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Formulation and Testing of In Vivo Anti-Inflammatory Activity of Ethanol Extract Cream from Plumeria rubra L. Leaves for Topical Skin Inflammation

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ABSTRACT

Skin inflammation in Indonesia has a prevalence of up to 6.8%, with corticosteroids being the primary treatment despite risking serious long-term side effects. This study aims to formulate and evaluate the topical anti-inflammatory and tissue-repair activity of an ethanol extract cream of *Plumeria rubra* L. leaves as a natural alternative. The extract was obtained through maceration with 96% ethanol and formulated into an oil-in-water (O/W) cream at concentration variations of 2% (F1), 4% (F2), and 6% (F3). Phytochemical screening revealed that the extract contains alkaloids, flavonoids, saponins, tannins, terpenoids, and steroids. Physical evaluation demonstrated that the preparations are stable, homogeneous, possess standard pharmaceutical spreadability and adhesion, and maintain an ideal skin-compatible pH range (4.5–5.24). *In vivo* anti-inflammatory evaluation in white rats showed a significant difference in healing by day 9 ($p < 0.05$). Formula F2 (4%) yielded the most optimal results, exhibiting the smallest average wound length of 0.64 ± 0.11 cm and a healing time of 9.00 ± 0.00 days, which was statistically equivalent to the positive control. Increasing the concentration to 6% (F3) did not provide a significant acceleration in healing, indicating a saturation concentration phenomenon. In conclusion, the *Plumeria rubra* L. leaf extract cream at a 4% concentration is the most efficient and effective formula for accelerating wound healing and treating skin inflammation.

Keywords: *Plumeria rubra* L., Topical Cream, Anti-Inflammatory, Cut Wounds, Phytochemistry

Introduction

Inflammatory skin diseases are one of the significant public health issues at the global level because they cause substantial clinical, psychosocial, and economic impacts. Among the group of non-infectious skin diseases, dermatitis has become one of the conditions with a continuously increasing prevalence in both developing and developed countries. In Indonesia, the prevalence of dermatitis in primary healthcare facilities has

been reported to range from 4.63% to 9.75% during the period of 2020–2024, with some reports in early 2025 indicating an incidence rate reaching 12.95%, thus placing dermatitis among the top ten skin diseases in primary healthcare facilities (Melati et al., 2025). These findings are supported by a study in the urban area of Makassar, which showed that more than half of patients with skin complaints were diagnosed with dermatitis, with poor personal hygiene as the dominant risk factor (Dewi et al., 2025). Additionally, a teledermatology study in remote areas of Indonesia reported that eczematous diseases were the second most common skin diagnosis after skin infections (Adella et al., 2024). Globally, the World Health Organization (WHO) reports that the prevalence of atopic dermatitis reaches around 18% in children and 5% in adults, while allergic contact dermatitis is estimated to affect about 7% of the general population, with a higher prevalence in the elderly (Dannia et al., 2024).

The high incidence of these conditions is closely related to the characteristics of dermatitis as a chronic inflammatory disease that not only causes erythema, edema, intense pruritus, and persistent pain, but also reduces the quality of life thru sleep disturbances, limitations in daily activities, and decreased social functioning. Pathophysiologically, exposure to allergens, irritants, or tissue damage activates various immune cells, which subsequently trigger the release of pro-inflammatory cytokines and chemokines, thereby prolonging the inflammatory phase if not adequately controlled (Eryani et al., 2025). Prolonged inflammatory response also leads to impaired skin barrier function, which can be evaluated using the Transepidermal Water Loss (TEWL) parameter. In the Indonesian population, patients with atopic dermatitis have an average TEWL value of 18.07 $g/h/m^2$, significantly higher than the healthy group, which only reaches 5.61 $g/h/m^2$ (Damayanti et al., 2025). Other studies also show that increased TEWL positively correlates with the severity of atopic dermatitis and psoriasis, making this parameter potentially useful as an objective indicator in evaluating therapy effectiveness (Montero-Vilchez et al., 2021).

Controlling inflammation is the main goal of dermatitis therapy. Until now, topical corticosteroids (TCS) remain the first-line therapy because they can suppress the inflammatory response by binding to cytoplasmic glucocorticoid receptors, which subsequently inhibit pro-inflammatory transcription pathways, including Nuclear Factor-kappa B (NF-κB), and enhance the expression of anti-inflammatory genes (DiRuggiero & DiRuggiero, 2025). Although it has high clinical efficacy, prolonged use of topical corticosteroids (TCS) is not without various local and systemic side effects. The most commonly found local side effects include skin atrophy, telangiectasia, striae, and purpura due to the inhibition of keratinocyte proliferation (Ricardo et al., 2023). Additionally, repeated use can lead to tachyphylaxis, resulting in decreased therapeutic response (Ricardo et al., 2023), while sudden cessation after long-term use can potentially trigger Topical Steroid Withdrawal Syndrome (TSWS), characterized by diffuse erythema, edema, and burning sensation (Lal et al., 2023). In children, higher percutaneous absorption also increases the risk of hypothalamic-pituitary-adrenal axis suppression (Ferreira et al., 2021).

Various limitations have prompted the development of topical anti-inflammatory agents based on natural ingredients that have adequate efficacy with lower risk of side effects. One of the plants that has attracted attention is Plumeria rubra L. (Apocynaceae),

which has long been utilized in traditional medicine as a wound healer, antipruritic, analgesic, and anti-inflammatory (Khan et al., 2022). Scientific validation of these empirical uses is further developing thru various phytochemical studies. LC-DAD-ESI-MS analysis shows that P. rubra leaves contain polyphenols (39%), iridoids (28%), terpenoids (13%), alkaloids (9%), and fatty acids (6%) (Malaspina et al., 2024). Until now, more than 110 bioactive compounds have been successfully identified from this plant, including fulvoplumierin, plumieride, plumericin, isoplumericin, lupeol, oleanolic acid, and stigmasterol [14]. Recent research also shows that methanol extract has the highest total phenolic content, while ethanol extract exhibits better antioxidant activity, thereby strengthening the potential of P. rubra as a phytopharmaceutical candidate (Elwakil et al., 2025).

The anti-inflammatory activity of P. rubra is primarily associated with its content of flavonoids and pentacyclic triterpenoids, particularly lupeol. Flavonoids inhibit the activity of the cyclooxygenase-2 (COX-2) enzyme, thereby reducing the biosynthesis of pro-inflammatory prostaglandins without disrupting the protective function of COX-1 (Suleman et al., 2025). Additionally, flavonoids are capable of neutralizing reactive oxygen species (ROS) produced during the neutrophil oxidative burst, thereby protecting fibroblasts from oxidative damage and supporting tissue regeneration (Suleman et al., 2025). Meanwhile, lupeol plays a role in suppressing the expression of pro-inflammatory cytokines, such as Tumor Necrosis Factor-α (TNF-α), Interleukin-6 (IL-6), and Interleukin-12 (IL-12), thru the inhibition of the NF-κB signaling pathway (Kim et al., 2025). The regulation of these inflammatory mediators has been proven to reduce neutrophil infiltration, increase fibroblast proliferation, accelerate collagen deposition, and hasten the transition from the inflammatory phase to the proliferative phase during the wound healing process (Zhao et al., 2024). These findings indicate that P. rubra has the potential to act as a multitarget topical anti-inflammatory agent on the inflammatory pathway.

The biological potential of the extract will not provide optimal therapeutic effects without being supported by an appropriate drug delivery system. Therefore, an oil-in-water (O/W) type cream was chosen as the delivery system because it provides a cooling effect, is easy to clean, is non-occlusive, and can enhance user comfort. The stability of the preparation is influenced by the combination of stearic acid and triethanolamine (TEA), which forms stearate soap in situ, thereby producing a stable emulsion. The ratio of these two emulsifiers determines the physical characteristics of the preparation, such as viscosity, homogeneity, adhesion, and pH. Maintaining pH within the physiological range of the skin (4.5–6.5) is also important to preserve the skin barrier function and prevent increased TEWL and the occurrence of irritant contact dermatitis (Murdiana et al., 2022).

Although the pharmacological activity of P. rubra has been widely reported, most studies still focus on the identification of bioactive compounds, antioxidant activity, or excision wound models. Research evaluating the variation in concentrations of P. rubra leaf extract in M/A type cream bases and linking the physicochemical characteristics of the formulation with anti-inflammatory activity in incision wound models is still very limited. Therefore, this study was conducted to evaluate the physical quality of P. rubra leaf extract cream at concentrations of 2%, 4%, and 6% and to determine the concentration that provides the most effective anti-inflammatory activity using an

incision wound model in male white rats (Rattus norvegicus L.). Overall, this research aims to support the use of plumeria leaves as a safe and natural method for treating skin inflammation. This research is expected to find new ways to reduce dependence on the use of topical corticosteroids, which carry significant risks of side effects for skin health in the long term. This will be achieved thru a thorough analysis of the physical parameters of the preparation as well as daily observations of the closure of the incision wound.

Methodology

Tools and Materials Equipment: blender, porcelain cup, measuring cup, glass beaker, Erlenmeyer flask, analytical balance, animal cage, rotary evaporator, funnel, filter paper, marker, scalpel, tissue, dropper, spatula, water bath, oven, baking tray, cream pot, towel, glass, mortar, pestle, scalpel, pH meter, alcohol cotton, glass bottle, cotton bud, camera. Materials: plumeria leaves (Plumeria spp.), 96% ethanol, stearic acid, TEA (Triethanolamine), lanolin, liquid paraffin, nipagin, distilled water, male white rats (Rattus norvegicus L) weighing 180-220grams.

Procedure: Sample Preparation 1 kg of dark green, intact, and undamaged frangipani leaves were collected, then washed with running water. The leaves are dried in an oven at 40°C until completely dry, then blended into a fine powder. A total of 150 grams of simplicia was extracted with 1.125 mL of 96% ethanol using the maceration method for 3 days with periodic stirring. The maceration result was filtered with filter paper, then the residue was re-macerated with 375 mL of 96% ethanol for 2 days and filtered again. All the macerates were evaporated using a rotary evaporator at 50°C, followed by a water bath at 50°C until a thick extract was obtained, then stored in glass bottles.

Phytochemical Screening

Alkaloid Test

Conducted using Dragendorff's reagent. 1 mL of the extract is placed into a test tube, and 0.5 mL of 2% HCl is added to the tube and shaken until homogeneous. Then, 2-3 drops of Dragendorff's reagent are added to the tube. if a brown precipitate forms in the tube, then the sample contains alkaloids.

Flavonoid Test

Conducted by adding 1 ml of extract into a test tube and adding enough hot water. The filtrate is taken in an amount of 5 mL and added with a 2 cm strip of Mg and 1 mL of concentrated HCl, then shaken. If a red, yellow, or orange color forms, it indicates the presence of flavonoids.

Saponin Test

In the extract, the Forth method is performed. 1 mL of the extract is placed into a test tube, then 2 mL of hot water is added. The sample will form foam, then 1 mL of 2N HCl is added. If the foam does not disappear within 30 seconds, the extract is positive for saponins.

Tannin Test

In the extract, several drops of 5% iron (III) chloride solution are added to 1 ml of the extract. If a dark blue or greenish-black precipitate forms, the extract is positive for tannins.

Triterpenoid and Steroid Test

In the extract sample, 1 ml of the extract is mixed with 2 ml of 98% chloroform in a test tube and shaken. After that, the formed chloroform layer is taken and dropped onto a drop plate and allowed to dry. Then, 5 drops of 98% anhydrous acetic acid and 3 drops of 98% H2SO4 are added. If red, orange, or yellow colors are formed, then the sample contains triterpenoids, and if a green color is formed, the sample contains steroids (Fikayuniar et al., 2023).

Formulation of Preparation

Tabel 1. Cream Base Formulation

Ingredients	Weight
Stearic Acid	14,64 grams
Triethanolamine	1,5 grams
Adeps lanae	3 grams
Liquid paraffin	25,2 grams
Nipagin	0,1 grams
Aquadest	Ad 100 ml
mf. cream	100 grams

The cream base being made consists of two phases, namely the oil phase (Stearic Acid, Lanolin, Liquid Paraffin) and the water phase (Triethanolamine and Nipagin). The oil phase is melted over a water bath at a temperature of 60-70°C until the oil phase is melted, then transferred to a hot mortar, and the water phase is added and stirred until cool, forming a cream mass. Next, the preparation of the ethanol extract cream from plumeria leaves (*Plumeria rubra* L.) involves adding the diluted extract with the aqueous phase into a mortar, with concentrations of 2%, 4%, and 6%, gradually until homogeneous. Then each formula is stored in cream containers.

Table 2. Cream Concentration Formula

Material	Formula		
	1	2	3
Ethanol extract of frangipani leaves (Plumeria rubra L.)	0,4 grams	0,8 grams	1,2 grams
Basis cream	ad 20 grams	ad 20 grams	ad 20 grams

The process of making ethanol extract cream from plumeria leaves (Plumeria rubra L.) begins with the preparation of a cream base. In the preparation of the cream type, an oil-water type is used, and the cream formulations for this study have ethanol extract concentrations of plumeria leaves (*Plumeria rubra* L.) at 2%, 4%, and 6%, each made in 20 grams.

Testing of plumeria leaf extract cream The cream testing includes organoleptic tests and physical properties tests, including form (texture), color, odor, pH test, homogeneity test, spreadability test, adhesion test, and anti-inflammatory test.

Organoleptic Test

The organoleptic examination is an initial parameter to assess the physical stability of the preparation thru direct visual and sensory observation. The parameters evaluated include texture (consistency), color, and aroma of the preparation. Cream preparations that meet esthetic and stability criteria should not show any phase separation (creaming or breaking), significant color changes, or rancid odors during the storage period. This is important because organoleptic changes often serve as early indicators of chemical degradation or microbiological contamination in both the active ingredient and the cream base (Sinaga et al., 2022; (Fikayuniar et al., 2023).

Homogeneity Test

The homogeneity test is conducted to ensure the even distribution of active ingredients within the cream base, so that the dose received by users remains consistent with each use. The procedure is carried out by applying a small amount of the sample onto a transparent glass slide or object glass. The preparation is considered homogeneous if it visually shows a uniform, smooth arrangement, and no coarse grains or separate particle clumps are found. The non-homogeneity of the preparation can affect user comfort (rough sensation on the skin) as well as therapeutic effectiveness due to uneven drug release (Primadina et al., 2019).

pH Test

pH measurement is an important stage in the evaluation of topical preparations because the pH value must match the physiological pH of the skin, which is around 4.5–6.5, to prevent irritation or dryness. Before the measurement, the digital pH meter is calibrated using standard buffer solutions of pH 4.01 and 7.00 until the device shows a stable value. The sample is then prepared by dissolving one gram of cream into ten milliliters of distilled water and stirring until homogeneous. The pH meter electrode is immersed in the solution and left until the reading stabilizes, then the displayed value is recorded as the pH of the preparation. Measurements are usually repeated to ensure accurate and consistent results (Rahmatillah et al., 2025; Sinaga et al., 2022).

Adhesion Test

0.5 grams of cream is applied on a glass slide with a known area. Another glass slide is placed on the cream and pressed with a 1 kg weight for 5 minutes. The glass slide is then mounted on a testing apparatus and subjected to an 80-gram weight, with the time recorded until the two glass slides separate (Tungadi et al., 2023). Spreadability Test 0.5 grams of the formulated cream is weighed and placed on a glass slide, then a petri dish is placed on top and left for 1 minute. The area covered by the preparation is measured. Next, each preparation was loaded with weights of 50, 100, and 250 grams respectively, left for 60 seconds, and then the area of the preparation produced was measured (Tungadi et al., 2023).

Anti-inflammatory test

Rats were divided into 5 groups, each consisting of 5 rats. Group 1 for testing the 2% plumeria leaf extract cream, group 2 for testing the 4% plumeria leaf extract cream, group 3 for testing the 6% plumeria leaf extract cream, group 4 for positive control testing

(ethanol extract plumeria leaf cream base), and group 5 for negative control testing (no treatment). Each rat was shaved sufficiently on the back, and then an incision was made using a scalpel with a length of 2 cm and a depth of ± 2 mm. The scalpel was previously marked along ± 2 mm with tape to ensure the incision depth could be measured. After the wound was created, each group of cream was applied thinly at ± 0.25 mg on the rats. The treatment was carried out until healing and applied once a day in the morning until healed, with daily observations using 4 assessment categories: 1 = very red, wet; 2 = red, somewhat wet; 3 = somewhat red, almost dry; and 4 = dry (healed) (Fauzia & Zuniarto, 2017).

Result and Discussion

Table 3. Results of Phytochemical Screening of Ethanol Extract of Plumeria Leaf

Phytochemical Test	Result	Explanation
Alcaloide	+	Brown precipitate formed with Dragendorff
Flavonoide	+	A red-orange color is formed
Saponin	+	Stable foam forms for more than 30 seconds.
Tannin	+	Bluish-black color
Terpenoide	+	Red-orange color
Esteroide	+	Green color formed

The results of the phytochemical screening shown in Table 3 indicate that the ethanol extract of plumeria leaves (*Plumeria rubra* L.) contains a number of secondary metabolite compounds, including alkaloids, flavonoids, saponins, tannins, terpenoids, and steroids. The fact that frangipani leaves have pharmacological properties, particularly anti-inflammatory and skin tissue healing properties, is based on the presence of these compounds. The formation of a brown precipitate with Dragendorff's reagent identifies alkaloids. This indicates the presence of organic basic compounds that often have analgesic and anti-inflammatory effects. The flavonoid content, marked by a red-orange color change, enhances this. Flavonoids are widely known as antioxidant agents that have the ability to inhibit the enzyme cyclooxygenase (COX), which stops the production of prostaglandins and other inflammatory mediators. Minimizing tissue damage in inflamed skin areas is very important (Devi & Chengamaraju, 2025).

The saponin content, characterized by its natural surfactant properties that can help active substances pass thru the skin's lipid layer, is indicated by the presence of saponin and stable foam for more than 30 seconds. Tannin, which can be identified by its bluish-black color, also has astringent properties that can shrink skin pores and harden the proteins present on the wound surface. This accelerates the wound closure process and prevents further infection by microorganisms. The synergy between terpenoids and steroids is also very important. Terpenoid compounds such as lupeol, characteristic of the Plumeria genus, have been proven to accelerate the proliferative phase of wound healing by promoting keratinocyte migration. According to research conducted by Shukla et al. (2023), the combination of complex secondary metabolites from frangipani leaves has greater synergistic benefits than single compounds in addressing inflammation symptoms (Shukla et al., 2023).

In the extraction process, the use of 96% ethanol solvent has proven effective in attracting compounds with various levels of polarity, ranging from polar flavonoids to non-polar steroids. To provide a therapeutic effect, the formulated cream preparation must contain representative chemical constituents. Overall, the in vivo test results showing that the treatment group healed faster than the control group are supported by this rich phytochemical profile. Therefore, the presence of these active compounds indicates that the *Plumeria rubra* L. leaf extract cream meets the physical requirements of the formulation and possesses chemical integrity that supports its anti-inflammatory claims (Tungadi et al., 2023).

Table 4. Physical Quality Profile of the Plumeria Leaf Extract Cream Formulation

Parameter	K- (Basis)	F1 (2%)	F2 (4%)	F3 (6%)	Standard
Organoleptic	White, odorless, semisolid	Light green, typical of frangipani, semisolid	Green, typical of Cambodia, semisolid	Dark green, typical of frangipani, semisolid	Semisolid, Homogen
pH	4,5	4,94	5,08	5,24	4,5 – 6,5
Spread Power (cm)	6,7	6,5	6,0	5,8	5 – 7 cm
Adhesive power(det)	4,0	4,2	4,8	5,3	> 4 second
Homogeneity Test	Homogeneity	Homogeneity	Homogeneity	Homogeneity	Homogeneity

The evaluation of the physical quality of cream preparations is an important step to ensure that the cream preparations are stable, safe, and comfortable to use. The three cream formulas of ethanol extract from *Plumeria rubra* L. leaves exhibit stable organoleptic characteristics, a soft texture, and a consistent color according to the concentration of the added extract, as shown in the data in Table 4. From F1 to F3, the green color becomes more intense, indicating that there are more chlorophyll and secondary metabolite compounds in the formulation. The results of the homogeneity test confirm that all formulas do not show phase separation or coarse granules. This indicates that, in the in situ saponification reaction, the use of stearic acid and triethanolamine (TEA) as emulsifiers has produced a stable dispersion system that effectively forms stearate soap to reduce interfacial tension. Homogeneity is crucial for the success of the therapy as it ensures an even distribution of active substances when the preparation is applied to the inflamed skin surface, thereby delivering the correct dose to the target tissue (Tungadi et al., 2023).

To reduce the likelihood of primary skin irritation, the pH parameter of the formulation is a determining factor for safety. The measurement results show that all three formulas have a pH value of 4.5–5.24, which is very ideal because it falls within the physiological pH range of human skin, namely 4.5–6.5. To maintain the function of the skin's acid mantle, which serves as a natural protection against pathogen colonization, it is very important to keep the pH within this weakly acidic range. According to Winarti et al. (2025), topical preparations with a pH that is too alkaline can disrupt the integrity of the skin barrier, causing dry skin, while a pH that is too acidic can lead to contact dermatitis (Winarti et al., 2025). Additionally, the pH stability of this study indicates that

the ethanol extract of plumeria leaves does not undergo significant chemical degradation in the cream base. Therefore, the pH profile in Table 4 ensures that the ethanol extract of plumeria leaves can be used as an alternative for long-term treatment of skin inflammation.

The results of the adhesion and spreadability tests show the mechanical characteristics of the formulation. This cream has good spreadability (ranging from 5-7 cm) so it can cover wounds or inflammation with low pressure, reducing pain or mechanical trauma when applied to sensitive skin. Due to the increase in viscosity, the increase in extract concentration from F1 to F3 seems to slightly reduce the spreadability. However, the concentration of the extract remains below the permissible pharmaceutical limits for semi-solid preparations. In addition, the optimal adhesion ensures that the preparation is not easily wiped off by clothing and remains attached to the skin for a longer period. Prolonged contact, or a longer contact time between the preparation and the skin, allows active ingredients such as flavonoids and terpenoids to penetrate deeper into the dermal layers. This helps inhibit inflammatory mediators (Devi & Chengamaraju, 2025).

The use of an M/A base, or oil-in-water, in this formulation has an additional benefit, namely a cooling effect, which is produced by the evaporation of the water phase on the skin surface, psychologically providing comfort to the inflammation that feels hot. Additionally, its non-sticky nature and ease of washing will enhance patient compliance in the use of topical medications. This product has good commercial and clinical potential as a competitive herbal anti-inflammatory agent thanks to the synergy between physical quality that meets physical stability standards and stable secondary metabolite content (Shukla et al., 2023).

Table 5. Results of Measurement of Changes in Incision Length (cm)

Treatment Group	Day 1(CM)	Day 6(CM)	Day 9(CM)
K-	2,00 ± 0,00	1,62 ± 0,13	1,04 ± 0,27
k+	2,00 ± 0,00	1,64 ± 0,11	0,98 ± 0,26
F1	2,00 ± 0,00	1,60 ± 0,12	1,36 ± 0,11
F2	2,00 ± 0,00	1,60 ± 0,10	0,64 ± 0,11
F3	2,00 ± 0,00	1,58 ± 0,19	0,78 ± 0,33

Based on the data presented in Table 5, a dynamic wound healing profile is observed in all treatment groups over the nine-day observation period. The complex biological process known as cut wound healing consists of the inflammatory, proliferative, and maturation phases. On the 6th day, the observation results showed that the average wound length for all treatment groups (K-, K+, F1, F2, and F3) still ranged from 1.58 ± 0.19 cm to 1.64 ± 0.11 cm. The significant value of the ANOVA statistical test at 0.965 ($p > 0.05$) indicates that there is no significant difference between the treatment groups at this stage. According to wound healing theory, granulation tissue begins to form in the first week and wound contraction has not yet reached its peak. According to the research conducted by Shedoeva et al. (2019), fibroblast migration is a sign of the early proliferative phase (Shedoeva et al., 2019). However, the reduction in wound size

macroscopically usually does not show significant differences between the concentrations of active substances in the early days of the proliferative phase.

The wound healing profile underwent significant changes on day 9, with a significance value of 0.001 ($p < 0.05$). This indicates that there is a significant impact of the formula changes on the wound closure rate. The Formula 2 (F2) group showed the best results with the smallest average wound length of 0.64 ± 0.11 cm, followed by the Formula 3 (F3) group with an average wound length of 0.78 ± 0.33 cm. Compared to the Negative Control (1.04 ± 0.27 cm) and Formula 1 (1.36 ± 0.11 cm), this indicates that the concentration of active substances in the formulas helps stimulate collagen formation and epithelialization. The content of active compounds, such as flavonoids or saponins, is responsible for this increase in effectiveness. These active compounds work by accelerating wound contraction thru the activation of myofibroblasts. According to Shukla et al. (2023), the ability of topical preparations to maintain moisture in the wound area and promote tissue growth factors significantly affects the acceleration of wound closure in the mid-phase (Shukla et al., 2023).

Interestingly, the research results show that, although both are in the same statistical subset, the increase in concentration in Formula 3 (0.78 ± 0.33 cm) did not lead to faster healing than Formula 2. This indicates the phenomenon of saturation concentration, where an increase in the dose of the active substance no longer correlates linearly with the tissue healing response. As shown by Shedoeva et al. (2019), the effectiveness of plant therapeutic agents highly depends on the correct dosage (Shedoeva et al., 2019) Concentrations that are too high can sometimes reach the threshold of cellular receptors or affect the viscosity of the preparation, which hinders the optimal absorption of the agent into the dermis. Overall, this study found that Formula 2 showed the most consistent and significant incision wound healing activity. This makes it the most promising formula for the development of topical therapy compared to other concentrations.

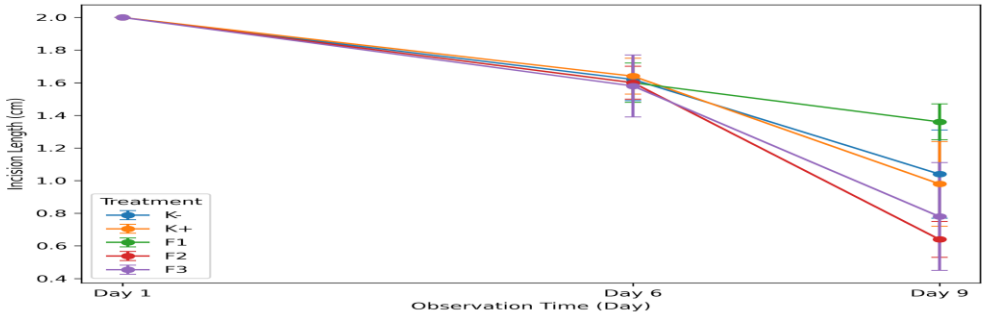

Figure 1. Graph of Wound Length Measurements for Each Treatment Group

Table 6. Effectiveness of Cream Formulation on the Healing Speed of Incised Wounds

Group	N	Average Recovery Days ± SD	Notation LSD (a=0,05)
K-	5	8,20 ± 0,45	a
K+	5	9,00 ± 0,00	b
F1	5	8,40 ± 0,55	a
F2	5	9,00 ± 0,00	b

F3	5	9,00 ± 0,00	b

Note: A wound is considered healed when it reaches a score of 4 (dry).

This study evaluates the effectiveness of cream preparations on the duration of healing of incision wounds in a white rat (Rattus norvegicus) test animal model, as shown by the data in Table 6. The parameters of macroscopic healing were calculated based on how long it took for the wound to reach a completely dry condition, which is described with a score of 4. With a p-value of 0.001 ($p < 0.05$), variations in the concentration of the preparation have a significant effect on the rate of healing of incision wounds. This is demonstrated by the results of the statistical analysis using the One-Way ANOVA test. This indicates that the pharmacological activity correlates with the dose of the active substance added to the cream. According to a study conducted by Shukla et al. (2023), the effectiveness of wound healing highly depends on the ability of the active substance to control pro-inflammatory cytokines and accelerate the transition to the proliferative phase, as evidenced by the drying of exudate and the formation of scabs (Shukla et al., 2023).

Further analysis using the Least Significant Difference (LSD) test found that the Formula 2 (4%) and Formula 3 (6%) groups had an average healing time of 9.00 ± 0.00 days, which was statistically identical to the positive control ($p = 1.000$). The success of Formula 2 (4%) indicates that this concentration is the minimum effective dose to achieve optimal wound closure. This is in accordance with the principles of moist wound healing developed in the past ten years, where the correct concentration of active substances supports tissue hydration and facilitates the migration of keratinocytes and fibroblasts to accelerate uniform epithelialization (Shedoeva et al., 2019). Compared to the negative control, which had a greater healing variation of 8.20 ± 0.45 days, the consistency of the data shows that the drug is capable of providing a stable biological response with a Standard Deviation (SD) of 0.00 at doses of 4% and 6%.

The results of the LSD test placed both in the same notation group (a), indicating no statistically significant difference ($p = 0.329$). This occurred despite the fact that numerically, the negative control (K-) and Formula 1 (2%) showed that the average wound drying time appeared to be faster. This phenomenon indicates that the wound healing process is highly dependent on the natural regeneration system and the unique physiological variables of the test animals without the support of active substances at sufficient concentrations(Iskandar et al., 2021). On the other hand, a cream with a minimum concentration of 4% helps control the duration of the tissue maturation phase. In accordance with the pharmacodynamic principle of receptor saturation kinetics, increasing the concentration beyond 4% no longer provides a significant acceleration in healing because the cellular capacity in the dermal remodeling process has reached the optimal threshold (Ikhsan et al., 2023). Thus, the use of cream preparations at a concentration of 4% is the most efficient formula in supporting the recovery of skin tissue integrity to meet the criteria for complete healing in this study.

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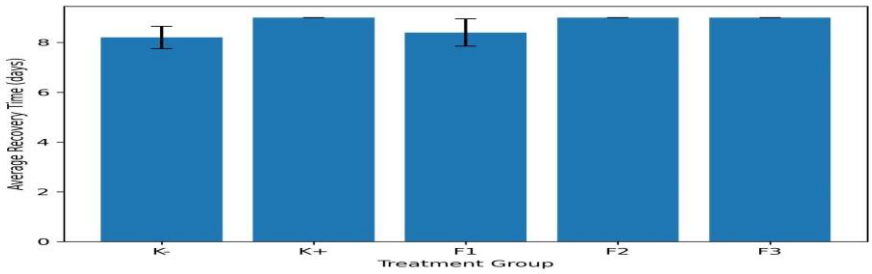
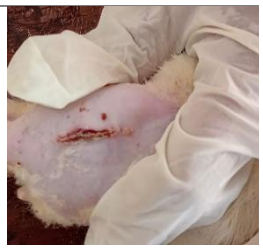
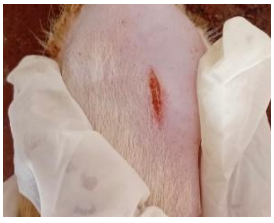
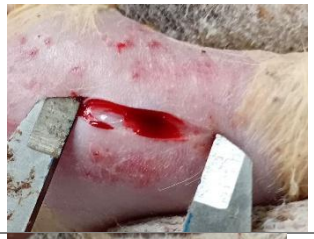
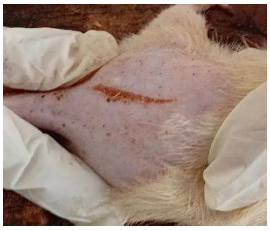
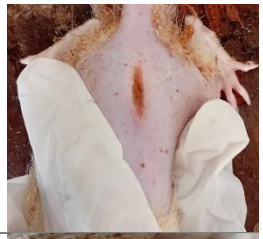
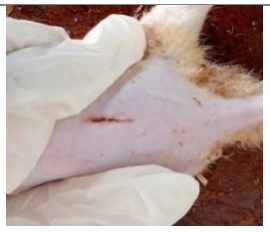
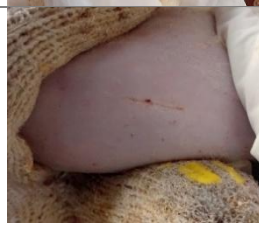
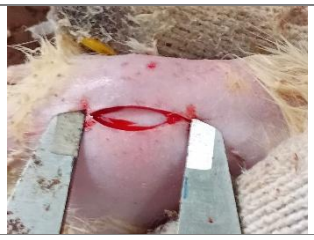
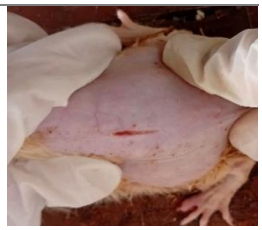


Figure 2. Graph of Average Wound Healing Time

Table 7. Appearance of The Incision Wound

Group	Wound Day-1	Wound Day-6	Wound Day-9
K+ (Without Treatment)			
K- (Crime Base)			
F1 (Concentration 2%)			
F2 (Concentration 4%)			
F3 (Concentration 6%)			

The Role of Fibroblasts, Collagen Deposition, and Epithelialization in Wound Healing The activity of ethanol extract cream from plumeria leaves (Plumeria rubra L.) in accelerating the reduction of wound length macroscopically is closely related to the

improvement of the histological architecture of the skin at the cellular level. Histologically, the skin is composed of epidermal and dermal tissues that must restore their mechanical barrier integrity after laceration trauma. The optimal acceleration of wound closure in the Formula 2 (4%) group indicates a positive modulation in the proliferative phase, characterized by increased fibroblast activity.

Fibroblasts are the main effector cells in the dermis layer that migrate to the wound area under the influence of growth factors such as Transforming Growth Factor-Beta (TGF-β) to form new granulation tissue. In the proliferative phase, fibroblasts become activated and differentiate into myofibroblasts that actively synthesize extracellular matrix (ECM) components, primarily collagen, as the main structural protein that provides tensile strength to the newly formed skin (Singh et al., 2023;Kim et al., 2025). Within the granulation tissue, fibroblasts actively synthesize collagen as the main extracellular matrix protein responsible for providing tensile strength to the newly formed skin (Diller & Tabor, 2022). In the early proliferative phase, type III collagen is synthesized in large amounts to provide a flexible provisional matrix, before being replaced by more mature and denser type I collagen fibers during the remodeling phase to prevent the formation of abnormal scar tissue (Singh et al., 2023). The content of secondary metabolites such as flavonoids and lupeol (triterpenoid) in the plumeria leaf extract is suspected to act as antioxidant agents that protect fibroblast cells from oxidative stress caused by neutrophil burst, allowing fibroblasts to optimally maintain their collagen biosynthesis capacity.

Synergistically with collagen deposition in the dermal layer, the process of epithelialization also occurs in the upper layer to restore the protective function of the epidermal barrier. This epithelialization involves the mobilization, proliferation, and lateral migration of keratinocyte cells from the wound edges across the moist surface of the granulation tissue (Mamun et al., 2024). The oil-in-water (O/W) cream formulation in this study provides ideal moist wound healing conditions, which hydrodynamically facilitate keratinocytes to migrate faster without the hindrance of thick dry scab tissue (Diller & Tabor, 2022). After the migration of keratinocytes from both sides of the wound meet and experience contact inhibition, the cells stop moving and begin the process of stratification and histological differentiation into a complete epidermal layer (Mamun et al., 2024). Thus, the efficiency of wound closure at a 4% dose (F2) is supported by the precise histological synchronization between collagen fiber formation by fibroblasts and the speed of the epithelialization process on the outer skin surface.

Conclusion

Based on the results of this study, it can be concluded that this research successfully formulated an oil-in-water (O/W) cream from ethanol extract of *Plumeria rubra* L. leaves that meets the physical quality standards of pharmaceutical preparations, including good homogeneity, ideal spreadability and adhesion, as well as a skin-safe acidity level (pH). The effectiveness test results show that the cream with a 4% extract concentration (F2) provides the most optimal anti-inflammatory activity in accelerating wound healing in white rats, with results statistically equivalent to the positive control. Thus, the formulation of this plumeria leaf extract cream has significant potential as a

natural topical treatment alternative for addressing skin inflammation and contributes to the development of phytopharmaceutical preparations based on local plants.

Declaration of Compenting Interest

The authors declare that there are no conflicts of interest in the conduct of this research or the publication of this manuscript. Conflict of Interest Statement

Reference

Adella, F. J., Ammah, H., Siregar, G. O., Harianja, M., Sundari, E. S., Sagara, R., Tarino, N., Hamers, R. L., Bøgh, C., Soebono, H., & Grijsen, M. L. (2024). Teledermatology to Improve Access to and Quality of Skin Care in Eastern Indonesia. *American Journal of Tropical Medicine and Hygiene*, *110*(2), 364–369. https://doi.org/10.4269/ajtmh.23-0218

Damayanti, Anggraeni, S., Umborowati, M. A., Adiba, A. F., & Prakoeswa, C. R. S. (2025). Profile of Transepidermal Water Loss in Non-Atopic Dermatitis and Atopic Dermatitis in Indonesian Adults. *Berkala Ilmu Kesehatan Kulit Dan Kelamin*, *37*(1), 28–33. https://doi.org/10.20473/bikk.V37.1.2025.28-33

Dannia, E. R., Habibi, J., Suyanto, J., & Fauzi, Y. (2024). Faktor-Faktor Yang Berhubungan Dengan Kejadian Dermatitis Kontak Di Wilayah Kerja Puskesmas Telaga Dewa Kota Bengkulu Factors Associated With The Occurrence Of Contact Ddermatitis In The Working Area Of Telaga Dewa Health Center, Bengkulu City. In *Journal Hygea Public Health* (Vol. 2, Number 2).

Devi, P. S. S. N., & Chengamaraju, N. S. (2025). Phytochemical Profile and Pharmacological Potentials of Plumeria Species: A Comprehensive Review. *International Journal of Pharmaceutical Research and Applications*, *10*(1), 1459–1468. https://doi.org/10.35629/4494-100114591468

Dewi, C., Syam, I., Syahrir, M., Sahida, M., Sarkol, M. C., Tinggi, S., Kesehatan, I., Teknologi, I., Tri, K., & Nasional, T. (2025). Incidence of Dermatitis in Urban Communities: A Study in Tallo Village, Makassar City Kejadian Dermatitis pada Masyarakat Urban: Studi di Kelurahan Tallo, Kota Makassar. *Miracle Journal of Public Health (MJPH)*, *8*(1). https://doi.org/10.36566/mjph/Vol8.Iss1/439

Diller, R. B., & Tabor, A. J. (2022). The Role of the Extracellular Matrix (ECM) in Wound Healing: A Review. In *Biomimetics* (Vol. 7, Number 3). MDPI. https://doi.org/10.3390/biomimetics7030087

DiRuggiero, D., & DiRuggiero, M. (2025). Beyond Skin Deep: The Systemic Impact of Topical Corticosteroids in Dermatology. *Journal of Clinical and Aesthetic Dermatology*, *18*(1–2 Suppl 1), 16–20. https://jcadonline.com/beyond-skin-deep-the-systemic-impact-of-topical-corticosteroids-in-dermatology/

Elwakil, B. H., Elsabrouty, M. H., Eskandrani, A., Paudel, K. R., & Moneer, E. A. (2025). Multifunctional Plumeria rubra nanoparticles: Antimicrobial, antiviral, antiparasitic and anti-inflammatory activities. *Heliyon*, *11*(2). https://doi.org/10.1016/j.heliyon.2025.e42044

Eryani, A., Mariyati, S. D. N., Naeluvar, L., Az-Zahra, G., & Saputri, V. S. (2025). Pemanfaatan Ekstrak Kulit Kayu Angsana (Pterocarpus indicus) sebagai Obat Luka pada Hewan Coba Tikus (Sprague-dawley) Model Luka Sayat. *Journal of Innovative and Creativity*, *5*(2), 2025.

Fauzia, R. R., & Zuniarto, A. A. (2017). Uji Efektivitas Antiinflamasi Suspensi Ekstrak Daun Bayam Duri (Amaranthus Spinosus L.) Terhadap Tikus Putih (Rattus Norvegicus) Yang

Diinduksi Karagenan. *Journal of Holistic and Health Sciences*, *1*(2). https://doi.org/https://doi.org/10.51873/jhhs.v1i2.11.

Ferreira, K. C. B., Valle, A. B. C. D. S., Paes, C. Q., Tavares, G. D., & Pittella, F. (2021). Nanostructured lipid carriers for the formulation of topical anti-inflammatory nanomedicines based on natural substances. In *Pharmaceutics* (Vol. 13, Number 9). MDPI. https://doi.org/10.3390/pharmaceutics13091454

Fikayuniar, L., Putri Valentina, D., Kurniawati, I., Fajriyatulhuda, S., Mudrikah, S., & Amelia, T. (2023). Literature Review Skrining Fitokimia Metode Tabung Pada Simplisia Bunga Kamboja (Plumeria Sp). *INNOVATIVE: Journal Of Social Science Research*, (2). https://doi.org/https://doi.org/10.51873/jhhs.v1i2.11.

Ikhsan, M. K., Saputro, S., & Asjur, A. V. (2023). Formulasi Sediaan Krim Ekstrak Etanol Biji Kalabet (Trigonella Foenum-Graecum) Dengan Basis Krim Tipe M/A Dan Uji Aktivitas Antibakteri Terhadap (Staphylococcus Aureus). *Kesehatan Tambusai*, *4*(3). https://doi.org/https://doi.org/10.31004/jkt.v4i3.18055.

Iskandar, B., Dian, Z. P., Renovita, F., & Leny. (2021). Formulasi dan evaluasi gel Lidah buaya (Aloe vera Linn) sebagai pelembab kulit dengan penggunaan carbopol sebagai gelling agent. *Health Sciences and Pharmacy Journal*, *5*(1), 1–8.

Khan, I. A., Hussain, M., Syed, S. K., Saadullah, M., Alqahtani, A. M., Alqahtani, T., Aldahish, A. A., Asiri, S., & Zeng, L. H. (2022). Pharmacological Justification for the Medicinal Use of Plumeria rubra Linn. in Cardiovascular Disorders. *Molecules*, *27*(1). https://doi.org/10.3390/molecules27010251

Kim, M. J., Yang, Y. J., Min, G. Y., Heo, J. W., Son, J. D., You, Y. Z., Kim, H. H., Kim, G. S., Lee, H. J., Yang, J. H., & Park, K. Il. (2025). Anti-inflammatory and antioxidant properties of Camellia sinensis L. extract as a potential therapeutic for atopic dermatitis through NF-κB pathway inhibition. *Scientific Reports*, *15*(1). https://doi.org/10.1038/s41598-025-86678-5

Lal, D. K., Kumar, B., Saeedan, A. S., & Ansari, M. N. (2023). An Overview of Nanoemulgels for Bioavailability Enhancement in Inflammatory Conditions via Topical Delivery. In *Pharmaceutics* (Vol. 15, Number 4). Multidisciplinary Digital Publishing Institute (MDPI). https://doi.org/10.3390/pharmaceutics15041187

Malaspina, P., Ingegneri, M., Betuzzi, F., Di Gristina, E., Cornara, L., Trombetta, D., & Smeriglio, A. (2024). Beyond the Scent: New Evidence about Micromorphological, Phytochemical and Biological Features of Plumeria rubra 'Tonda Palermitana' (Apocynaceae). *Plants*, *13*(17). https://doi.org/10.3390/plants13172479

Mamun, A. Al, Shao, C., Geng, P., Wang, S., & Xiao, J. (2024). Recent advances in molecular mechanisms of skin wound healing and its treatments. In *Frontiers in Immunology* (Vol. 15). Frontiers Media SA. https://doi.org/10.3389/fimmu.2024.1395479

Melati, R., Aryanti, E., Alfaqih, M. S., Ferianasari, I. W., & Purwoko, R. Y. (2025). Science Midwifery Prevalence and characteristics of dermatitis cases in Dermatology Clinics (2020-2024): A retrospective analysis. In *Science Midwifery* (Vol. 12, Number 6). Online. www.midwifery.iocspublisher.orgJournalhomepage:www.midwifery.iocspublisher.org

Montero-Vilchez, T., Segura-Fernández-nogueras, M. V., Pérez-Rodríguez, I., Soler-Gongora, M., Martinez-Lopez, A., Fernández-González, A., Molina-Leyva, A., & Arias-Santiago, S. (2021). Skin barrier function in psoriasis and atopic dermatitis: Transepidermal water loss and temperature as useful tools to assess disease severity. *Journal of Clinical Medicine*, *10*(2), 1–12. https://doi.org/10.3390/jcm10020359

Murdiana, H. E., Kristariyanto, Y. A., Kurniawaty, A. Y., Putri, M. K., & Rosita, M. E. (2022). Optimasi Formula Sediaan Krim Beras (Oryza Sativa L.) Tipe M/A Dengan Variasi Asam

Stearat, Setil Alkohol Dan Trietanolamin. *Pharmamedica Journal*, *7*(2), 55–63. https://doi.org/https://doi.org/10.47219/ath.v7i2.161.

Primadina, N., Basori, A., & Perdanakusuma, D. S. (2019). Proses Penyembuhan Luka Ditinjau dari Aspek Mekanisme Seluler dan Molekuler. *Qanun Medika - Medical Journal Faculty of Medicine Muhammadiyah Surabaya*, *3*(1), 31. https://doi.org/https://doi.org/10.30651/jqm.v3i1.2198.

Rahmatillah, B. K., Pelupessy, A. H., Putri, A., Pertiwi, A. E., Majid, A. R., & Rhamadianto, M. I. (2025). Studi in Silico dari Senyawa Turunan Flavonoid pada Bahan Alam sebagai Terapi Antinyeri dan Inflamasi dalam Menghambat COX 2. *JURNAL RISET RUMPUN MATEMATIKA DAN ILMU PENGETAHUAN ALAM*, *4*(2), 142–153. https://doi.org/10.55606/jurrimipa.v4i2.6373

Ricardo, J., Gosch, M., & Wang, Y. (2023). Risk of skin atrophy induced by short-term topical corticosteroid use in atopic dermatitis lesional skin: A systematic review. *JAAD International*, *12*(7), 165–167. https://doi.org/10.1136/bmjopen-2020-046476

Shedoeva, A., Leavesley, D., Upton, Z., & Fan, C. (2019). Wound healing and the use of medicinal plants. In *Evidence-based Complementary and Alternative Medicine* (Vol. 2019). Hindawi Limited. https://doi.org/10.1155/2019/2684108

Shukla, K., Odedra, K. N., Jadeja Scholar, B., & professor, A. (2023). AN EXTENSIVE REVIEW ON THE GENUS PLUMERIA. *TIJER*, (3). https://doi.org/http://doi.one/10.1729/Journal.33428.

Sinaga, E. M., Ambarwati, N. F., Aritonang, B., & Ritonga, A. H. (2022). Making Antiseptic Solid Soap Ethanol Extract Lemon Peel (Citrus Limon (L.) Burm. F.). *Jurnal Multidisiplin Madani (MUDIMA)*, *2*(2), 877–888. https://journal.yp3a.org/index.php/mudima/index

Singh, D., Rai, V., & K Agrawal, D. (2023). Regulation of Collagen I and Collagen III in Tissue Injury and Regeneration. *Cardiology and Cardiovascular Medicine*, *07*(01). https://doi.org/10.26502/fccm.92920302

Suleman, M., Sayaf, A. M., Moltrasio, C., Tricarico, P. M., Giambuzzi, F., Rimondi, E., Melloni, E., Secchiero, P., Marcuzzi, A., Marzano, A. V., & Crovella, S. (2025). Phytocompounds in Precision Dermatology: COX-2 Inhibitors as a Therapeutic Target in Atopic-Prone Skin. *Biomolecules*, *15*(7). https://doi.org/10.3390/biom15070998

Tungadi, R., Sy. Pakaya, M., & D.as'ali, P. W. (2023). Formulasi dan Evaluasi Stabilitas Fisik Sediaan Krim Senyawa Astaxanthin. *Indonesian Journal of Pharmaceutical Education*, *3*(1). https://doi.org/10.37311/ijpe.v3i1.14612

Winarti, L., Gazzali, A. M., Arafika, W. W., Ameliana, L., Ulfa, E. U., Nurahmanto, D., & Paramayuda, F. (2025). Optimization study on palm fat base (HAMIN®) and purified water proportion in diphenhydramine hydrochloride cream formulation. *Pharmaciana*, *15*(2), 264–274. https://doi.org/10.12928/pharmaciana.v15i2.30790

Zhao, Y., Wu, J., Liu, X., Chen, X., & Wang, J. (2024). Decoding nature: multi-target anti-inflammatory mechanisms of natural products in the TLR4/NF-κB pathway. In *Frontiers in Pharmacology* (Vol. 15). Frontiers Media SA. https://doi.org/10.3389/fphar.2024.1467193