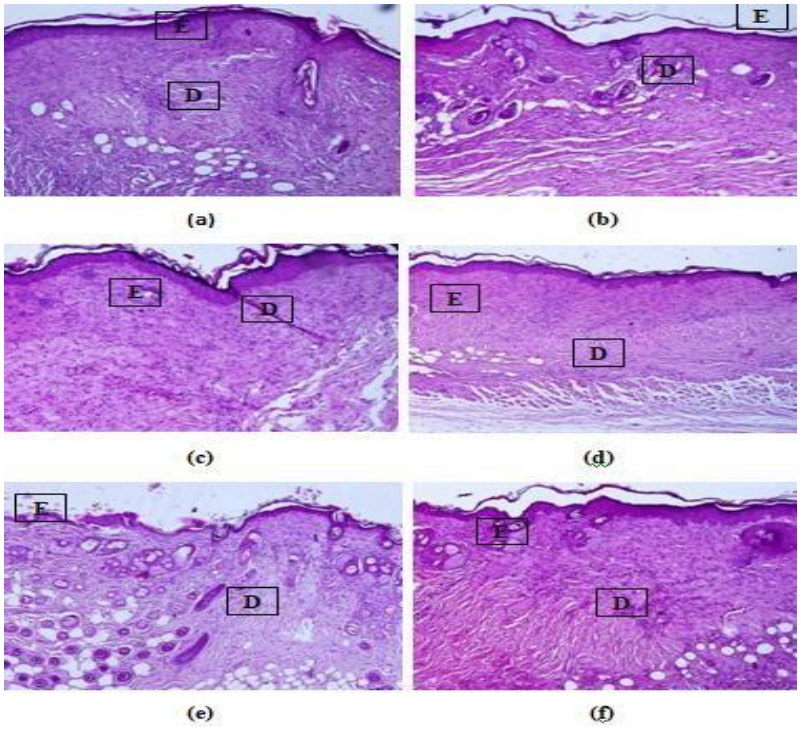

Figure 5. Histopathology of Epithelial Cells, where: (a) ethanol extract 15%, (b) n-hexane fraction 15%, (c) ethyl acetate fraction 15%, (d) n-Butanol fraction 15%, (e) positive control, (f) comparator.

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From the histopathological results of the epithelialized cells of the skin tissue of experimental animals, the epidermal epithelium (E), and dermis (D) areas showed better growth of the epidermal epithelium, especially in the group of 15% n-butanol fraction and 15% ethyl acetate fraction compared to the comparison of epithelium that completely covered the surface of the burn scar area. Meanwhile, in the ethanol extract group of 15%, the n-hexane fraction of 15% and the positive control showed epithelium that covered the surface of the scar area was thin and incomplete. Where the average score of ethanol extract 15% (group I) was 2.67, the n-hexane fraction 15% (group II) 1.66, the ethyl acetate fraction 15% (group III) 3, the n-Butanol fraction 15% (group IV) 3, the positive control (group V) 1.33, the comparator (group VI) 3. The score results obtained showed that the formation of epithelial cells was the most shown by the group of 15% n-Butanol fraction equivalent to the 15% ethyl acetate fraction and the comparator followed by 15% ethanol extract, 15% n-hexane fraction and positive control.

Fibroblast cells are stem cells that can stimulate collagen, these cells play a role in forming and laying fibers on the matrix, especially collagen fibers The histopathology results of fibroblast cells are seen on a 100x objective lens, which can be seen in Figure 6.

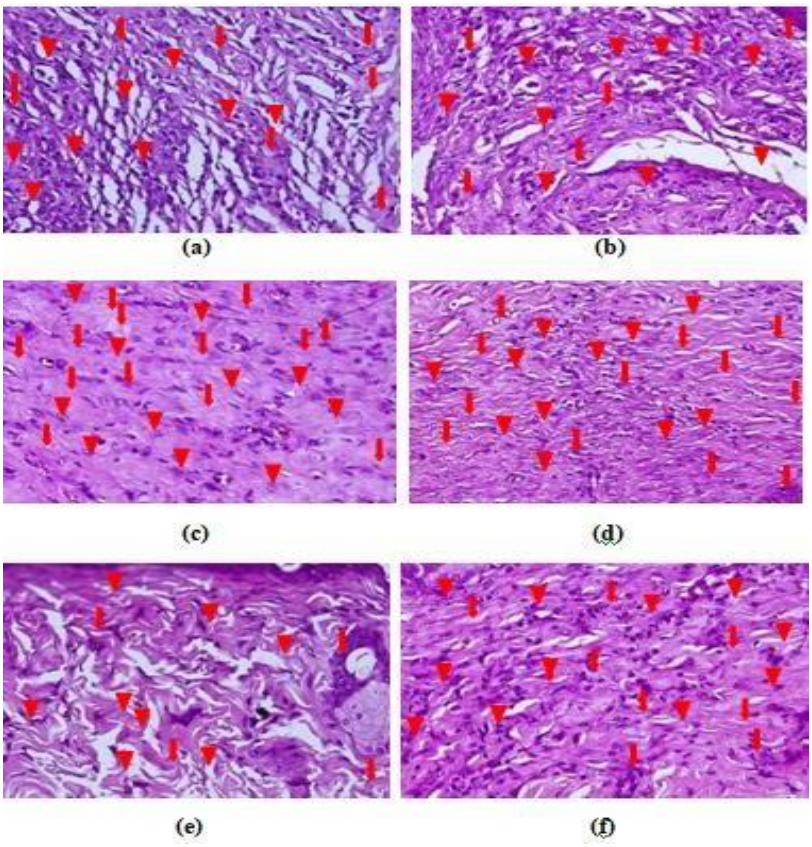

Figure 6. Histopathology of Fibroblast Cells, where: (a) ethanol extract 15%, (b) n-hexane fraction 15%, (c) ethyl acetate fraction 15%, (d) n-Butanol fraction 15%, (e) positive control, (f) comparator

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The fibroblast tissue in the image above is shown by an arrow, from the results above it shows that the n-butanol fraction group is 15%, the ethyl acetate fraction is 15% and the comparator group has a lot of fibroblast growth, while in the ethanol extract group 15%, the n-hexane fraction 15% and the positive control show a little fibroblast growth. The average score obtained by each group were, 15% ethanol extract (group 1) 1.667, n-hexane fraction 15% (group II) 1.667, ethyl acetate fraction 15% (group III) 3, n-Butanol fraction 15% (group IV) 3, positive control (group V) 1.333, comparator (group VI) 3. From the results of the scores, it can be concluded that the group with the most fibroblast cells is shown by the group of n-Butanol fraction 15% equivalent to the ethyl acetate fraction of 15% and the comparator followed by ethanol extract 15%, n-hexane fraction 15% and positive control.

Flavonoids are a compound that has the ability to stimulate fibroblasts to produce shows that the n-butanol fraction group is 15%, the ethyl acetate fraction is 15% and the comparator group has a lot of fibroblast growth, while the ethanol extract group is 15%, the n-hexane fraction is 15% and the positive control shows little fibroblast growth. The average score obtained by each group were, 15% ethanol extract (group 1) 1.667, n-hexane fraction 15% (group II) 1.667, ethyl acetate fraction 15% (group III) 3, n-Butanol fraction 15% (group IV) 3, positive control (group V) 1.333, comparator (group VI) 3. From the results of the scores, it can be concluded that the group with the most fibroblast cells is shown by the group of n-Butanol fraction 15% equivalent to the ethyl acetate fraction of 15% and the comparator followed by ethanol extract 15%, n-hexane fraction 15% and positive control.

Flavonoids are compounds that have the ability to stimulate fibroblasts to produce collagen fibers (Afifah, 2020). flavonoid adalah suatu antioksidan memiliki kemampuan yang baik secara in vitro (Hassan, 2024). From each group of extracts and fractions obtained contain flavonoids in it. The content of flavonoids has the ability as an antioxidant that is able to ward off free radicals, this is in line with research conducted by (Nada, 2020) stated that tibarau root has a very strong antioxidant level with an IC50 value of 12.29 ppm. Flavonoids are a group of phenolic compounds that have antioxidant properties that play a role in preventing damage to cells and their cellular components by reactive free radicals (Anggarani, 2023). Flavonoids have anti-inflammatory, antioxidant effects and also flavonoid content is believed to have benefits in the wound healing process. Flavonoids as compounds that have an important role in wound healing have a mechanism of action by reducing the effect of the inflammatory period, namely acting as an anti-inflammatory that helps the blood flow process throughout the body work properly and prevents blockages in blood vessels, in other words it has a function as an anti-oxidant and is able to reduce pain if swelling and bleeding occur. Flavonoids as antioxidants have pharmacological activity as a burn healing by inhibiting the activity of the enzymes cyclosiginase (COX) and lipooxygenase, inhibiting the accumulation of leukocytes, slowing the release of histamine, and inhibiting neutrophil degranulation (Maria, 2022).

From the results of the wound healing measurement data, it was continued with hypothesis testing with *One-way* ANOVA SPSS 16.0. Tests conducted on day 7, day 14, and day 21 showed results that varied significantly between groups with a value of ($P<0.05$), meaning that each group had a meaningfully different percentage of wound healing. The ANOVA test was then followed by the Duncan test. On the 7th, 14th, and 21st days showed

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that the 15% n-Butanol fraction group had a significant difference with the 15% ethyl acetate fraction group, the comparison group with the ethyl acetate fraction group did not differ significantly from the other groups, the 15% ethanol extract group had a significant difference with the other groups, the n-Hexan fraction group had a significant difference with the other groups, and the positive control group also had significant differences with the other groups, Each group had a noticeable difference in results.

Histopathological tests to see the density of collagen fibers were tested statistically with *One-way* ANOVA SPSS 16.0. On the 21st day, the results of each group variation were obtained which showed a value ($P<0.05$), where each group showed a significant difference except for the 15% ethyl acetate fraction group did not differ significantly from the comparator.

In the test, looking at the formation of epithelialized cells statistically from each group, a source of variation was obtained that had a value ($P<0.05$). Each group showed significantly different results except for the 15% ethyl acetate fraction group which did not have a significant difference from the comparison group. In statistical testing, fibroblast cells from each group were obtained as a source of value variation ($P<0.05$). The 15% ethyl acetate fraction group did not have a significant difference with the comparator, but with the other group there was a significant difference.

The test with *One-way* ANOVA SPSS 16.0 and followed by the Duncan test showed significant differences between groups except for the 15% ethyl acetate fraction group with no significant difference between the comparators. It can be seen that the highest significant values are shown by the n-Butanol fraction group which means it has the best wound healing rate, collagen fiber density, epithelialized cells, fibroblast cells and epithelialization time of all groups.

CONCLUSION

The administration of 15% n-Butanol fraction containing the roots of Tibarau (Saccahrum spontaneum L.) made in the form of an ointment using vaseline flavum base with treatment once a day has the best activity in accelerating the healing of burn wounds in male white mice, where the results show the percentage of wound healing. optimum, optimum epithelialization time, optimum collagen fiber density, optimum epithelialization cells, and optimum fibroblast cells were seen on the 21st day. And in general, among all the fractions tested, the n-Butanol fraction had the best results. Followed by statistical testing and Duncan's test with ($P<0.05$), showing that the 15% n-butanol fraction group had significantly different values from the other groups. Meanwhile, the ethyl acetate fraction group did not differ significantly from the comparison group

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