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Research Article

Formulation of 70% Ethanol Extract Cream of Ketapang Leaf (*Terminalia catappa* L.) and Efficacy Test for Cut Wound in Mice (*Mus muscullus*)

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ABSTRACT

A laceration (*vulnus scissum*) is characterized by straight, regular wound edges, typically resulting from sharp object trauma. Ketapang leaves (*Terminalia catappa* L.) contain bioactive compounds, including flavonoids, saponins, tannins, sterols, alkaloids, and terpenoids, which possess anti-inflammatory properties and the potential to accelerate wound recovery. This study aimed to evaluate the effectiveness of an extract cream derived from *T. catappa* leaves on laceration healing in mice (*Mus musculus*). The extract was obtained via maceration using 70% ethanol, followed by phytochemical screening. The cream base was optimized to meet pharmaceutical standards, with evaluations encompassing organoleptic properties, pH, dispersibility, adhesion, viscosity, and freeze-thaw stability. Three formulations were developed with varying extract concentrations: F1 (15%), F2 (20%), and F3 (25%). The in vivo efficacy test involved five groups of mice. Results indicated that all three formulations met the criteria for physical stability. In terms of healing duration, the negative control group required 21 days, while the positive control group achieved healing in 10 days. Among the treatment groups, F1 (15%) and F2 (20%) showed healing times of 18 and 16 days, respectively. Notably, F3 (25%) exhibited the highest efficacy with a healing time of 10 days, comparable to the positive control. Statistical analysis using One-Way ANOVA confirmed these findings with a p-value < 0.05.

Keywords: lacerations; *Terminalia catappa* L. leaf extract; cream; *Mus musculus*

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INTRODUCTION

Indonesia boasts a vast diversity of flora, with thousands of plant species serving as potential raw materials for both traditional and modern medicine. Historically, local communities have utilized traditional concoctions to treat various ailments, including the use of ketapang leaves (*Terminalia catappa* L.). This species is well-known for its rich content of bioactive compounds, such as flavonoids, alkaloids, tannins, triterpenoids, steroids, resins, and saponins. These secondary metabolites contribute to a broad spectrum of pharmacological activities, including antioxidant, antibacterial, antifungal, and anti-inflammatory effects, as well as hepatoprotective properties.

Previous studies have highlighted the potential of *T. catappa* L. leaf extract in accelerating tissue regeneration and reducing inflammation. Specifically, the flavonoid and tannin content in 70% ethanol extracts are suspected to play a crucial role in antibacterial activity. Phenolic compounds, such as flavonoids, work by denaturing cellular proteins and damaging bacterial cell walls. Furthermore, research by Nugroho et al. (2017), demonstrated that concentrated ethanol extracts of *T. catappa* L. leaves have a significant anti-inflammatory effect on collagen fibers, which is essential for wound closure in mice (*Mus musculus*).

Despite these findings, research regarding the practical application of *T. catappa* L. extract in wound care remains limited. Most existing studies focus on in vitro assays or basic pharmacological screenings without advancing toward stable topical dosage forms. Moreover, the optimal concentration required for effective wound healing has not been definitively established. This creates a significant research gap in the development of standardized herbal products.

Wounds are defined as disruptions in the protective function of the skin, characterized by a break in the continuity of epithelial tissue due to trauma, such as incisions or lacerations (*vulnus scissum*) (Arifin 2014). As the body's largest organ and primary barrier, the skin is highly vulnerable to injury in daily activities, posing risks of infection and delayed healing (Qomariah, Lisdiana, and Christijanti 2014). Consequently, developing an effective and practical delivery system for wound healing agents is imperative.

In this study, a semi-solid cream preparation of the oil-in-water (O/W) type was developed using a 70% ethanol extract of *T. catappa* L. leaves. Creams are preferred topical delivery systems because they allow for even drug release, are easy to apply, and can be rinsed off with water. Furthermore, O/W creams provide a non-sticky texture and a localized therapeutic effect on dermal tissues (Widodo 2013). Therefore, this research aims to formulate and evaluate the stability of *T. catappa* L. leaf extract cream and test its efficacy in accelerating the healing of lacerations in mice (*Mus musculus*), providing scientific evidence for its use as a safe, practical, and economical alternative therapy.

MATERIALS AND METHODS

Materials

The primary equipment used in this study included an analytical balance, a Brookfield viscometer, a pH meter, a rotary evaporator, a water bath, a 40-mesh sieve, and various laboratory glassware (beakers, glass slides, and stirring rods).

The botanical materials consisted of *T. catappa* L. leaves collected from West Dalapuli Village, North Bolaang Mongondouw, North Sulawesi. Chemical reagents included 70% ethanol, distilled water (aquadest), triethanolamine (TEA), stearic acid, liquid paraffin, adeps lanae, methylparaben, and propylparaben. Betadine cream served as the positive control. The experimental subjects were male mice (*Mus musculus*), aged 6–8 weeks and weighing 20–40 g.

All experimental protocols involving animal subjects were reviewed and approved by the Health Research Ethics Committee of Universitas Negeri Gorontalo (Ethical Approval No: 271/UN47.7/7/KE/2024).

Methods

Preparation of Simplicia

Approximately 2,000 g of fresh green *T. catappa* L. leaves were thoroughly washed under running water and sun-dried. The dried leaves (simplicia) were pulverized using a blender to increase the surface area and sieved through a 40-mesh screen. The resulting powder was stored in a dry, airtight container protected from humidity until further use.

Extraction Process

The extraction was performed via maceration. Briefly, 500 g of *T. catappa* L. leaf powder was submerged in 5 L of 70% ethanol for three days with periodic stirring. The mixture was filtered through a flannel cloth. The filtrate was subsequently concentrated using a rotary evaporator at 50°C. To obtain a standardized thick extract, the residue was further evaporated in a water bath at 50°C until a constant weight was achieved.

Phytochemical Screening

Phytochemical analysis was conducted on the concentrated *T. catappa* L. extract to identify the presence of secondary metabolites, specifically alkaloids, flavonoids, saponins, tannins, and terpenoids, using standard qualitative reagents.

Experimental Animal Selection

The study utilized healthy male mice (*Mus musculus*) selected based on predefined criteria to ensure experimental uniformity. The inclusion criteria comprised male mice aged 6–8 weeks, weighing between 20–40 g, which demonstrated active behavior and showed no physical abnormalities or clinical signs of illness. Conversely, the exclusion criteria focused on mice exhibiting lethargy, pre-existing infections, or those falling outside the specified age and weight ranges. Furthermore, any animals that died or sustained injuries unrelated to the experimental procedure during the course of the study were excluded from the final data analysis.

Cream Formulation and Evaluation

Table 1. Formulations of *Terminalia catappa* L. leaf extract cream with varying concentrations.

Materials	F1 (%)	F2 (%)	F3 (%)
T. catappa L. leaf extract	15	20	25
Triethanolamine	3	3	3
Stearic acid	14.5	14.5	14.5
Propyl paraben	0.02	0.02	0.02
Methyl paraben	0.18	0.18	0.18
Liquid paraffin	15	15	15
Adeps lanae	3	3	3
Aquadest	Ad 100	Ad 100	Ad 100

The 70% ethanol extract of *T. catappa* L. was incorporated into an optimized cream base at three distinct concentrations: F1 (15%), F2 (20%), and F3 (25%). The physical stability and physicochemical characteristics of these formulations were comprehensively evaluated through a series of standardized tests. Organoleptic properties were assessed via visual inspection of color, odor, and consistency, while homogeneity was determined by smearing 0.5 g of the preparation between two glass slides to confirm the absence of coarse particles. To evaluate rheological and application properties, the adhesion (stickiness) test was conducted by pressing 0.5 g of cream between glass slides under a 1 kg load for 5 minutes, subsequently measuring the time required for separation under an 80 g tensile load. Spreadability was quantified by measuring the spreading diameter of 0.5 g of cream between glass plates under incremental loads of 50, 100, and 250 g applied at 60-second intervals. Furthermore, the pH was determined at 25°C using a calibrated digital pH meter after diluting 1 g of the formulation in 9 mL of distilled water, and viscosity was measured using a RION Viscometer VT-04E with spindle No. 2. Finally, the formulations were subjected to a freeze-thaw stability study consisting of six cycles—alternating between 4 ± 2°C and 40 ± 2°C every 24 hours—to monitor for potential phase separation or physical degradation.

Animal Acclimatization and Maintenance

Fifteen male mice (*Mus musculus*) were acclimatized for seven days to laboratory conditions. They were housed in sanitized cages with husk bedding (replaced every two days) and provided with standard pellet feed and distilled water *ad libitum*. Following acclimatization, the mice were observed for 14 days during the treatment period.

Wound Healing Efficacy Test

In this study, the experimental subjects were randomly assigned to five distinct groups, with each group consisting of three mice (n=3). Group 1 served as the negative control, receiving no treatment to establish a baseline for comparison. Group 2 functioned as the positive control and was treated with a standard commercial povidone-iodine (Betadine) cream. The remaining three groups were administered the experimental formulations containing varying concentrations of *T. catappa* L. extract: Group 3 (F1) received the 15% extract cream, Group 4 (F2) was treated with the 20% extract cream, and Group 5 (F3) was given the 25% extract cream.

Wound Induction

The dorsal region was shaved (4 × 2 cm area) and disinfected. A linear incision (laceration) measuring 1.5 cm in length and 0.3 cm in depth was created using a sterile scalpel. The wound was cleaned with 0.9% NaCl.

Treatment and Monitoring

The respective creams were applied topically twice daily for 14 days. Healing progress was evaluated by monitoring scab formation and tissue regeneration. The wound length was measured daily using a digital caliper (0.01 mm accuracy). The percentage of wound closure was calculated to determine the healing rate.

Statistical Analysis

The data, including wound closure time and reduction in wound length, were expressed as Mean ± SD. Data were analyzed for normality and homogeneity using the Shapiro-Wilk and Levene's tests, respectively. Significant differences between groups were determined using One-Way Analysis of Variance (ANOVA) followed by a post-hoc test, with a significance threshold of $p < 0.05$.

RESULTS

Extraction of *Terminalia catappa L.* Leaves

The extraction of *T. catappa* L. leaves was performed by macerating 500 g of the powdered sample in 5000 mL of 70% ethanol solvent. This process yielded 53.6 g of thick extract, corresponding to a yield percentage of 10.7%.

Table 2. Extraction of *T. catappa* L. Leaves

Solvent	Solvent Volume (mL)	Sample (g)	Extract (g)	Yield (%)
70% ethanol	5000	500	53.6	10.7

Phytochemical Screening

The qualitative phytochemical screening of the 70% ethanol extract of *T. catappa* L. leaves revealed the presence of several classes of secondary metabolites. As summarized in Table 3, the extract tested positive for alkaloids, flavonoids, saponins, tannins, and terpenoids.

Table 3. Phytochemical Screening

Phytochemical Testing	Reagent(s)	Observation	Result Description
Alkaloids	Dragendorff's reagent	Formation of a reddish precipitate	Positive (+)
Flavonoids	Magnesium powder and concentrated HCl	Development of a red coloration	Positive (+)
Saponins	Warm water and HCl	Stable foam formation	Positive (+)
Tannins	Ferric chloride ($FeCl_3$)	Green coloration observed	Positive (+)
Terpenoid	Liebermann–Burchard reagent	Color change to red	Positive (+)

Formulation of 70% Ethanol Extract Cream of *Terminalia catappa L.* Leaves

The cream formulations incorporated 70% ethanol extract of *T. catappa* L. leaves at three varying concentrations. These were designated as Formula 1 (F1), containing 15% extract; Formula 2 (F2), containing 20% extract; and Formula 3 (F3), containing 25% extract.

Table 4. Formulation of Ketapang Leaf Ethanol Extract Cream Preparations

Material	Concentrations		
	F1 %	F2 %	F3 %
Terminal catappa L. leaves extract	15	20	25
Triethanolamine	3	3	3
Stearic acid	145	14,5	14,5
Propyl paraben	0,02	0,02	0,02
Methyl paraben	0,18	0,18	0,18
Liquid paraffin	15	15	15
Adeps lanae	3	3	3
Purified water	q.s. ad 100	q.s. ad 100	q.s. ad 100

Physicochemical Evaluation and Stability of *Terminalia catappa* L. Cream Formulations

The evaluation test on the cream preparation was carried out to determine the stability of the cream preparation of 70% ethanol extract of *T. catappa* L. Evaluation tests carried out include organoleptic test, homogeneity test, adhesion test, spreadability test, pH test, viscosity test, and Freeze Thaw test.

Table 5. Organoleptic properties and homogeneity of *T. catappa* cream formulations at room temperature

Formula	Physical Form	Color	Odor	Homogeneity
F1 (15%)	Semi-solid	Brownish	Characteristic	Homogeneous
F2 (20%)	Semi-solid	Brownish	Characteristic	Homogeneous
F3 (25%)	Semi-solid	Brownish	Characteristic	Homogeneous

The organoleptic assessment of the *T. catappa* L. leaf extract creams is summarized in Table 5. All formulations (F1, F2, and F3) exhibited a consistent semi-solid texture and a characteristic herbal odor. A visual color gradient was observed, ranging from light brown to dark brown, correlating with the increasing concentration of the 70% ethanol extract. Furthermore, all preparations demonstrated excellent homogeneity with no detectable coarse particles or phase separation.

Table 6. Adhesion, Spreadability, pH, and Viscosity of *T. catappa* L. extract cream formulations at room temperature

Formula	Adhesion (sec)	Spreadability (cm)	pH Value	Viscosity (cP)
F1 (15%)	06.82	6.5	6.0	4019
F2 (20%)	05.07	6.0	6.0	3089
F3 (25%)	05.70	6.5	6.1	4407

Based on Table 6. the adhesion times ranged from 5.07 to 6.82 s, exceeding the minimum requirement (≥ 4 s), with F1 (15%) showing the highest adhesion, indicating stronger surface interaction at lower extract concentrations. All formulations exhibited acceptable spreadability (6.0–6.5 cm), skin-compatible pH values (6.0–6.1), and viscosities within the acceptable range for topical creams (3089–4407 cP). Notably, F3 (25%) showed the highest viscosity, likely due to increased solid content. Overall, all formulations demonstrated satisfactory physical properties and stability suitable for topical application.

Table 7. Organoleptic properties and homogeneity of *T. catappa* cream formulations after the Freeze-Thaw stability test

Formula	Physical Form	Color	Odor	Homogeneity
F1 (15%)	Semi-solid	Brownish	Characteristic	Homogeneous
F2 (20%)	Semi-solid	Brownish	Characteristic	Homogeneous
F3 (25%)	Semi-solid	Brownish	Characteristic	Homogeneous

The accelerated Freeze-Thaw cycling test was used to further assess the *T. catappa* L. leaf extract creams' physical stability. All three formulations (F1, F2, and F3) retained their structural integrity over the course of the cycles, as shown in Table 7. There were no discernible changes in physical characteristics, color, or odor. Most significantly, there was no indication of phase separation, syneresis, or the development of coarse particles in any of the preparations; they all stayed homogeneous. According to these findings, the cream base is extremely stable and able to shield the *T. catappa* L. extract from severe temperature changes, which is a crucial sign of a robust formulation and extended shelf life.

Table 8. Physicochemical properties of cream formulations after Freeze–Thaw cycles (Mean ± SD, n = 7)

Parameter	F1 (15%)	F2 (20%)	F3 (25%)
Adhesion (s)	6.19 ± 0.15	5.49 ± 0.30	5.63 ± 0.37
Spreadability (cm)	6.07 ± 0.43	6.07 ± 0.41	6.19 ± 0.26
pH value	6.13 ± 0.11	6.16 ± 0.20	6.07 ± 0.09
Viscosity (cP)	3989 ± 463	4199 ± 426	3945 ± 328

The long-term physical and chemical stability of the *T. catappa* L. leaf extract creams was assessed through a seven-cycle freeze–thaw test, with the summarized results presented in Table 8. The adhesion times for all formulations (F1: 6.19 ± 0.15 s; F2: 5.49 ± 0.30 s; F3: 5.63 ± 0.37 s) consistently exceeded the 4-second pharmaceutical requirement. This indicates that despite extreme temperature fluctuations, the creams retained their ability to adhere effectively to the skin, ensuring sustained delivery of the active phytochemicals. Furthermore, the spreadability was exceptionally stable across all concentrations, with values ranging from 6.07 to 6.19 cm. This uniformity suggests that the internal matrix of the cream base successfully maintained its flexibility and ease of application, falling well within the ideal range of 5–7 cm. Similarly, the pH values (6.07 ± 0.09 to 6.16 ± 0.20) remained within the physiologically safe range for human skin (4.5–6.5), confirming that the chemical integrity of the extracts and additives was preserved throughout the thermal stress cycles. The rheological robustness was further confirmed by the viscosity measurements, which ranged 3945 ± 328 cP to 4199 ± 426 cP. Although minor fluctuations occurred—as indicated by the standard deviations—the viscosity remained well within the standard limits (2,000–50,000 cP). The relatively low SD values across all parameters demonstrate that the 70% ethanol extract of *T. catappa* L. can be formulated into a stable cream preparation that resists phase separation and physical degradation.

Effect of Treatments on Wound Diameter Reduction

Table 9. Effect of *T. catappa* L. leaf extract cream on wound diameter reduction in mice (Mean ± SD, n = 3)

Treatment Group	Day 1 (mm)	Day 3 (mm)	Day 6 (mm)	Day 9 (mm)	Day 12 (mm)	Day 14 (mm)
Negative Control (Untreated)	15.00 ± 0.00	14.53 ± 0.06	13.27 ± 0.06	12.03 ± 0.06	9.23 ± 0.06	8.03 ± 0.06
Positive Control (Povidone Iodine)	15.00 ± 0.00	13.20 ± 0.10	9.83 ± 0.06	2.63 ± 0.12	0.03 ± 0.06	0.00 ± 0.00
F1 (15% Extract Cream)	15.00 ± 0.00	13.97 ± 0.06	12.50 ± 0.00	10.13 ± 0.12	7.00 ± 0.00	2.23 ± 0.29
F2 (20% Extract Cream)	15.00 ± 0.00	13.53 ± 0.06	11.83 ± 0.06	7.77 ± 0.06	4.20 ± 0.17	0.07 ± 0.12
F3 (25% Extract Cream)	15.00 ± 0.00	1327 ± 0.06	10.57 ± 0.40	4.57 ± 0.12	0.67 ± 0.06	0.00 ± 0.00

Table 9 shows the results, out of all the extract-treated groups, the F3 (25%) formulation demonstrated the strongest healing effect. By Day 9, F3's wound diameter had dropped to 4.57 ± 0.12 mm, while the negative control group's wound diameter was 12.03 ± 0.06 mm. Complete wound closure (0.00 ± 0.00) was attained by Day 14 with the F3 formulation, demonstrating clinical results that were on par with the positive control (Povidone Iodine).

Statistical Data Analysis Results

Table 10. One-way ANOVA results for wound healing evaluation across observation periods

Observation Period	p-value	Statistical Inference
Day 3	< 0.001	Significant
Day 6	< 0.001	Significant
Day 9	< 0.001	Significant
Day 12	< 0.001	Significant
Day 14	< 0.001	Significant

Significant at $p < 0.05$ level.

Table 11 presents the results of the statistical analysis performed using one-way ANOVA to evaluate differences in wound diameter among the five treatment groups. The analysis revealed statistically significant differences at all observation time points (days 3, 6, 9, 12, and 14), with p-values < 0.05. These findings indicate that the treatments had a significant effect on wound healing progression. Furthermore, the *T. catappa* L. leaf extract cream demonstrated a significant enhancement in wound healing compared to the untreated control group. Among the tested formulations, the 25% extract concentration showed the greatest wound healing effect, as evidenced by the most pronounced reduction in wound diameter over the observation period.

DISCUSSION

Preparation and Extraction of *Terminalia catappa* L. Leaves

The *T. catappa* L. leaf samples were sourced from West Dalapuli Village, North Sulawesi. Initial preparation involved wet sorting and thorough washing under running water to eliminate exogenous impurities. To optimize the drying kinetics, the samples were comminuted into smaller fragments, thereby increasing the surface area-to-volume ratio and facilitating faster moisture evaporation. The drying process was conducted via air-drying in a shaded environment to prevent the degradation of thermolabile bioactive compounds caused by direct ultraviolet (UV) exposure.

Following dehydration, dry sorting was performed to ensure the purity of the simplicia, which was subsequently pulverized into a fine powder to maximize the contact area during extraction. A total of 500 g of the powder was extracted using the maceration method for 3 x 24 hours with periodic agitation. Maceration was specifically selected to avoid thermal degradation of sensitive secondary metabolites, ensuring the chemical integrity of the extract.

70% ethanol was utilized as the meniscus due to its optimal polarity, which allows for the simultaneous extraction of both polar and semi-polar active compounds. Ethanol is a preferred solvent in pharmaceutical research because of its high safety profile (low toxicity) and low boiling point (approx. 78°C), which simplifies the concentration process using a rotary evaporator.

The extraction yielded 50.6 g of thick extract, corresponding to a percent yield of 10.5%. This result complies with the Kementerian Kesehatan RI (2017) standards, which stipulate a minimum yield of 10% for thick extracts. This high yield suggests that the maceration parameters and solvent volume (1:10 ratio) effectively exhausted the plant matrix, ensuring a robust concentration of phytochemicals for the subsequent cream formulation.

Phytochemical Screening of *Terminalia catappa* L. Secondary Metabolites

Phytochemical screening serves as a fundamental preliminary analysis to identify the classes of bioactive compounds within a plant extract, providing a chemical profile that correlates with its pharmacological potential. The qualitative analysis of the 70% ethanol extract of *T. catappa* L. leaves revealed the presence of alkaloids, flavonoids, saponins, tannins, and terpenoids (Table 3).

The detection of alkaloids using Dragendorff's reagent resulted in a reddish-brown precipitate. The addition of HCl during this test was essential to convert the alkaline alkaloids into their salt forms, increasing their solubility for better reaction with the reagent (Harborne 1996). Similarly, flavonoids were identified by a distinct red color change upon the addition of Mg powder and concentrated HCl. This reaction involves the reduction of the benzopyrone nucleus, where the acid facilitates the hydrolysis of flavonoid glycosides into their aglycones (Robinson

1995). Flavonoids are well-documented for their potent antioxidant and anti-inflammatory activities, which are crucial for mitigating oxidative stress in the wound bed.

The presence of saponins was confirmed by the formation of stable foam after vigorous agitation with hot water. This phenomenon occurs due to the amphiphilic nature of saponin molecules, which possess both lipophilic (sapogenin) and hydrophilic (sugar) moieties, significantly reducing the surface tension of the aqueous solution. Furthermore, the tannin test yielded a characteristic blue-green coloration after the addition of $FeCl_3$. This color shift results from the formation of coordination complexes between the ferric ions (Fe^{3+}) and the phenolic hydroxyl groups of the tannins (Harborne 1996). Tannins play a vital role in wound healing through their "astringent" effect, which promotes tissue contraction and protein precipitation, forming a protective layer over the injured area.

Lastly, terpenoids were identified using the Liebermann-Burchard reagent, indicated by a green coloration. This reaction involves the acetylation of the hydroxyl group by acetic anhydride, followed by the oxidation and dehydration of the steroid/terpenoid nucleus by concentrated sulfuric acid to form a conjugated chromophore system (Anam 2015). The synergistic presence of these metabolites—particularly tannins and flavonoids—provides the scientific basis for the extract's efficacy in accelerating the wound healing process observed in the in vivo trials.

Formulation and Preparation of *Terminalia catappa* L. Extract Creams

The development of the *T. catappa* L. cream involved the optimization of an emulsion-based vehicle to ensure physical stability and efficient drug delivery. The base optimization utilized a combination of stearic acid and triethanolamine (TEA) as the primary emulsifying system. When reacted, these components form an anionic soap (triethanolamine stearate), which facilitates the creation of a stable and aesthetically pleasing oil-in-water (O/W) emulsion. This system is particularly advantageous for topical applications as it is easily washable and provides a non-greasy, "shiny" effect upon skin application.

To ensure microbiological stability, a synergistic combination of methylparaben and propylparaben was employed. This binary preservative system offers broad-spectrum antimicrobial and antifungal activity, providing a more robust defense against contamination than single agents.

The lipid phase was composed of Adeps lanae and liquid paraffin. Adeps lanae functions as a potent emollient and hydrophobic agent that, when combined with mineral oils like liquid paraffin, enhances the penetration of bioactive compounds through the stratum corneum (Rowe, Sheskey, and Quinn 2009). Liquid paraffin also serves as a humectant and the primary oil phase, contributing to the cream's spreadability and occlusive properties.

The creams were prepared using the conventional fusion method by separating the formulation into oil and aqueous phases. The oil phase, consisting of stearic acid, adeps lanae, and liquid paraffin, was melted in a water bath at 60–70 °C until a clear and homogeneous mixture was obtained. Separately, the aqueous phase containing triethanolamine, methylparaben, and propylparaben was dissolved in distilled water and heated to the same temperature. The heated aqueous phase was then gradually incorporated into the oil phase in a pre-heated mortar with continuous trituration until a uniform cream base formed during cooling. Finally, the 70% ethanolic extract of *T. catappa* L. was incorporated into the cream base at concentrations of 15% (F1), 20% (F2), and 25% (F3) using the geometric dilution method to ensure homogeneous distribution of the bioactive constituents throughout the formulation.

Physicochemical Evaluation of *Terminalia catappa* L. Extract Creams

Organoleptic Properties and Homogeneity

The organoleptic assessment (Table 5) confirmed that all formulations (F1, F2, and F3) maintained a consistent semi-solid texture, a characteristic herbal odor, and a uniform brownish color throughout the study period. The high degree of homogeneity observed in all preparations suggests that the 70% ethanol extract was successfully integrated into the O/W cream base without phase separation. Maintaining these sensory attributes is crucial for patient compliance and ensures that the bioactive metabolites are distributed evenly within the matrix.

Physicochemical and Rheological Properties

The mechanical properties of the *T. catappa* L. cream formulations (F1–F3) ensure both ease of application and prolonged residence time on the skin. Adhesion values (5–6 s) and spreadability (6.0–6.5 cm) were within the ideal pharmaceutical ranges (≥ 4 s and 5–7 cm, respectively). High adhesion facilitates the continuous absorption of tannins and flavonoids at the wound site, while optimal spreadability allows for uniform coverage with minimal friction, preventing further mechanical trauma to the injured tissue.

Furthermore, the formulations exhibited excellent dermal compatibility with pH values ranging from 6.0 to 6.5. This aligns with human skin physiology (4.5–6.5), ensuring that the cream is non-irritating and safe for repeated application on compromised skin. Rheological analysis using a Brookfield DV-E viscometer (Spindle 5, 50 rpm) confirmed that all creams met the standard viscosity requirements (2,000–50,000 cP). This consistent viscosity indicates a robust emulsion structure that balances physical stability with an efficient active ingredient release rate, ensuring the preparation is thick enough to remain on the wound bed while remaining user-friendly.

Accelerated Stability via Freeze-Thaw Cycling

The physical and chemical stability of the *T. catappa* L. cream formulations were rigorously evaluated through a six-cycle freeze-thaw test (4°C and 40°C for 24 hours each). Post-test organoleptic assessments (Table 8) showed no significant alterations in shape, color, or odor, with all preparations remaining homogeneous and free from phase separation. Furthermore, the pH levels remained stable within the skin-compatible range, indicating that the chemical integrity of the bioactive components was preserved despite thermal stress.

However, fluctuations were observed in the mechanical and rheological parameters. Specifically, the formulations exhibited a slight decrease in viscosity after the final cycle, which was conducted at a high temperature (40°C). This reduction in viscosity is attributed to the increased kinetic energy and distance between particles at elevated temperatures, which weakens the inter-particle cohesive forces.

Consequently, this decrease in viscosity led to a proportional increase in spreadability. A larger spreadability area enhances the contact surface between the cream and the skin, potentially improving the distribution of active substances. Despite these minor rheological shifts, all parameters—including adhesion and spreadability—remained within the established pharmaceutical requirements, confirming that the cream base is robust enough to withstand extreme temperature fluctuations during storage and distribution.

In Vivo Wound Healing Efficacy of *T. catappa* L. Extract Creams

The therapeutic potential of *T. catappa* L. leaf extract creams was evaluated using an excision wound model in rats, focusing on wound contraction rates and the duration of the healing process. Following a one-week acclimatization period, the test animals were divided into five treatment groups. To ensure statistical reliability and minimize experimental error, all analyses were conducted in triplicate (n=3), a standard practice in quantitative pharmacological research to achieve high confidence in the observed results.

The wound induction was performed under standardized surgical conditions. Prior to the procedure, the dorsal region of each animal was shaved and anesthetized with 2% lidocaine to ensure a painless induction. A longitudinal incision of 2.0 cm in length and 0.3 cm in depth was created using a sterile scalpel, extending to the subcutaneous layer without penetrating the underlying muscle tissue. This controlled wounding technique allows for a precise comparison of the regenerative capacities of the different formulations.

Daily topical administration of the respective treatments (Negative Control, Positive Control, and F1–F3) allowed for the observation of the inflammatory and proliferative phases of healing. As noted in the results (Table 10), the extract-treated groups demonstrated a significant acceleration in tissue repair compared

to the untreated negative control. This efficacy is attributed to the presence of high-concentration phytochemicals in the 25% formulation (F3), which successfully mimicked the healing trajectory of the positive control (Povidone Iodine) by promoting faster re-epithelialization and wound closure.

Therapeutic Efficacy of *T. catappa* L. Extract on Wound Healing

In the Negative Control group, wound closure relied solely on natural physiological recovery, which took the longest duration, reaching a diameter of 8.03 ± 0.06 mm by Day 14. Although the body can initiate self-repair through a complex sequence of inflammation, proliferation, and remodeling, the absence of therapeutic intervention leaves the wound vulnerable to microbial infection and prolonged inflammation.

In contrast, the Positive Control (Povidone Iodine) and Formula F3 (25%) groups achieved complete epithelialization (0 mm) by Day 12 and Day 14, respectively. The efficacy of Povidone Iodine is attributed to its broad-spectrum bactericidal activity, which prevents secondary infections, particularly from *Staphylococcus aureus*, thereby streamlining the transition from the inflammatory to the proliferative phase. Remarkably, the high-concentration extract (F3) exhibited a comparable healing trajectory, demonstrating its potent regenerative capacity.

The accelerated wound healing observed in the extract-treated groups (F1, F2, and F3) may be attributed to the synergistic activity of the identified secondary metabolites. Flavonoids act as potent antioxidants and inhibitors of matrix metalloproteinase-1 (MMP-1), thereby preventing premature collagen degradation and promoting the conversion of procollagen into mature collagen fibers (Elliot et al. 2007). In addition, flavonoids enhance vascular endothelial growth factor (VEGF) expression, which plays a crucial role in angiogenesis and nutrient supply to regenerating tissues (Purnama et al. 2018). Tannins contribute to collagen stabilization and exert an astringent effect by precipitating proteins on the wound surface, forming a protective scab that shields granulation tissue from external trauma and microbial invasion (Thomas et al. 2025). Meanwhile, saponins are involved in the early inflammatory phase, functioning as natural antiseptics and stimulating VEGF expression to accelerate the proliferative phase. They also regulate collagen synthesis, thereby minimizing excessive scar formation (Rahmawati 2014).

Macroscopic observations revealed earlier scab formation and detachment in the extract-treated groups, indicating enhanced progression through the normal phases of wound healing. As granulation tissue matured and myofibroblasts initiated wound contraction, the wound edges migrated centripetally, leading to natural scab detachment (Sjamsuhidajat 2017). Among the tested formulations, the 25% extract concentration demonstrated the most pronounced effect, suggesting a concentration-dependent enhancement of fibroblast proliferation and

epithelialization. Overall, these findings support the potential of *T. catappa* L. extract cream, particularly at a 25% concentration, as a promising herbal alternative for promoting tissue regeneration in incision wounds.

Statistical Data Analysis

Quantitative wound diameter reduction data were statistically analyzed using SPSS software to assess the significance of treatment effects. One-way analysis of variance (ANOVA) was applied to evaluate the influence of extract concentration as the independent variable on wound healing rate as the dependent variable across five experimental groups, with a confidence level of 95% ($\alpha = 0.05$). As presented in Table 11, the ANOVA results demonstrated highly significant differences among groups at all observation time points, with p-values < 0.001. To further identify intergroup differences, a Least Significant Difference (LSD) post hoc test was conducted. The analysis revealed that all extract-treated formulations (F1, F2, and F3) differed significantly from the negative control ($p < 0.05$), confirming the pharmacological activity of the *T. catappa* L. leaf extract. Among the tested formulations, F3 (25%) exhibited the highest wound healing efficacy and showed no significant difference ($p > 0.05$) compared with the positive control (povidone iodine). In contrast, F3 differed significantly ($p < 0.05$) from the 15% (F1) and 20% (F2) extract formulations, indicating a concentration-dependent enhancement of wound healing. These findings demonstrate that *T. catappa* L. leaf extract cream produces a consistent, dose-dependent wound healing effect, and the comparable efficacy of the 25% formulation to the commercial positive control highlights its potential as a viable herbal alternative for topical wound therapy.

CONCLUSIONS

The 70% ethanolic extract cream of ketapang leaves (*Terminalia catappa* L.) effectively accelerated the healing of incisional wounds in male mice, as evidenced by a faster reduction in wound length and diameter compared to the untreated control group. Among the tested formulations, the 25% extract concentration exhibited the greatest wound healing efficacy and showed comparable performance to the positive control. These results indicate that *T. catappa* L leaf extract possesses significant wound healing activity and holds promise as an active ingredient for topical wound care formulations.

Nevertheless, this study was limited to preclinical evaluation using an animal model. Therefore, further investigations are required, including comprehensive toxicity assessments, formulation stability studies, and detailed elucidation of the underlying cellular and molecular mechanisms. Ultimately, well-designed clinical trials are necessary to confirm the safety, efficacy, and therapeutic potential of ketapang leaf extract for human topical wound treatment.

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