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Evaluation of Starfruit Leaf Extract (*Averrhoa carambola* L.) in the Incised Wound Healing Process in Mice (*Mus musculus*)

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ABSTRACT

Incision wounds are a form of skin tissue damage that affects the body's anatomical structure and function, especially when caused by sharp objects. Starfruit leaves are an alternative plant-based remedy that can be used to heal incision wounds. This study aimed to analyze the effect of starfruit leaf extract (*Averrhoa carambola* L.) on male mice (*Mus musculus*) with incision wounds. A total of 20 male mice (n = 4/group) were randomly assigned to five treatment groups: K- (injection water), K+ (10% povidone-iodine), P1 (25% starfruit leaf extract), P2 (50% starfruit leaf extract), and P3 (75% starfruit leaf extract). A 2-cm-long incision was made at the subcutaneous level, and the observation period lasted 14 days. The parameters observed included wound length, erythema, edema, and leukocyte differential count. Shorter wound length, lower erythema and edema scores, and lower neutrophil and lymphocyte counts were observed in the extract-treated groups than in the control groups; however, these differences were not statistically significant ($p > 0.05$). Further studies are required to determine the efficacy of starfruit leaf extract in incision wound healing.

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INTRODUCTION

A wound can be defined as damage to the skin tissue that affects the function and anatomical structure of the body due to several factors, such as exposure to chemicals, sharp trauma, electric shock, and other causes. Wounds are classified as acute or chronic based on the healing process and time (Purnama et al., 2017). Incision wounds, also known as surgical wounds, are examples of

acute wounds. Incision wounds generally occur due to sharp objects that can cause bleeding owing to damage to the skin tissue (Parinduri, 2020).

Wound healing is an important and complex process in which cellular and biochemical activities are interrelated. Several processes, such as vascular response, cellular activity, and the formation of chemical compounds that act as mediators in the wound area, are interrelated in the wound healing process. When a wound occurs, the body forms new functional structures to restore damaged tissue components through several stages, including the homeostasis, inflammation, proliferation, and maturation phases (Purnama et al., 2017). The effectiveness of wound healing is influenced by the availability of bioactive substances that can regulate inflammation, stimulate cell proliferation, and promote tissue remodeling. Therefore, medicinal plants rich in phytochemicals have attracted significant interest as potential wound-healing agents.

Natural medicinal products and various plant extracts have been widely used in topical wound healing. Phytochemicals or secondary metabolites such as alkaloids, flavonoids, saponins, tannins, and steroids found in plants can act as natural substances capable of interacting with biological macromolecules to promote wound healing. Alkaloids play an active role in wound healing as antimicrobials by inhibiting the formation of bacterial cell walls, leading to cell death (Priamsari & Yuniawati, 2019). Flavonoids in wound healing can promote granulation tissue formation and accelerate the epithelialization process (Qamarani & Aryani, 2023). Tannins and saponins have the same function, namely as antibacterial agents (Wulandari et al., 2019). The starfruit is a common plant, and its fruit is widely consumed for its sweetness. However, various parts of the starfruit plant are also frequently used in traditional medicine, particularly the leaves. Starfruit leaves are often used to alleviate various ailments (Lubis, 2023). Furthermore, starfruit leaves also contain several secondary metabolites, a finding supported by several studies, such as those conducted by Abtian et al. (2019) and Mulyati et al. (2020), which indicate that starfruit leaves contain alkaloids, flavonoids, saponins, tannins, and steroids.

Research on the healing of incisions using starfruit leaf extract (*Averrhoa carambola* L.) is still limited. One study conducted by Laili et al. (2023) tested the activity of starfruit leaf extract (*Averrhoa carambola* L.) on the healing of incision wounds in New Zealand rabbits and found that starfruit leaf extract at a concentration of 75% had an optimal effect on the healing of incision wounds in New Zealand rabbits. However, no research has been conducted on the effect of starfruit leaf extract (*Averrhoa carambola* L.) as an incision wound healer in rodents, namely mice. Therefore, research is needed to test the effect of starfruit leaf extract (*Averrhoa carambola* L.) on the healing of incision wounds in male mice (*Mus musculus*). Mice were selected as the experimental

animals based on their ability to represent the wound healing process through a combination of wound contraction and re-epithelialization mechanisms. Based on Chen et al. (2015), the excisional wound model in mice is consistent, readily reproducible, and allows objective assessment of wound healing parameters, making it a suitable model for evaluating wound healing efficacy.

RESEARCH METHODS

This study used was a completely randomized design with five treatment groups: K- (Aqua pro Injection), K+ (Povidone Iodine 10%), P1 (starfruit leaf extract 25%), P2 (starfruit leaf extract 50%), and P3 (starfruit leaf extract 75%). The male mice used in this study were of the ddY strain, approximately 3-4 months old and weighing around 30–40 grams, sourced from a breeder in the Bandar Lampung, Indonesia. Exclusion criteria include mice showing signs of illness or infection prior to treatment, or those with wounds, physical defects or anatomical abnormalities before the start of the study. Each treatment group consisted of four mice as replicates. The study protocol was approvedby the Sekolah Tinggi Ilmu Kesehatan Nasional (STIKESNAS) Research Ethics Committee with number : 313/EC/KEPK/X/2025.

Extraction of Starfruit Leaves (*Averrhoa carambola* L.)

Starfruit leaves were collected from South Lampung, specifically selecting leaves that were fully matured yet still fresh. The starfruit plants were then subjected to taxonomic identification tests at the Botany Laboratory, Department of Biology, University of Lampung, and were confirmed to be of the species *Averrhoa carambola* L. The starfruit leaves were subsequently washed, air-dried, and ground using a blender (Laili et al., 2023). After that, 400 g of starfruit leaves were weighed and macerated with 96% ethanol at a 1:5 ratio for 24 hours, stirred occasionally, and remacerated under the same conditions. The resulting solution was then filtered and evaporated using a rotary evaporator (Hamdhani et al., 2023).

Phytochemical Screening of Starfruit Leaf Extract (*Averrhoa carambola* L.)

Phytochemical screening tests of starfruit leaf extract were conducted based the methods described by Aminah et al. (2024) and Rini et al. (2020).

1. Flavonoid Test

Starfruit leaf extract (0.5 g) was dissolved in 10 ml of distilled water, and 2 ml was taken. Then, 0.1 g of magnesium powder, 2 ml of amyl alcohol, and 1 ml of concentrated hydrochloric acid were added. A positive flavonoid reaction was indicated by an orange-yellow color.

2. Saponin Test

Starfruit leaf extract (0.5 g) was added to distilled water and heated, followed by the addition of HCL. Positive saponins formed foam.

3. Alkaloid Test

Starfruit leaf extract (0.5 g) was mixed with 1 ml of HCl and 9 ml of distilled water, and 0.5 ml of the mixed extract was placed in three test tubes. Each test tube was treated with a different reagent: Mayer, Wagner, and Dragendorff. A positive alkaloid reaction with Mayer's reagent produced a white precipitate, Wagner's reagent produced a brown precipitate, and Dragendorff's reagent produced a reddish-brown precipitate.

4. Tannin Test

Starfruit leaf extract (0.5 g) was added to 10 ml of distilled water and heated, followed by the addition of a few drops of 1% FeCl3. A positive result was indicated by the formation of a brownish-green color.

5. Steroid and Terpenoid Test

Starfruit leaf extract (0.5 g) was added to 10 ml of distilled water, then add 2-3 Lieberman-Burchad reagents. A positive steroid reaction was indicated by a bluish-green color, whereas a positive terpenoid reaction formed a brownish or violet ring.

Preparation of Starfruit Leaf Extract Concentrate (*Averrhoa carambola* L.)

Starfruit leaf extracts were prepared with extract concentrations in the solvent of 25%, 50%, and 75% W/V by diluting them with Aqua Pro injection (Septiawati et al., 2023). The extracts were prepared once a week.

The Acclimatization Process of Experimental Animals

Male mice were prepared and acclimatized before being treated for 7 days while being fed and given water, and their litter was replaced every other day. This acclimatization was intended to allow the animals to adjust to their environment (Dewi et al., 2017).

Wounding Procedure

Prior to conducting the study, preliminary tests were performed, particularly regarding the anesthesia process before making the incision. Topical anesthesia was chosen to ensure greater safety for the test animals without affecting their physiology. During the preliminary tests of the topical anesthesia process, the condition and response of the test animals were closely monitored. When the incision was made after anesthesia, the test animals showed no signs of pain or discomfort. One day before the incision wound treatment, the hair on the back of the mice was shaved using an electric razor, and the remaining hair was cleaned with Veet. On the day of the incision, the skin on the back was disinfected using an alcohol swab to ensure sterility, and the mice were anesthetized using topical anesthesia (Estesia) and waited for approximately 10 min

(Wijayantini et al., 2018). Subsequently, the back skin of the mice was incised using a sterile surgical knife with a length of 2 cm and a depth up to the subcutaneous layer (Susanto et al., 2023).

Treatment of Incision Wounds

The backs of the mice, which had been incised, were treated with five different treatments: K- (Aqua pro Injection), K+ (10% Povidone-iodine) by Onemed, which contains iodine and polyvinylpyrrolidone (PVP), and starfruit leaf extract at concentrations of 25%, 50%, and 75% W/V. Each test material was administered at a dose of 1 ml per application twice daily for 14 days (Sari et al., 2019).

Parameters

The parameters observed in this study were the length of the incision wound, erythema, edema, and leukocyte differentials in each group.

1. Observation of Incision Wound Healing Activity, Erythema, and Edema

The healing process of these incisions was observed by measuring the length of the wounds using calipers and observing the condition of the wounds daily. The healing process was evaluated daily by the same investigator to maintain consistency in wound assessment. Wound length was measured using a digital caliper, and erythema and edema were scored (Table 1 and 2) based on predefined criteria based on the literature (Sumarni, 2022) and (Purwanto, 2017) throughout the observation period.

Table 1. Erythema Scores

Skin Reactions	Scores
No Erythema	0
Very slight erythema (barely visible)	1
Erythema clearly visible (25,1-30 mm)	2
Moderate erythema (30,1-35 mm)	3
Severe erythema (dark red, >35 mm)	4

Table 2. Edema Scores

Skin Reactions	Scores
No Edema	0
Very slight edema (barely visible)	1
Edema clearly visible (thickness < 1 mm)	2
Moderate edema (edges raised ± 1 mm)	3
Severe edema (edges raised > 1 mm and spreading)	4

2. Differential Leukocyte Observation in Mice

A differential leukocyte count was performed using blood drawn from the tail vein. Blood samples were collected on day 1 after the incision was made; the samples were then placed on a microscope slide, and a smear was prepared by positioning another slide on the left side of the sample at a 45° angle. The slide was then pulled to the right and quickly pulled back to the left to form a thin smear. The smear was fixed with methanol for 5 minutes and air-dried. Next, the cells were stained with Giemsa stain for 5 minutes, washed with water, and air-dried. The results were observed under a microscope at 40x magnification, and 60 fields of view were examined (Sari & Masrillah, 2022).

Data Analysis

The data obtained were statistically analyzed using ANOVA. The results of the one-way ANOVA included normality, homogeneity, and ANOVA. The Shapiro-Wilk test was used to test normality, with a p-value > 0.05 indicating that the data were normally distributed. Then, the homogeneity test used the Homogeneity of Variances Test with a significance value of $p>0.05$ (Alvita et al., 2023). A follow-up test was conducted using the least significance Different) test if there were significant differences (Bawotong et al., 2020).

RESEARCH RESULT

The study began with testing the phytochemical content to identify the secondary metabolites in starfruit leaf extract (*Averrhoa carambola* L.). Based on the test results, starfruit leaf extract (*Averrhoa carambola* L.) was found to contain flavonoids, saponins, steroids, tannins, and alkaloids, as indicated by specific color changes or conditions in each test. The phytochemical test results for starfruit leaf extract (*Averrhoa carambola* L.) are shown in Table 3.

Table 3. Results of Phytochemical Screening of Starfruit Leaf Extract

No	Test	Reaction	Results (+/-)
1.	Flavonoid	Yellow in color	+++
2.	Saponin	Foamy	+
3.	Steroid	Blue-green in color	++
4.	Tanin	Greenish brown	+
5.	Alkaloid	Brown sediment	+++

Descriptions: (+) Positive for metabolite compounds, (-) Negative for metabolite compounds, color intensity scale (e.g., + for low, ++ for moderate, and +++ for high).

The first parameter of incision wound healing observed was the average reduction in incision length based on observations in each treatment group over 14 days (Table 4).

Table 4. Average Length of Incision (mm) and Standard Deviation

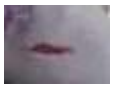
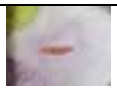
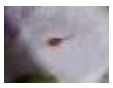
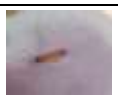
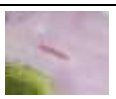
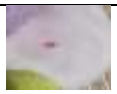
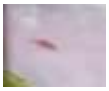
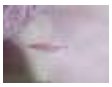
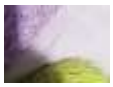
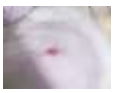
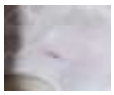
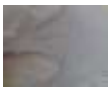
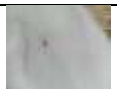
Days	K-	K+	P1	P2	P3
0	20 ± 0	20 ± 0	20 ± 0	20 ± 0	20 ± 0
2	17.1 ± 1.1	14.7 ± 2.9	14.9 ± 1.5	14.9 ± 1.5	14.4 ± 1.0
4	14.5 ± 0.4	12.4 ± 3.0	13.4 ± 1.6	12.9 ± 1.1	10.5 ± 1.6
6	12.6 ± 0.3	10.4 ± 3.8	9.9 ± 2.5	9.4 ± 0.4	8.6 ± 1.6
8	10.4 ± 0.6	7.8 ± 2.5	7.6 ± 1.1	7.3 ± 0.9	6.4 ± 1.4
10	7.8 ± 0.3	5.5 ± 1.5	5.5 ± 0.5	5.6 ± 1.3	4.4 ± 0.7
12	4.8 ± 0.6	3.9 ± 2.5	4.1 ± 0.8	4.2 ± 0.8	2.8 ± 0.8
14	3.2 ± 0.7	1.8 ± 1.8	1.8 ± 1.6	1.3 ± 0.2	0.9 ± 0.9

Descriptions: (K-) mice were given aqua pro injection, (K+) mice were given Povidone iodine 10%, (P1) 25% starfruit leaf extract, (P2) 50% starfruit leaf extract, and (P3) 75% starfruit leaf extract.

Measurements of the average incision length in four mice per group showed that each treatment group experienced a reduction in incision length. Final measurements on day 14 showed that group P3 had the lowest average incision length compared to the other treatment groups. However, the results of a One-way ANOVA statistical test showed no significant difference in the average reduction in incision length among the groups, with a mean p-value of 0.273 ($p > 0.05$).

Observations of incision wound closure in mice were conducted to observe the development of incision wounds every other day for 14 days. The incision wound closure process in mice is presented in Table 5.

Table 5. The Process of Closing Incision Wounds in Mice

Days	Treatment Groups				
	K -	K+	P1	P2	P3
2					
4					
6					
8					
10					
12					
14					

Descriptions: (K-) mice were given aqua pro injection, (K+) mice were given Povidone iodine 10%, (P1) 25% starfruit leaf extract, (P2) 50% starfruit leaf extract, and (P3) 75% starfruit leaf extract.

The results of the incision wound healing process shown in Table 5 indicate that by day 2, the wounds in all treatment groups had begun to dry out and scabs had formed. Complete wound closure was observed in some mice receiving starfruit leaf extract during the observation period, whereas no complete wound closure was observed in the K+ and K− groups by day 14. Although wound closure appeared earlier in some animals from the P3 group, the differences among treatment groups were not statistically significant.

The next parameter observed was erythema (redness) and edema (swelling), which were assessed visually on a daily basis to detect signs of inflammation. The erythema and edema scores are presented in Tables 6 and 7, respectively.

Table 6. Average Erythema (Redness) Score in Mice

Days	K -	K+	P1	P2	P3
1	1 ± 0	0.3 ± 0.5	0.3 ± 0.5	0.6 ± 0.5	0.3 ± 0.5
2	1 ± 0	0.3 ± 0.5	0.3 ± 0.5	0.6 ± 0.5	0.3 ± 0.5
3	1 ± 0	0.3 ± 0.5	0 ± 0	0.3 ± 0.5	0 ± 0
4	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0

Descriptions: (K-) mice were given aqua pro injection, (K+) mice were given Povidone iodine 10%, (P1) 25% starfruit leaf extract, (P2) 50% starfruit leaf extract, and (P3) 75% starfruit leaf extract.

The average erythema score did not exceed 1 in any of the treatment groups. This indicates that erythema in all groups was very slight and almost invisible. Erythema was observed only up to day 3 in the K+, K-, and P2 treatments, whereas P1 and P3 showed erythema only up to day 2.

Table 7. Average Edema (Swelling) Score in Mice

Days	K -	K +	P1	P2	P3
1	1.6 ± 0.5	2 ± 1	1.6 ± 0.5	1 ± 0	0.6 ± 0.5
2	1.6 ± 0.5	2 ± 1	1.6 ± 0.5	1 ± 0	0.3 ± 0.5
3	1.6 ± 0.5	1.6 ± 0.5	1.3 ± 0.5	0.3 ± 0.5	0.3 ± 0.5
4	1.6 ± 0.5	1.3 ± 0.5	0.3 ± 0.5	0 ± 0	0 ± 0
5	1.6 ± 0.5	0.6 ± 0.5	0.3 ± 0,5	0 ± 0	0 ± 0
6	1 ± 1.7	0 ± 0	0 ± 0	0 ± 0	0 ± 0
7	0.6 ± 1.1	0 ± 0	0 ± 0	0 ± 0	0 ± 0
8	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0

Descriptions: (K-) mice were given aqua pro injection, (K+) mice were given Povidone iodine 10%, (P1) 25% starfruit leaf extract, (P2) 50% starfruit leaf extract, and (P3) 75% starfruit leaf extract.

The average edema score in all treatment groups was low because the edema thickness was less than 1 mm. Edema in K- was visible until day 7, while K+ and P1 were visible until day 5, and edema in groups P2 and P3 was only visible until day 3.

The last parameter observed was differential leukocyte observation, which was conducted to determine the types of leukocytes that played the most dominant role in the early phase of incision wound healing. The results of the average differential leukocyte observation in mice are presented in Table 8.

Table 8. Average Differential Leukocyte in Mice

Cell Types	K -	K +	P1	P2	P3
Segmented neutrophils	102.3 ± 63.8	102.5 ± 24.6	44 ± 20	43 ± 33.2	25.3 ± 14
Lymphocytes	276 ± 83.5	133 ± 79.5	152 ± 94.5	141.6 ± 77.5	96 ± 54.6
Basophils	3 ± 4.3	3.6 ± 3.2	1.6 ± 2	5 ± 4.5	3.3 ± 3
Eusinophils	2.3 ± 1.5	3.6 ± 3.2	1.3 ± 1.5	1.6 ± 1.5	1.6 ± 1.5
Monocytes	1.6 ± 0.5	0.6 ± 0.5	0.6 ± 1.1	0 ± 0	0.6 ± 1.1
Stab neutrophils	3 ± 1	1 ± 1	1.3 ± 1.5	1 ± 1.7	2.6 ± 2.8

Descriptions: (K-) mice were given aqua pro injection, (K+) mice were given Povidone iodine 10%, (P1) 25% starfruit leaf extract, (P2) 50% starfruit leaf extract, and (P3) 75% starfruit leaf extract.

The differential leukocyte count results showed that the most abundant leukocyte type in all treatment groups was lymphocytes. The groups administered the test substance (K+, P1, P2, and

P3) had lower lymphocyte counts compared to the K- group. The other dominant leukocyte type was segmented neutrophils. Groups P1, P2, and P3 had lower segmented neutrophil counts than the K- and K+ groups. However, these data indicate that administration of starfruit extract showed a non-significant trend, followed by the results of a one-way ANOVA statistical test showing no significant differences in leukocyte differentials among the treatment groups ($p > 0.05$; Segmented neutrophils $p = 0.158$, Lymphocytes $p = 0.137$, Basophils $p = 0.846$, Eusinophils $p = 0.633$, Monocytes $p = 0.249$, and Stab neutrophils = 0.546). The morphology of each type of leukocyte has distinct features that serve as the basis for cell identification and leukocyte differential counting. Images of leukocyte morphology in mice observed during the study are presented in Table 9.

Table 9. Leukocyte Morphology in Mice

No.	Types of Leukocytes	Image
1.	Segmented neutrophil	
2.	Lymphocytes	
3.	Monocyte	
4.	Band neutrophils	

DISCUSSION

The ethanol extract of starfruit leaves (*Averrhoa carambola* L.) used in this study showed that the extract contained flavonoids, saponins, steroids, tannins, and alkaloids. Several studies have also shown that starfruit leaf extract contains several similar compounds. Laili et al. (2023) reported that the ethanol extract of starfruit contained flavonoids, alkaloids, saponins, and tannins, whereas Abtian et al. (2019) reported that the water extract of starfruit leaves contained only flavonoids, alkaloids, and tannins. The differences in the phytochemical test results may be influenced by the type of solvent used in the extraction process. Ethanol is a solvent often used in the extraction process and has a lower polarity than water; therefore, ethanol can dissolve almost all organic compounds in the extract (Kurniawati et al., 2016).

The effect of starfruit leaf extract (*Averrhoa carambola* L.) on the healing of incision wounds in mice over 14 days was observed from the length of the wound, erythema, edema, and leukocyte

differential. The length of the incision wound was used to observe the rate of incision wound healing at 2-day intervals. As shown in Table 4, the average incision length was shorter in group P3 on day 14. Descriptively, the P3 group showed the shortest mean wound length on day 14. However, the differences among treatment groups were not statistically significant; therefore, this observation should be interpreted as a descriptive trend. The observed trend toward shorter wound length may be related to the phytochemical constituents of starfruit leaf extract.

The extract which contains flavonoids, saponins, steroids, tannins, and alkaloids as shown in Table 3. Although no statistically significant differences were observed in the present study, these phytochemicals have been reported to possess biological activities associated with wound healing. Flavonoids play an anti-inflammatory role in wound healing, preventing the inflammatory phase of the healing process from becoming prolonged (Irawan et al., 2023). In addition, flavonoids act as antioxidants by suppressing excess free radicals that cause oxidative stress, which has a negative effect on cells and tissues, as well as wound healing (Arief & Widodo, 2016). Saponins play a role in wound healing by forming type 1 collagen to accelerate epithelialization, thereby speeding up the wound healing process (Thaib et al., 2021), while steroids help the structure of the injured area by increasing fibroblasts that synthesize collagen (Akhmadi et al., 2022). Tannins and alkaloids act as antibacterials during wound healing. Tannins disrupt the internal metabolism of bacteria by passing through the bacterial cell wall, causing bacterial death (Beltsazar et al., 2024), whereas alkaloids disrupt peptidoglycan cell components, causing bacterial cell death (Nayaken et al., 2023).

The results in Table 5. showed differences in wound conditions and morphology within a 2-day interval. The closure of incision wounds occurs in several phases, such as the hemostasis, inflammatory, proliferation, and maturation phases. The hemostatic phase is the earliest phase of wound healing, characterized by blood clotting, after which the wound enters the inflammatory phase. The inflammatory phase is characterized by erythema (redness) and edema (swelling) (Wisnasari et al., 2021). Erythema (redness) occurs when blood vessels dilate, resulting in increased blood flow to the wound area (vasodilation), whereas edema (swelling) occurs when capillary permeability increases due to blood flow in the wound area (Astuti et al., 2024). During this phase, a scab forms from blood clotting, which protects the underlying dermis and is reddish-brown in color, remaining until the proliferation phase (Hutauruk et al., 2020).

The proliferation phase is characterized by the disappearance of inflammatory signs, such as erythema (redness) and edema (swelling), and the formation of pink or red granulation tissue rich in blood vessels, which can be seen when the scab begins to peel off gradually. The scab falls off

completely at the end of the proliferation phase when re-epithelialization is complete (Saputra et al., 2024). Finally, the wound undergoes a maturation phase characterized by the formation of scar tissue with only 80% tensile strength of normal skin (Ritonga et al., 2024). Based on descriptive observations, the extract-treated groups tended to exhibit features consistent with earlier progression through the wound-healing phases, by shortening the hemostatic and inflammatory phases. This observation is supported by the absence of erythema since day 3 and edema since day 4, so that mice in the extract groups appeared to enter the proliferation phase faster than mice in the control groups (K- and K+). This observation was also consistent with the finding that the incision wounds in the extract groups, which were completely closed by day 14.

Differential leukocyte counts were performed to observe the inflammatory response to incision wounds. Based on Table 8, the average differential leukocyte count in all groups showed that lymphocytes were more dominant than other types of leukocytes, with neutrophils being the second most abundant. This is consistent with the research by Siegel and Walton (2020), which states that lymphocytes are the most common white blood cells found in mice, accounting for about 75% of the blood, followed by neutrophils.

At the onset of injury, neutrophils which are part of the innate immune system increase and migrate to the injury site to fight infection (Nugrahaningsih et al., 2023). Based on the observations, K- and K+ had higher average neutrophil counts compared to the extract groups. The higher neutrophil count in K- and K+ may indicate a stronger inflammatory response in mice in these groups compared to mice in the extract groups. This may suggest a potential anti-inflammatory effect of starfruit (*Averrhoa carambola* L.) leaf extract, although this mechanism was not directly evaluated in the present study.

Lymphocytes during the inflammatory phase play a role in releasing lymphokines that will bring macrophages to the wound area and continue to activate them for the phagocytosis process (Malaha et al., 2023). The results of the observation showed that the K- group had a higher average lymphocyte count compared to other treatment groups. This may be because the treatment given to K- (*Aqua pro injection*) did not have a good antiseptic or anti-inflammatory effect, making it less effective in fighting microbes and causing a greater antigen load, which resulted in an increase in lymphocytes in the blood as a form of a more active adaptive immune response compared to other treatment groups (Zayyan et al., 2016).

The creation of a 2-cm-long incision with subcutaneous depth can elicit observable biological responses, such as wound condition and length, erythema, and edema. Additionally, microscopic changes in leukocyte differentiation can be observed on the first day following the

incision. The application of starfruit leaf extract, particularly at a concentration of 75%, showed a favorable descriptive trend in wound-healing parameters, including shorter durations of erythema and edema and lower neutrophil predominance. However, these observations should be interpreted cautiously because no statistically significant differences were detected among treatment groups.

CONCLUSION

This study shows that starfruit leaf extract (*Averrhoa carambola* L.) contains flavonoids, saponins, steroids, tannins, and alkaloids. Administration of starfruit leaf extract (*Averrhoa carambola* L.), particularly at a 75% W/V concentration, showed a descriptive trend toward shorter wound length, lower erythema and edema scores, and lower neutrophil and lymphocyte counts compared with the control groups. However, these differences were not statistically significant. Therefore, further studies are required to determine the efficacy of starfruit leaf extract and to clarify its potential role in incision wound healing.

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CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper.

CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

N. A. Putri: conceptualization, methodology, project administration, resources, writing – original draft, visualization, and writing – review & editing**, I. A. Khairani:** formal analysis, supervision, project administration, investigation, resources, data curation, validation, writing – original draft, and writing – review & editing**, L. H. Imaniar:** investigation, formal analysis, validation, data curation, and writing – original draft & editing.

DECLARATION OF THE USE OF AI

During the preparation of this manuscript, the authors used DeepL to assist with the translation of the manuscript, Paperpal to improve the structure and grammar of the sentences, and ChatGPT (OpenAI) to assist in improving the clarity or readability of the text. After using those tools, the authors thoroughly reviewed, edited, and verified the entire content to ensure it accurately represents their own ideas and interpretations. The authors take full responsibility for the integrity and originality of the published work.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author.

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