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Potential of *Averrhoa bilimbi* Leaf in Wound Healing of Diabetic Rat

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Abstract: *Diabetic wounds are a serious problem and need to be treated appropriately. One of the wound healing efforts is using the leaves of the Averrhoa billimbi (Abl). This study aims to examine the reduction in wound healing and histopathological images of wound-healing rats with Abl extract. The rat in the study were 2-3 months old, weighed 200-250 grams and were 12 male. The rat were divided into 3 groups: the control group (G1) received only distilled water, the group 10 µl extract (G2) and the group of diabetic mice received 10 l extract (G3). The treatment and skin tissue samples were obtained on day 8. The rats were digested with ether solution and skin incisions were made, followed by povidine idone administration. The study data were analyzed by analysis of variance (ANOVA). Statistical test results showed that the incision reduction was highest in G2, followed by G3 and G1 at 1.120 ± 0.156, 1.405 ± 0.007, 1.510 ± 0.028, respectively. Histopathological images of proliferative fibroblast cell counts and collagen density were higher in K3 than in K1. Based on the study results, it can be concluded that using 10 µl extract can heal cuts in diabetic rats.*
Keywords: *Incision wound, starfruit leaves, decrease in wound length, hispathology*.

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1. INTRODUCTION

Cases of diabetes melitus (DM) are on the rise and this phenomenon has the effect of increasing the incidence of diabetic wounds. Up to 15-25% of people with diabetes will develop a diabetic ulcer in their lifetime as a result of a chronic complication of diabetes. Chronic and difficult-to-heal wounds are subject to infection, ischemia, and risk of amputation or even life [1].

The World Health Organization (WHO) predicts that the number of cases of diabetes in Indonesia will continue to increase from 8.4 million people in 2000 to 21.3 million people with diabetes in 2030 [2]. The risk of infection and amputation remains quite high, namely 40-80% for infected DM wounds, 14-20% for amputation, 66% for recurrence and 2% for amputation. wound after 5 years of recovery [3],[4]. According to data from the Agency for Basic Health Research (Riskesdas) 2013, the prevalence of diabetes increased from 1.1% in 2007 to 1.5% in 2013. The prevalence of diabetes in women tends to increase compared to men. In addition, increased incidence also occurs with age, and people with diabetes have a higher prevalence in urban communities than in rural areas [5].

Research regarding diabetic wound management continues. Society's tendency to return to nature encourages the study of medicinal plants. One of the plants used as traditional medicine for diabetic wounds is the leaves of *Averrrhoa bilimbi*.

Against this background, the aim of this study was to determine the effect of *Averrrhoa bilimbi* leaf extract on reducing wound length and skin histopathological features of diabetic rats.

2. MATERIALS AND METHODS

Determination of Sample

Leaf collected from the Kajhu site were placed in clear plastic for identification at the USK FMIPA Biology Laboratory.

Sample Preparation.

Fresh leaves were harvested up to 5 kg, wet graded, cut into 3 mm and dried at room temperature. The dried samples were pulverized with a blender and soaked in 70% ethanol in a glass bottle for 3 days. The obtained macerate was concentrated using a rotary evaporator.

Plant Screening Tests.

The phytochemical screening method is performed by testing the color with color reagents. Phytochemical screening includes testing for alkaloids, steroids and terpenoids, saponins, tannins, phenols and flavonoids [6].

GC-MS test

1 g of the ethanolic extract of A. bilimbi leaves was dissolved in methanol (pa) and aspirated 1 ml plus 9 ml of methanol. The solution was then withdrawn with a syringe for filtration and ready for GC analysis. Rtx-5MS stationary phase detector (30m x 0.25mm) and helium gas mobile phase detector. Running conditions were used at constant pressure, column temperature 60°C-10°C/min 240°C (15 min), pump temperature 200°C, and injection volume 3 µL. Chromatographic analysis of the compounds obtained was performed by comparing them with the library.

Animal testing

Rat testing was performed at the UPT on Experimental Animals and the USK Department of Veterinary Medicine Physiology Laboratory. The study began in April 2023. Animals used were Wistar rats weighing 200-250 grams, 2-3 months old, including 12 males. The rats were divided into 3 treatment groups. Group one (G1) served as the control, i.e. the group was cut and treated, the wound was cleaned with distilled water. Group two (G2), that is, the incision group, received star fruit extract with an extract content of 10%. Group three (G3), namely a group of cut-cut diabetic rats, received carom leaf extract containing 10%. Rats were acclimatized for 7 days and on day 8 they were cut. Incisions were processed for 14 days and skin samples were fixed with 10% NBF. Samples fixed with 10% NBF were chopped and placed in a cloth basket and labeled. Dewater, clear, infiltrate paraffin I, II, III then dip in paraffin block then cut with microtome, place on glass object and leave for 24 hours.

HE staining begins with deparaffinization with xylol. Followed by rehydration with alcohol solution of decreasing concentration for 5 minutes, then rinse with distilled water for 10 minutes. Next, the tissue was stained with hemactocillin for 5 min and rinsed again under running water for 10 min, then the tissue was stained with eosin for 2 min, followed by graded alcohol, cleaned with xylol and overlay procedure with an adhesive. Rat skin histology was observed mi-

croscopically and the data obtained was processed by ANOVA.

3. RESULTS AND DISCUSSION

Phytochemical screening of ethanolic leaf extracts of A. bilimbi.

Table 1: *Results of screening phytochemicals of A. bilimbi*

Compounds	Methods	Result
Flavonoid	NaOH	+
Alkaloid	Mayer	+
Saponin	Foam	+
Fenol	FeCl3	+
Terpenoid	Cloroform + asetat anhidrat + sulfate acid	-
Steroid	Cloroform + asetat anhidrat + sulfate acid	+
Tanin	FeCl3	-

Analyze the content of alkaloids, tannins, polyphenols, saponins, terpenoids, flavonoids and steroids in the ethanol extract of ABL in Table 1 by color test using multiple reagents. The specific reagent is polar and can interact with the leaf extract according to the "like to dissolve" principle. The phytochemical content of the leaves has pharmacological effects. Precipitation formation in the Mayer test demonstrated that the ethanolic extract of *A. bilimbi* contains alkaloids. The Mayer reagent contains a heavy metal salt, specifically potassium mercuric iodide, which reacts with the nitrogenous alkaloids to form a precipitate [7].

The results of the tannin test are negative, the presence of tannins will precipitate the protein in the gelatin. Tannins react with gelatin to form stable, water-insoluble copolymers. This reaction is more sensitive when NaCl is added to increase the tannin-gelatin salinity. The appearance of foam in the fourth test indicates the presence of glycosides that are capable of foaming in the water and hydrolyzed to glucose and other compounds. In addition to the Forth test, the Lieberman-Burchard test was also performed, which is specific for unsaturated sterols and triterpenoids [8].

GC-MS test

The GC-MS test results obtained for the chemical compounds are 2-methylpropanal propyl hydrazone (1,41%); 2,3-dihidrobenzofuran (3,52%); 2-propenoic acid (3,46%); n-hexadecanoic acid (6,83%); phytol (9,36%); 9,12,15-octadecatrien-1-ol (6,96%); geranylgeraniol (10,31%).

Flavonoid compounds have the ability to stabilize free radicals by donating hydroxyl groups, methoxyl or supporting the electron conjugation system. In addition to flavonoids, alkaloids and tannins are also present in the phenolic component, the phenolic component is a powerful antioxidant. Active compounds such as flavonoids and alkaloids have antioxidant activity. Flavonoids can scavenge ROS and free radicals, which are reactive mediators capable of slowing wound healing. Flavonoids, as potent antioxidants, can reduce lipid peroxidation, thereby promoting re-epithelialization and antimicrobial activity [9], [10]. Saponins and terpenoids enhance the biological effects of many medicinal plants. Saponins increase the binding capacity of the TGF-β fibroblast receptor to TGF-β. TGF-β is a growth factor required for fibroblasts to synthesize collagen, while tannins are thought to play a role in the regulation of transcription and translation of vascular endothelial growth factor (VEGF). VEGF acts in a parasecretory fashion not only in the vascular endothelium of the skin but also in keratinocytes and immune cells, exerting a re-epithelial effect and simultaneously restoring angiogenesis and oxygen perfusion. In addition, flavonoids, tannins and saponins can act as hemostatic agents, which can accelerate wound healing [11], [12], [13]

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Reducing the length of the rat's incision

The results showed that the injury rate decreased from day 2 to day 8 ($P < 0.05$), as shown in Table 2. The study results showed

that recovery days were marked by a decrease in wound length and closure in groups G2 and G3. Wound closure in the normal group and the glycemic control group showed increased wound length at day 8, although there was no difference between the two groups. The difference in wound length in the control and treated groups was due to the presence of bioactive compounds in the leaf extract of A. bilimbi. The results of this study are consistent with reports [14] that wound length was reduced after the use of Chinese castor resin.

Table 2: *Mean of wounds length*

Groups	Length of Rat Wounds (cm)			
	X ± SD (Day 2)	X ± SD (Day 4)	X ± SD (Day 6)	X ± SD (Day 8)
G1	1.985 ± 0.021	1.855 ± 0.064	1.660 ± 0.071	1.510 ± 0.028
G2	1.905 ± 0.021	1.695 ± 0.007	1.505 ± 0.106	1.120 ±0.156
G3	1.900 ± 0.000	1.750 ± 0.071	1.660 ± 0.141	1.405 ± 0.007

Wound healing goes through three stages, namely hemostasis/inflammation, proliferation and regeneration/maturation. These stages are governed by a range of different factors and mediators. Immediately after an injury, an inflammatory response occurs and cells beneath the dermis (the deepest layer of the skin) begin to increase collagen (connective tissue) production. Then epithelial tissue (external skin) is regenerated. Hemostasis is triggered by the constriction of blood vessels to stop blood loss. Platelets aggregate to form fibrin or fibrous tissue. Different types of cells such as neutrophils, monocytes, keratinocytes and fibroblasts migrate to the wound site [15],[16].

The proliferative phase is characterized by angiogenesis, collagen deposition, granulation tissue formation, epithelialization, and wound shrinkage. Angiogenesis involves the growth of new blood vessels from endothelial cells. During the formation of fibrous and granulosa tissue, fibroblasts secrete collagen and fibronectin to form a new temporary extracellular matrix. The epithelial cells then crawl through the wound to seal and the wound is contracted by myofibroblasts, which adhere to the wound edges and contract by a mechanism similar to that of muscle cells 17],[18].

Histology of Rat Skin

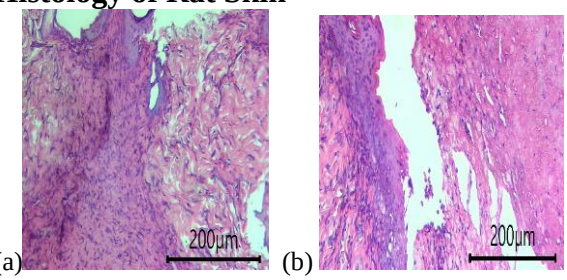

Figure 1: Histopathology of skin Rat (a) G3 (b) G1

From the Figure 1, it can be seen that there is a difference in collagen and fibroblast density between the control group and the diabetic mouse group. The G3 group had better collagen and fibroblast density than the control group. The use of leaf extract of *A. bilimbi* showed higher collagen density compared to the control, indicating improved wound healing. Observing the histopathology of the incision on the 8th day after the application of star fruit extract, the density of collagen fibers in the wound area was lower than that of the incision in the control group. When wound healing begins, fibroblasts have the ability to contract and are called myofibroblasts, they will cause the wound edges to pull apart and then close together, so that the wound edges adhere. together. As wound healing progresses, fibroblasts increase. These cells produce collagen, give rise to granulomatous tissue, then gradually collect the connective tissue matrix, eventually leading to dense fibrosis [19], [20].

According to Masir et al. [21] cited by Balqis et al. [22] Fibroblasts play a role in the synthesis, deposition, and remodeling of the extracellular matrix. After migrating to the wound site, the fibroblasts initiate the synthesis of the extracellular matrix.

Proliferating fibroblasts accompanies these blood vessels and begins to accumulate collagen.

During the proliferative phase, after 3 to 5 days, a special type of scar tissue appears, called granulomatous tissue. The term granulomatous tissue derives from its histological shape which is characterized by proliferation of fibroblasts and new fine thin-walled capillaries in a loose extracellular matrix. The granulosa tissues will then gradually accumulate a matrix of connective tissue, eventually leading to dense fibrosis, which may have to undergo further remodeling over time [23].

The therapeutic activity of the leaf extract of *A. bilimbi* is probably due to the contribution of the group of chemical compounds contained in it, such as flavonoids, phenolics, saponins, and steroids [24], [25]. Several mechanisms of this class of compounds as therapeutic agents have been widely reported.

4. CONCLUSION

Based on the study results, it can be concluded that the administration of *A. bilimbi* extract for 8 days accelerates wound healing in G2 mice. This extract also has a good diabetic wound healing effect on G3. Histopathological image What was observed was an increase in the number of fibroblast cells and collagen density in the wound in the G3-healing Rat skin.

5. CONFLICT OF INTEREST

No conflict of interest with any institution and/or any person related with the research and publication.

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