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Bilimbi (*Averrhoa bilimbi* L.) Leaf Extract Cream: Formulation and Efficacy in Accelerating Wound Healing in Male White Mice

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ABSTRACT

Bilimbi (*Averrhoa bilimbi* L.) is known for its wound-healing potential due to its flavonoids, saponins, triterpenoids, and tannins. This study aims to develop a practical and stable dosage form of Bilimbi leaf extract, specifically a cream, and evaluate its efficacy as a wound-healing agent. Bilimbi leaf extract at 2% concentration was formulated into a cream with two variations of triethanolamine concentration as an emulsifier, F1 (2%) and F2 (4%). Data were collected through observation and measurement scales during the cream formulation stage. The effectiveness of the cream as a cut wound healer was assessed using a randomized control group pre-test and post-test design. Physical properties, including organoleptic properties, homogeneity, pH, and mechanical stability, were observed, and spreadability was measured. Both formulations met the criteria for creams with good physical quality and effectively reduced the wound length to 0 cm within the 7-day observation period. In contrast, in the control group, wounds still appeared to be 0.4-1 cm in size. In conclusion, the findings in this study indicate that the cream containing 2% Bilimbi leaf extract (*Averrhoa bilimbi* L.) has favorable physical characteristics and effectively heals incision wounds in male white mice. This formulation shows promise for further development regarding efficacy, safety, stability, and market acceptability.

***Keywords*:** *Averrhoa bilimbi*; Cream; Formulation; Incision wound; Triethanolamine

INTRODUCTION

The skin serves several functions, including acting as a barrier against foreign pathogens, protecting against harmful physical or chemical substances, and preventing the loss of water and extracellular fluid.[1,2] The skin is directly influenced by environmental exposure, a feature distinct from most other organs in the body.[3] The proper functioning of the skin is compromised when disrupted, such as in the case of wounds. A wound refers to a damaged skin tissue condition caused by factors like exposure to heat, injury from sharp or blunt objects, changes in physiological conditions, or medical procedures. Wounds can manifest in various forms, including both open and closed types.[4,5] A sharp object, such as metal or wood, can cause a straight, small,

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and thin wound known as a cut or *Vulnus scissum*. Cuts can also result from deliberate medical treatment.[6] Wound healing occurs in three phases: inflammation, proliferation, and remodeling.[7] Optimal treatment of wounds is essential to prevent the progression of infection and trauma, thereby avoiding the development of more severe medical conditions.[8]

The field of wound science, including wound healing and management, has experienced rapid growth. Medicinal plants are being explored for wound treatment, such as the use of extracts from the Bilimbi plant (*Averrhoa bilimbi* L.). This plant has been traditionally believed to aid in wound healing within communities. Bilimbi leaf extract contains secondary metabolites, including flavonoids, alkaloids, saponins, steroids, and tannins.[9] The wound-healing properties of Bilimbi leaf extract are attributed to flavonoids, tannins, and saponins. In male mice, a dosage of 200 mg/kg body weight of Bilimbi leaf extract demonstrated effectiveness in healing incision wounds.[10] Several studies have demonstrated that Bilimbi exhibits anti-inflammatory, antioxidant, and antibacterial properties. Additionally, it has been shown to increase the number of fibroblasts involved in the wound healing process.[11,12] A 2% concentration of bilimbi leaf extract in gel dosage form has effectively healed cut wounds in white rats.[13]

The use of crude extracts is limited due to poor solubility and bioavailability, instability to pH changes, absorption constraints in the body, and impracticality for direct medicinal use. These limitations hinder their utilization in the community.[14,15,16] To ensure that patients can easily use the active ingredients in the form of Bilimbi leaf extract, it is necessary to develop a topical pharmaceutical preparation, such as a cream.

Creams are semi-solid preparations that are applied externally to the skin and mucous membranes and often contain drugs. Pharmaceutical and cosmetic creams are typically oil-in-water emulsions or aqueous creams. O/W creams can increase the concentration of active ingredients that penetrate the skin, leading to improved absorption and optimal effects.[17] Creams for wound care offer easy application, even distribution, moisture retention, barrier protection against infections, and versatility with various active ingredients, making them an effective and adaptable choice for managing different types of wounds and patient needs.[18,19] Creams are more effectively absorbed into the skin than gels, resulting in better efficacy.[20]

Triethanolamine and stearic acid are commonly used in cream-based formulations as polymers. Combining stearic acid with triethanolamine can be used as an emulsifier to neutralize the cream. When mixed in equal proportions with fatty acids such as stearic acid, triethanolamine becomes an emulsifier that produces smooth and stable dispersed phase oil-in-water (O/W) emulsions.[21] Stearic acid can be used as an emulsifier to create a cream with a consistent viscosity. The concentration of triethanolamine added determines the level of viscosity.[22] The appropriate combination and concentration of emulsifiers are crucial for achieving a cream with high quality and stability.[23]

Given the available information, formulating Bilimbi leaf extract into a cream with an optimal concentration of triethanolamine emulsifier is crucial. This formulation aims to achieve two key objectives: (1) ensuring good physical quality of the cream and (2) enhancing its efficacy in promoting wound healing. Previous studies have demonstrated the efficacy of Bilimbi leaf extract in the formulation of cream preparations with properties as an anti-mosquito, antioxidant, and astringent, anti-inflammatory, and antibacterial agent.[11,24,25,26,27] It is noteworthy that no prior studies have developed a cream formulation using Bilimbi leaf extract with a specific focus on wound healing, especially incised wounds.

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This research aligns with Sustainable Development Goal 3, which aims to ensure healthy lives and promote well-being for all at all ages.[28] Specifically, the results of this study were expected to contribute to good health, particularly in treating skin cuts using active ingredients derived from natural sources.

METHODS

Source of plant material

The *Averrhoa bilimbi* L. (Bilimbi) plant sample was collected from Kerambitan Village, Kerambitan District, Tabanan Regency, Bali, Indonesia. The plants were identified by the Characterization Laboratory of the Bali "Eka Karya" Botanical Garden at the National Research and Innovation Agency, and voucher specimen no. B-347/IPH.7/AP/XII/2020 for *Averrhoa bilimbi* L. were assigned.

Equipment and Materials

Materials include triethanolamine (PT. Brataco, Indonesia), stearic acid (PT. Brataco, Indonesia), cetyl alcohol (PT. Brataco, Indonesia), glycerin (PT. Brataco, Indonesia), propylene glycol (PT. Brataco, Indonesia), methylparaben (PT. Karunia Sejahtera Abadi SABA KIMIA, Indonesia), propylparaben (PT. Karunia Sejahtera Abadi SABA KIMIA, Indonesia), ethanol 96% (PT. Brataco, Indonesia), distilled water or aquadest (UD. Sekawan Bali Sejahtera, Indonesia), and male white mice (*Mus musculus* L.).

The study employed various instruments including a rotary evaporator (BUCHI R-300), oven (MEMMERT GmbH+Co., KG, Germany), Büchner funnel, Elma Sonic S 40 H Ultrasonicator (Hans Schmidbauer GmbH & Co.KG, Germany), analytical balance (Ohaus pioneer, PA 224C), Digital weighing Balance (ACIS BC-500), Blender (Philip), Universal pH indicator strip (Macherey-Nagel, Germany), water bath (MEMMERT GmbH+Co., KG, Germany), various laboratory glassware (Pyrex), mortar-stamper, extensometer set of 10 x 10 cm^2 glass, scalpel, ruler, razor, syringe, cotton button, and injection syringe.

Extraction of Bilimbi Leaf

The collected Bilimbi leaves were wet sorted, and washed with running water. They were then dried in an oven at 50°C for 24 hours before being pulverized with a blender.[9] The extraction of Bilimbi leaves involved weighing 45 grams of dry powder, which was then dissolved in 450 ml (1:10) of 96% ethanol solvent. The extraction process was carried out at 40°C for 20 minutes using an ultrasonic bath (Elma Sonic®).[29]

Next is filtration which is conducted using filter paper. The filtrate obtained is then evaporated to separate bioactive compounds using solvents. Evaporation is carried out using a rotary vacuum evaporator (40°C) with a pressure of 100 mbar and a speed of 45 rpm.[30]

Formulation of Bilimbi Leaf Extract Cream

The Bilimbi leaf extract cream preparations were manufactured in accordance with the formulas presented in Table 1. In separate Beaker glasses, the oil phase (containing stearic acid, cetyl alcohol, and propylparaben) and the water phase (containing triethanolamine, glycerin, methylparaben, propylene glycol, and distilled water) were each melted at 70°C.

After melting both phases, the water phase was carefully combined with the oil phase while stirring continuously until a uniform mixture was obtained. Next, the ethanol extract of Bilimbi leaves was adequately diluted with 96% ethanol. The diluted extract was poured into a preheated mortar and meticulously crushed to eliminate any lumps. Subsequently, the cream base was incrementally added to the extract, ensuring thorough homogenization. Finally, the resulting cream was carefully transferred to an appropriate container.

The entire process was repeated three times for each formula with varying concentrations of triethanolamine (TEA),

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Table 1. The Formula of Bilimbi Leaf Extract Cream

Ingredients	Concentration (%w/v)		Function
	F1	F2	
Bilimbi leaf extract	2	2	Active ingredient
Triethanolamine	2	4	Emulsifying agent, alkalizing agent
Stearic acid	8	8	Creamy base, emulsifying agent
Propylene glycol	7	7	Humectant
Cetyl alcohol	4	4	Emulsifying agent, stiffening agent
Glycerine	4	4	Humectant
Methylparaben	0,2	0,2	Preservative
Propylparaben	0,02	0,02	Preservative
Ethanol 96%	qs.	qs.	Extract solvent
Aquadest	ad 100	ad 100	Vehicle

namely F1 with a 2% TEA concentration and F2 with a 4% TEA concentration. This is intended to validate the consistency and reproducibility of the manufacturing methods. Experimenting multiple times with different formulations allows the assessment of the consistency of the observed effects across variations. This approach ensures that any observed effects are not due to chance or random fluctuations but rather reflect true differences related to the tested formulas.

Physical Quality Evaluation of Cream

The Physical Quality testing was conducted with three replications. This means that the test was repeated three times to ensure consistency and reliability of the results. Each replication is intended to verify that the outcome is reproducible under the same conditions, which is an important aspect of quality control in product evaluation.

Organoleptic Test

The organoleptic test examination encompasses the assessment of the cream's odor, color, and texture, which are evaluated through the five senses of the researcher.[23]

Homogeneity Test

One gram of cream is applied to a piece of transparent glass. The preparation must then be observed to show a homogeneous composition, and no coarse grains should be visible.[23]

pH Test

The pH test aims to determine the safety of the cream preparation when used, ensuring it does not irritate the skin. One gram sample of the cream extract was weighed and diluted with 10 mL of distilled water. The pH strip was immersed in the diluted cream and then compared with the universal indicator color standard available on the pH strip packaging. The pH of the cream was recorded.[31,32]

Spreadability Test

To assess spreadability, a test using a 10 cm long glass slide was conducted. First, an adequate amount of cream was applied to the slide, which was then covered with another glass slide. A 1000 g weight was placed on top of the upper glass slide for 5 minutes, allowing any excess cream to settle. Next, a 120 g mass was attached to the upper glass slide. The time (in seconds) it took for the glass to move across a 10 cm distance was recorded as the spreadability measure.[33] Notably, the bottom glass remained stationary during this test. The spreadability was calculated using the following formula:

$$S = (M \times L)/T \quad \text{.....(1)}$$

Where S is the spreadability, M is the mass tied to the top glass in grams (120 g), L is the length of the glass (10 cm = $10x10^{-2}$ m), and T is the time in seconds required to move the glass across a distance of 10 cm. The spreadability was recorded in g.m/s.[34]

Mechanical Stability Test

A 10 g sample of the preparation was placed into a device called a centrifuge,

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which was then spun at a speed of 3000 rpm for 30 minutes. Afterward, it was observed whether the preparation separated or not.[23]

Animal Preparation

The experiments involving male mice in this study have been approved by the Animal Ethics Commission of the Faculty of Veterinary Medicine, Udayana University, and have been assigned the Animal Ethics Approval Certificate No.IX:B/60/UN14.2.9/PT.01.04/2021.

This study utilized white male mice (*Mus musculus* L.) as research subjects. The inclusion criteria were male mice aged 2–3 months, weighing between 25 and 35 g, and healthy and active. The exclusion criteria included abnormal or overly aggressive behavior, while the drop-out criteria encompassed mice that became ill or died during the study.

All mice were randomly assigned to one of four treatment groups. Group 1 (P1) was a negative control group, receiving only the cream base. Group 2 (P2) was a positive control group receiving Povidone Iodine ointment. Group 3 (P3) was a treatment group receiving F1 (Bilimbi leaf extract cream with a 2% triethanolamine concentration), and Group 4 (P4) received F2 (Bilimbi leaf extract cream with a 4% triethanolamine concentration).

The sample size for this study was determined using the Federer formula, given by $(n - 1)(t - 1) \geq 15$, where n represents the number of samples per treatment group and t denotes the number of treatment groups.[35] With t set to 4 treatment groups, the sample size calculation proceeded as follows:

$$(n - 1)(t - 1) \geq 15$$
$$(n - 1)(4 - 1) \geq 15$$
$$(n - 1)(3) \geq 15$$
$$3n - 3 \geq 15$$
$$3n \geq 18$$
$$n \geq 6$$

The calculations indicated that each treatment group required a minimum of 6 white male mice. To account for potential attrition, the number of mice per group was increased to 8. Thus, with four treatment groups, a total of 32 white male mice were needed for the study.

Animal Treatment

Before the creation of wounds in mice, the animals are initially anesthetized using 10% ketamine, which is administered intramuscularly (i.m.) in mice.[36] The hair on the backs of the mice was shaved and cleaned with 70% alcohol, after which an incision was made in the back area with a 2 cm long scalpel.[37]

Following the incision, each mouse was administered the appropriate treatment according to its group. The negative control group received a cream base, the positive control group received povidone iodine ointment, and the treatment groups I and II received Bilimbi leaves ethanol extract cream. The concentration of the solution was 2%, with variations of 2% and 4% in triethanolamine.

All treatment groups were administered once daily for 7 consecutive days. The incision wound was assessed daily for 7 days using a ruler for measurement. Observations of the incision wound were made daily over the same period. Wound assessment involved monitoring indicators such as the absence of erythema, reduction in wound dimensions, and progression toward closures. Once all predefined indicators were met, the wound was considered to have attained healing status.[37]

Data Analysis

The data obtained were statistically analyzed using SPSS 20 for Windows with a 95% confidence level. Spreadability data in the physical quality test were analyzed using One-way ANOVA. The development of incision wound healing within each treatment group was analyzed using the Wilcoxon test, while differences in incision wound healing between groups were tested using the Kruskal-Wallis test, followed by the Mann-Whitney post hoc test.

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RESULTS AND DISCUSSION

Formulation and Physical Quality Evaluation of Bilimbi Leaf Extract Cream

Initially, 280 g of Bilimbi leaf (*Averrhoa bilimbi* L.) simplisia powder were used to prepare a thick extract of Bilimbi leaves, resulting in 25.5 g of blackish-green extract with a yield of 9.107%. Notably, this yield meets the requirements specified in the Indonesian Herbal Pharmacopoeia, which mandates a minimum yield of 4.5% for thick Bilimbi leaf extracts.[38]

The organoleptic tests revealed that both cream formulas exhibited a light green color and a distinctive aroma characteristic of Bilimbi leaf extract (Figure 1). This aroma likely stems from the inclusion of Bilimbi leaf extract, known for its light green color and unique scent. Interestingly, Formula 2 (F2) demonstrated a thicker consistency compared to Formula 1 (F1), attributed to the higher concentration of triethanolamine (TEA) in F2. Notably, as an emulsifier, the concentration of TEA directly influences the viscosity of stearic acid—the cream base.[21]

Figure 1. Bilimbi leaf extract cream

The homogeneity test results for Bilimbi leaf extract creams in both F1 and F2 demonstrated uniformity. Homogeneity in a cream refers to the absence of fine grains, an even color distribution, and the absence of visible spots.[23] The resulting cream exhibited consistent characteristics, and the homogeneity test serves to verify whether the formulation's ingredients are thoroughly mixed. This test is critical to ensure that the active ingredient is uniformly dispersed throughout the preparation, preventing particle aggregation and maximizing its therapeutic effect.[39] The homogeneity of these Bilimbi leaf extract creams aligns with previous studies.[31,40] Rohmani and Putri's study on creams containing varying concentrations of triethanolamine also reported the same result. In that research, all three cream formulations exhibited favorable homogeneity.[41]

The pH test results for the Bilimbi leaf extract cream revealed that the average pH ± standard deviation (SD) of F1 was 6±0, whereas F2 exhibited a pH of 7±0. The elevated pH value observed in F2 can be attributed to the variation in TEA concentration employed, where the TEA concentration in F2 is higher than that in F1. Notably, TEA is an alkaline substance with a pH of 10.5.[21] The results of this pH test on the Bilimbi leaf extract cream were consistent with previous research findings that demonstrated a correlation between the concentration of TEA in the cream formula and the pH value of the cream.[41,42] The measurement of a cream's pH is essential to assess its compatibility with the skin's natural pH. Maintaining an appropriate pH level in skincare products is crucial for skin health. Creams with a low or acidic pH can irritate the skin. They disrupt the acid mantle, leading to redness, itching, and discomfort. Prolonged use of acidic creams weakens the skin barrier, making it susceptible to external factors (e.g., pollutants and microbes). On the contrary, creams with a high pH (alkaline) can strip the skin of natural oils. This disrupts the skin's moisture balance, causing dryness. Alkaline conditions affect the skin's microbiome, potentially leading to imbalances.[32,43,44] The pH levels across different areas of the skin range from 4.1 to 7.4,[43] so the Bilimbi leaf extract cream meets the requirements of a good cream pH range.

The spreadability test plays a crucial role in evaluating the quality of cream preparations. Spreadability pertains to a

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cream's capacity to distribute evenly across the skin's surface. It directly affects the application of a standard dosage of a medicated formulation and affects the effectiveness of topical treatment. Topical creams formulated with oil and water content exhibit superior spreadability compared to moist dressings, thereby promoting effective wound healing. These creams facilitate skin repair by maintaining optimal hydration and ensuring adequate coverage of active ingredients over the wound area, thereby accelerating the healing process.[33,45] The results of the test indicate that the cream preparation exhibits good spreadability, with values ranging from 0.397 to 1.664 g.m/s. The spreadability value of F2 is higher than that of F1, indicating that the two glasses require less time to release (Figure 2). This is likely due to the softer consistency of F2 cream. This finding is consistent with the research conducted by Lestari et al.,[46] which indicates that the addition of TEA can reduce the consistency of the cream, resulting in a softer texture and increased spreadability (Table 2). The smaller the coefficient (g.m/s) of spreadability obtained, the easier it is to apply the resulting cream to the skin.[34,47]

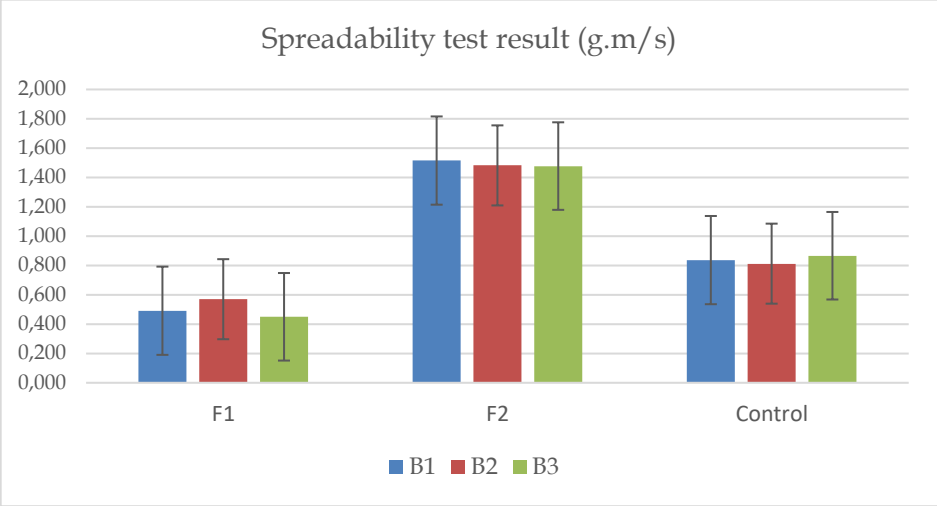

Figure 2. Graph of spreadability test results per formula compared to control (marketed formula).

Table 2. Spreadability test results

Formula		$\bar{x}$±SD
F1	B1[a]	0.491±0.102
	B2[a]	0.570±0.127
	B3[a]	0.450±0.018
F2	B1[b]	1.515±0.184
	B2[b]	1.482±0.160
	B3[b]	1.478±0.162
Control	B1[c]	0.837±0.141
	B2[c]	0.812±0.132
	B3[c]	0.866±0.082

Analysis of variance (ANOVA) revealed a p-value > 0.05, indicating no significant difference among the three batches of each cream formula, which were B1, B2, and B3, respectively. Different superscript letters represent significant differences between each formula (F1, F2, and Control) at p <0.05.

The spreadability data of Bilimbi leaf extract cream (F1 and F2) and a commercially available cream (control) were compared. The normality test for the spreadability data, replicated three times from each batch in formulas F1, F2, and Control, was conducted using the Shapiro-Wilk test and showed that all data were normally distributed. This was followed by a homogeneity test using Levene's test, which resulted in a p-value greater than 0.05, indicating that the data were homogeneous and suitable for further analysis through ANOVA. The ANOVA results on the spreadability data for each batch of formulas F1, F2, and Control showed that the p-values for all groups were above 0.05 (Table 2), indicating no significant differences between the batches. These results suggest that the manufacturing process for creams F1 and F2 was reproducible.

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The ANOVA results on the spreadability data for the three formulas (F1, F2, and Control) showed a p-value below 0.05, indicating a significant difference between the formulas. This suggests that the variation in triethanolamine (TEA) concentration—2% in F1 and 4% in F2—affects the spreadability of the creams. Stearic acid, used as an emulsifier in the cream formula, forms a cream base when partially neutralized with alkali or triethanolamine. The amount of triethanolamine significantly influences the visual appearance and flexibility of the resulting cream, which in turn determines its spreadability.[21,23]

The comparison of the formula developed in this study with commercially available products is based on the premise that products with a marketing license have undergone rigorous supervision and quality control processes, thereby ensuring high-quality standards. In the pharmaceutical industry, stringent quality control measures are essential to ensure that consumers receive the highest quality medications. The indiscriminate production of products intended to save lives or promote health is both unjustifiable and potentially harmful.[48]

A mechanical stability test was conducted at a speed of 3000 rpm over 30 minutes. The objective was to assess the durability and stability of the cream preparation about its shelf life. This was done by simulating the effect of gravity on the cream's storage for 10 months.[39] The results of the mechanical stability test on F1 and F2 indicated the presence of foam at the top in each replication. This is likely due to the absence of excipients containing antifoaming, which would otherwise prevent the centrifugation force from causing the preparation to foam. Antifoaming is a common ingredient in topical preparations, serving as a barrier to prevent the formation of foam. It is also used to create a water-repellent film, ensuring that the preparation does not become easily lost or erased by sweat during application on the skin.[21] Observation of incision wounds showed evident healing in all groups, as detailed in Table 3. On day 7, wound healing was observed in eight mice from both groups, P3 (F1 treatment) and P4 (F2 treatment). In contrast, no fully healed mice were found in groups P1 (negative control) and P2 (positive control). The extent of healing was indicated by wound closure and the absence of redness and bleeding. These results demonstrate the efficacy of the 2% Bilimbi leaf ethanol extract cream in promoting cut wound healing in mice, corroborating existing research on its effectiveness in white rats.13 Additionally, other studies have demonstrated that Bilimbi leaf ethanol extract at a dose of 200 mg/kgBW is capable of healing cuts. The Wilcoxon test was conducted on paired data of day 0 and day 7 for each treatment group to evaluate significant wound healing progression. A significant difference with a p-value below 0.05 was found for all four treatment groups,

Table 3. The wound length measurement results in mice

Groups	Average Wound Length in Mice (cm) ± SD								Difference between day 7 and 0 (cm)
	day 0	day 1	day 2	day 3	day 4	day 5	day 6	day 7	
P1*	2.00±0	1.93±0.15	1.70±0.17	1.45±0.15	1.25±0.18	1.08±0.22	1.03±0.21	0.88±0.15	**1.12**[a]
P2*	2.00±0	1.85±0.16	1.72±0.16	1.37±0.24	1.23±0.27	1.15±0.28	0.97±0.34	0.78±0.23	**1.22**[a]
P3*	2.00±0	1.58±0.11	1.42±0.22	1.23±0.19	1.00±0.24	0.67±0.27	0.52±0.27	0.17±0.24	**1.83**[b]
P4*	2.00±0	1.57±0.07	1.33±0.11	1.20±0.15	0.92±0.09	0.68±0.13	0.43±0.20	0.12±0.19	**1.88**[b]

*There were significant differences within each group (p-value < 0.05) as determined by the Wilcoxon test.
Different superscript letters represent significant differences between each groups at p <0.05.
P1: Negative control group
P2: Positive control group
P3: treatment group receiving cream F1
P4: treatment group receiving cream F2

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indicating notable wound healing in the negative group (P1), positive group (P2), and both cream formulas (P3 and P4). Subsequently, the difference in wound length between day 7 and day 0 for each group was analyzed using the Kruskal-Wallis test to assess differences between treatment groups. Significant differences with p-values below 0.05 were observed, suggesting variations between treatment groups.

Further analysis with the Mann-Whitney post hoc test revealed no It is important to note that different bases have distinct properties. The partition coefficient of an active substance in one base differs from the coefficient of the active substance in another, resulting in disparate drug release rates from different bases. The viscosity of the base has a significant impact on the diffusion coefficient of the drug within the base, which in turn affects the rate of drug release from the base. A high-viscosity base will result in a low diffusion coefficient, leading to a slower release of the drug from the base.[49] However, the results of this study indicate that variations in triethanolamine levels did not significantly impact the efficacy of Bilimbi leaf extract. This is demonstrated by the statistical analysis results of the two formulas, which yielded no significant difference (p-value > 0.05).

Wound healing can be influenced by the content of secondary metabolites present in Bilimbi leaf extract, namely flavonoids, tannins, and saponins. Flavonoids exhibit anti-inflammatory, antioxidant, vasodilatory, and analgesic properties, which collectively contribute to their beneficial effects on wound healing.[10] Tannins with astringent properties help in protein precipitation, forming a protective layer and stopping bleeding to accelerate healing. Tannins also act as bacteriostatic, inhibiting the growth of bacteria by disrupting the permeability of cell membranes. significant difference between groups P1 and P2, as well as between P3 and P4 ($p > 0.05$). However, significant differences were found between the P3 and P4 groups compared to the P2 as the positive control group ($p < 0.05$). This difference does not imply that the F1 and F2 creams are less effective than the positive control; rather, the shorter wound lengths in the P3 and P4 groups on day 7 suggest greater efficacy of these creams. The absence of significant difference between P3 and P4 indicates that the variation in TEA concentration (2% and 4%) did not significantly affect the Bilimbi leaf extract cream's effectiveness in aiding wound healing in mice over the 7-day observation period.

This is achieved by the formation of wrinkles in the cell walls or membranes, which impede the passage of bacteria into the cell. Tannin also has several health benefits such as antioxidant, anti-allergy, and anti-inflammatory, which will certainly in the wound healing process.[50]

Figure 3. The dorsal views of P3 and P4 mice, highlighting a 2 cm wound on day 0 (A) and the near-complete wound healing observed on day 7 (B)

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Furthermore, saponins are agents that accelerate wound healing and prevent scar formation by reducing fibroblast accumulation and α-SMA (*Smooth Muscle Actin*) expression in the wound.[51] Saponins facilitate collagen formation, a structural protein that plays a role in the wound-healing process. Furthermore, saponins act as cleansing and antiseptic agents, with the capacity to kill or prevent the growth of microorganisms.[52]

CONCLUSION

The study's limitations include a 7-day wound healing observation period and assessment based solely on visual observation, without skin tissue analysis. Despite these limitations, the findings indicate that the cream containing 2% Bilimbi leaf extract (*Averrhoa bilimbi* L.) has favorable physical characteristics and effectively heals incision wounds in male white mice. This formulation shows promise for further development regarding efficacy, safety, stability, and market acceptability.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors affirm that the content of this article is original, and they will take full responsibility for any claims arising from it.

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