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Wound Healing Potential of *Citrus Maxima* (Rutaceae) Methanolic Leaf Extract Cream and Ointment Formulation on Excised Cutaneous Wounds in Mice

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Abstract: Wound healing is a crucial aspect of healthcare, particularly for individuals with chronic wounds or those at risk of infection and delayed tissue repair. Conventional treatments, including antiseptics and synthetic medications, can be costly, less accessible in low-resource settings, and may cause side effects. As a result, there is growing interest in plant-based alternatives with traditional medicinal applications. *Citrus maxima* (pomelo), from the Rutaceae family, is traditionally used for various therapeutic purposes. Its leaves contain phytochemicals such as flavonoids, alkaloids, tannins, terpenoids, and phenolic compounds, which exhibit antioxidant, anti-inflammatory, and antimicrobial properties. These attributes may enhance wound healing by promoting tissue regeneration, reducing infection, and controlling inflammation. This study investigates the wound-healing activity of a 50% methanolic leaf extract of *C. maxima* formulated into cream and ointment preparations. A full-thickness excision wound model was used in male albino mice. The test groups were compared to a negative control (petroleum jelly) and a positive control (10% povidone-iodine). Safety of the formulations was evaluated following OECD 402 acute dermal toxicity guidelines. Results showed that the 50% *C. maxima* ointment group achieved the most rapid wound healing, with complete closure observed by Day 14. The Area Under the Curve (AUC) for wound contraction was lowest in this group (97.6 mm²·days), compared to 109.3 mm²·days for the positive control and 187.1 mm²·days for negative control. Statistical analysis using two-way ANOVA and Tukey's post hoc test revealed significant improvement over the negative control and comparable efficacy to the standard treatment. These findings highlight *C. maxima*'s potential as a safe, effective, and accessible natural agent for wound healing.

Keywords: *Citrus Maxima, Pomelo, Wound Healing, Methanolic Extract, Cream, Ointment, Albino Mice*.

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I. INTRODUCTION

Wound healing is a dynamic and complex biological process involving hemostasis, inflammation, proliferation, and remodeling phases, all of which are essential for restoring tissue integrity following injury [5]. Impaired wound healing remains a significant challenge in clinical settings, particularly among individuals with chronic wounds, diabetes, or compromised immunity [3]. These conditions often lead to prolonged inflammation, infection, and tissue damage, increasing the risk of serious complications such as sepsis [6]. Consequently, there is growing interest in plant-derived treatments that offer antimicrobial, antioxidant, and anti-inflammatory benefits to support the healing process.

Citrus maxima (Burm.) Merr., commonly known as pomelo, is a tropical fruit tree widely cultivated in Southeast Asia, including the Philippines. Traditionally used to manage various ailments such as cough, digestive disorders, and inflammation [8], the plant is rich in bioactive compounds, including flavonoids, terpenoids, phenolics, and tannins [9]. These phytoconstituents are known to exhibit wound-healing properties by modulating oxidative stress, reducing inflammation, and promoting tissue regeneration [1,7].

Despite its ethnomedicinal use, there is limited scientific evidence evaluating the wound-healing efficacy of *C. maxima* leaf extracts, especially in topical formulations. This study aims to investigate the wound-healing potential of *C. maxima* methanolic leaf extract formulated as a cream and ointment using an excision wound model in male albino mice.

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The extract's safety is also evaluated through acute dermal toxicity testing.

By integrating traditional knowledge with pharmacological validation, this research contributes to the development of plant-based therapeutic alternatives for wound management. The outcomes may provide foundational evidence supporting the clinical potential of *C. maxima* leaf extract as a natural wound-healing agent.

II. MATERIALS AND METHODS

➢ *Plant Collection and Extraction*

Fresh leaves of *C. maxima* (Rutaceae) were collected and subjected to authentication by a certified botanist, Muhmin Manting. The authenticated plant material was air-dried under shade and pulverized using an electric powder grinder machine. Extraction was performed using the maceration method. The powdered leaves were soaked in 80% methanol for 72 hours at room temperature with occasional stirring. Afterward, the mixture was filtered using Whatman No. 1 filter paper, and the filtrate was concentrated using a rotary evaporator at 40°C. The resulting extract was stored in a clean, amber-colored bottle at 4°C until further use.

➢ *Percentage Analysis*

Citrus maxima leaves (25 g) were dried, ground (60 µm), and macerated in 125 mL methanol for 72 hours at room temperature with 50 rpm agitation [7]. The filtrate was concentrated at 40–50 °C under reduced pressure and stored in amber bottles [5]. A 2.5% yield (0.625 g) was obtained and stored in a cool, dry place to preserve stability [6].

$$\%\ (w/w)\ yield = \frac{weight\ of\ crude\ extract\ (g)}{weight\ of\ dried\ leaf\ sample\ (g)} \times 100$$

➢ *Confirmatory Phytochemical Test*

The methanolic leaf extract of *C. maxima* was subjected to confirmatory tests to detect the presence of secondary metabolites including flavonoids, tannins, alkaloids, terpenoids, and phenolics. These tests followed standard procedures and results were recorded.

➢ *OECD 402: Acute Dermal Toxicity*

Prior to conducting the wound healing study, the methanolic leaf extract of *Citrus maxima* was evaluated for acute dermal toxicity following a modified OECD 402 guideline (2017)[ ]. The test aimed to determine the safety of the extract when applied to the skin. Swiss albino male mice (8–12 weeks old, 20–30 g) were housed individually in ventilated, transparent plastic containers with sterilized rice hull bedding under standardized conditions: 22–24°C temperature, 50% ± 10% humidity, and a 12-hour light/dark cycle. The mice had free access to filtered drinking water and a nutritionally balanced pellet diet. They were acclimatized for at least 7 days before testing.

Each mouse received two full-thickness excisional wounds (6 mm in diameter) on the dorsal region—one on each side of the midline. The left wound received the cream formulation, while the right wound received the ointment, both containing the same concentrations of *C. maxima* extract. Three dose levels (200 mg/kg, 1000 mg/kg, 2000 mg/kg) were tested with three mice per dose group, totaling 12 mice. An Elizabethan collar was used for 24 hours to prevent interference with the application. Mice were monitored daily for 14 days for local and systemic toxicities, scored on a 0–3 scale for parameters such as body weight, dehydration, posture, coat condition, movement, and respiration. This ensured consistent, objective detection of any adverse effects and confirmed the formulations' dermal safety.

➢ *Formulation of Cream and Ointment*

The methanolic extract of *Citrus maxima* (Rutaceae) leaves was formulated into topical cream and ointment preparations to evaluate its efficacy in wound healing. The formulations were prepared at a 50% concentration of the crude extract, chosen based on preliminary studies and aligned with common topical phytopharmaceutical preparations.

- *Cream Formulation*

The cream base was an oil-in-water (O/W) emulsion, offering a non-greasy and cosmetically acceptable form ideal for daily application. The oil phase included stearic acid (6%), cetyl alcohol (2%), and glyceryl stearate (3%) as emulsifiers and emollients. The aqueous phase contained glycerin (5%) as a humectant and purified water as the primary solvent. Triethanolamine was added in minimal quantities to adjust pH and stabilize the emulsion.

To incorporate the extract, 50 g of the methanolic extract was first dissolved and homogenized into the aqueous phase under continuous stirring and mild heating (not exceeding 70°C). The oil phase was separately melted, and the two phases were combined gradually while maintaining constant stirring to achieve uniform consistency. The resulting cream was poured into sterile containers and stored at room temperature.

- *Ointment Formulation*

The ointment was a hydrophobic, occlusive formulation ideal for retaining moisture and extending skin contact time, which is beneficial for wound healing. Petroleum jelly (85%) served as the base, providing a smooth and stable matrix. The 50 g of crude extract was directly incorporated into the base using geometric dilution and trituration until uniform distribution was achieved.

The ointment base was melted at low heat (40–50°C), followed by the gradual addition of the extract with continuous mixing. Once homogeneous, the mixture was allowed to cool to room temperature before transferring into sterile containers.

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Both cream and ointment formulations were prepared in batches of 100 g and subjected to organoleptic evaluation for color, texture, and spreadability. All containers were labeled and stored in a cool, dry place for subsequent wound-healing activity testing in animal models. The formulations remained stable over the short-term storage period with no signs of phase separation, crystallization, or microbial contamination.

➢ *Animal Grouping and Wound Induction*

Twenty-four healthy male Swiss albino mice (20–30 g; 6–8 weeks old) were obtained from the AMCC Animal House and randomly divided into six groups (n=4 per group). The treatment groups were as follows: (1) negative control – petroleum jelly; (2) positive control – 10% povidone-iodine; (3) 50% *C. maxima* cream; (4) 50% *C. maxima* ointment; (5) 10% povidone-iodine cream; and (6) 10% povidone-iodine ointment. Under light anesthesia, a full-thickness excision wound (1 cm in diameter) was created on the dorsal thoracic region of each mouse. Treatments were topically applied once daily for 14 consecutive days. Wound contraction was monitored on Days 0, 3, 7, 10, and 14 to assess the healing progress across all groups.

➢ *Wound Area Measurement*

Wound area was measured using a non-digital vernier caliper on Days 0, 3, 7, and 14. Full-thickness excision wounds were created using a 6-mm biopsy punch. The length and width of each wound were measured in millimeters to estimate the surface area. The measurements were taken by gently placing the caliper across the longest and shortest diameters of the wound without causing further trauma. This method allowed consistent tracking of wound size at each time point.

➢ *Statistical Analysis*

Wound area measurements were statistically analyzed using two-way Analysis of Variance (ANOVA) to assess the effects of treatment and time, followed by Tukey's post hoc test to determine significant differences among groups. Wound healing rates were compared using the Area Under the Curve (AUC) method, which represented cumulative wound reduction over the 14-day study period. Results were expressed as Mean ± Standard Error of the Mean (SEM), and a p-value of less than 0.05 was considered statistically significant.

III. RESULTS AND DISCUSSIONS

➢ *Percentage Yield*

To determine the Percentage yield of the extract, triplicate analysis was performed using three 25-gram samples of powdered *C. maxima* (Rutaceae) leaves. Each sample underwent methanolic maceration and subsequent solvent evaporation. The evaporating dish (ED) was weighed before and after the extraction process to measure the amount of crude extract obtained. The percentage yield for each trial was calculated accordingly. Table 10 below presents the individual yields obtained from each trial. The triplicate analysis yielded an average percentage yield of 3.27 ± 0.42% for the methanolic leaf extract of *C. maxima*.

Table 1 Percentage Yield of the Methanolic leaf Extract of *C. Maxima* (Rutaceae)

TRIAL	WEIGHT OF DRIED LEAVES	WEIGHT OF CRUDE EXTRACT	% YIELD
1	25 g	0.69 g	2.76%
2	25 g	0.87 g	3.48%
3	25 g	0.89 g	3.56%
		MEAN VALUE	**3.27%**

➢ *Phytochemical Confirmation test of C. maxima (Rutaceae)*

Phytochemical confirmation of *C. maxima* methanolic leaf extract revealed the presence of flavonoids, alkaloids, tannins, phenolics, and terpenoids. These secondary metabolites are associated with various pharmacological activities that support wound healing. Flavonoids, in particular, exhibit antioxidant and anti-inflammatory properties that enhance tissue regeneration, while tannins promote wound contraction and form a protective layer over the wound surface. The results confirmed the therapeutic potential of the extract in wound healing applications.

Table 2 Phytochemical Confirmation Test of the Methanolic leaf Extract of *C. maxima* (Rutaceae)

Plant Constituents	Tests	Visible Result		Interpretation
Alkaloids	Wagner's Test		Brown Coloration	Positive (++)
Flavonoids	Shinod's Test		Red Coloration	Positive (+++)

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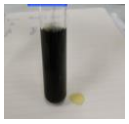
Phenols	Ferric Chloride Test		Blue-green Coloration	Positive (+++)
Hydrolysable Tannins	Ferric chloride Test		Blue-black coloration	Positive (+++)
Terpenoids	Liebermann-Burchard Test		Green coloration	Positive (+)

➢ *Acute Dermal Toxicity*

The acute dermal toxicity of the test substance was assessed using the fixed dose procedure at 50, 200, 1000, and 2000 mg/kg. Dosing began at 50 mg/kg and increased stepwise, with each group observed over a 14-day period for signs of toxicity. No behavioral changes, toxicity signs, or mortality were observed at 50, 200, and 1000 mg/kg. At 2000 mg/kg, mild to moderate dermal irritation, such as erythema at the application site, and some mortality were noted. No systemic toxicity was observed in surviving mice. Except for the effects noted at 2000 mg/kg, there were no significant physical or behavioral abnormalities recorded. Overall, the substance exhibited low dermal toxicity at doses up to 1000 mg/kg.

➢ *Wound Healing Evaluation*

The excision wound model showed that topical application of *C. maxima* methanolic extract significantly accelerated wound healing in male albino mice. The 50% ointment formulation showed the most effective healing, with complete wound closure observed by day 14. Compared to the negative control (petroleum jelly), both the cream and ointment extract formulations reduced the wound area substantially. Area Under the Curve (AUC) analysis further confirmed that the 50% *C. maxima* ointment (AUC: 97.6 mm²·days) exhibited superior wound contraction compared to the positive control (10% povidone-iodine, AUC: 109.3 mm²·days) and the negative control (AUC: 187.1 mm²·days). These findings support the extract's efficiency in promoting tissue regeneration.

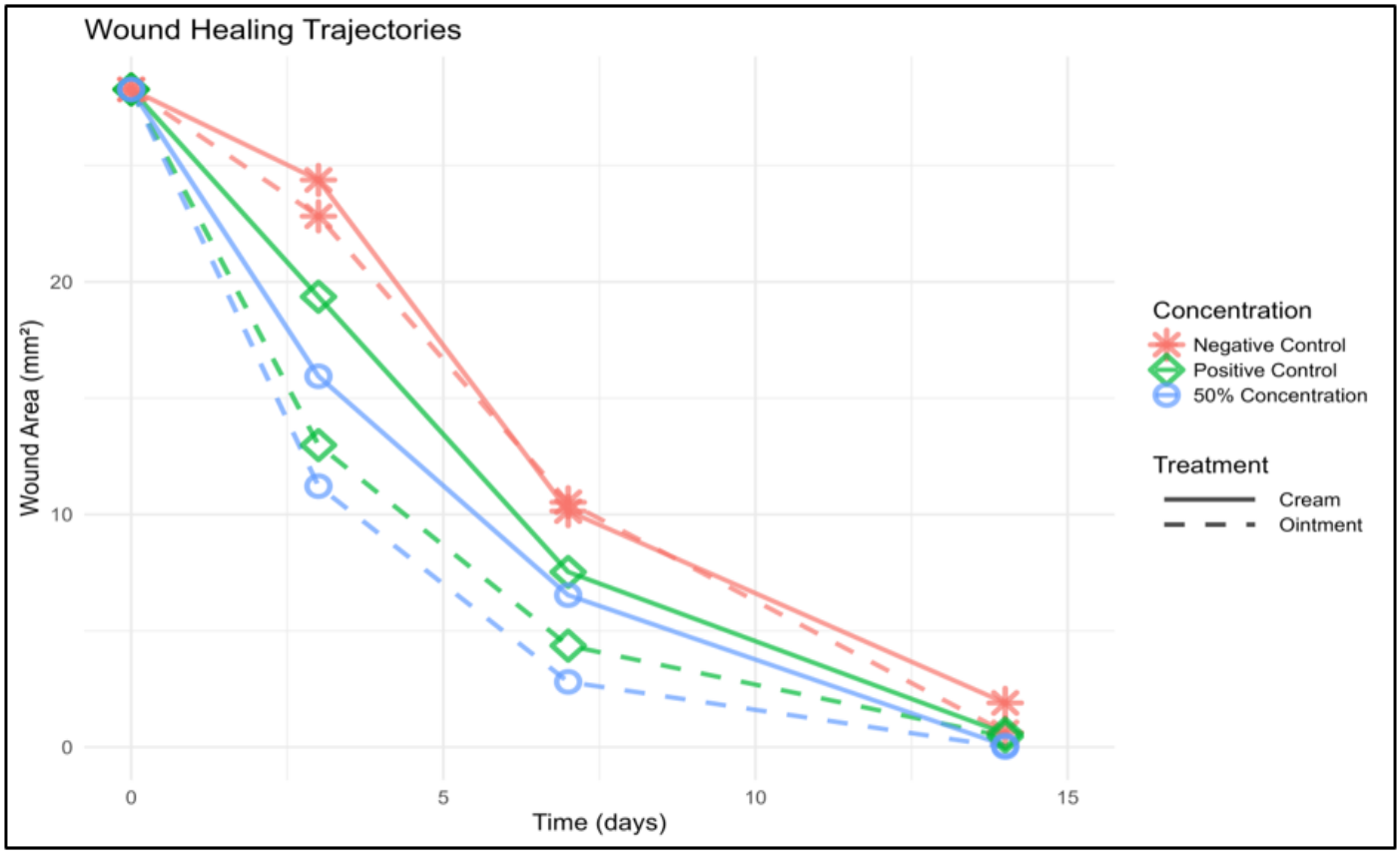


Fig 1 AUC analysis showing wound area reduction during healing.

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➢ *Statistical Analysis of Treatment Effects*

Two-Way ANOVA revealed that treatment type, concentration, and time had statistically significant effects on wound healing ($p < 0.05$). Tukey-adjusted pairwise comparisons showed that the 50% *C. maxima* ointment differed significantly from the negative control ($p < 0.001$) and was comparable to the positive control ($p > 0.05$). This statistical result confirms the extract's potential as a natural wound healing agent.

Table 3 Statistical Comparison of Wound Closure Across Treatment Groups

Treatment	Contrast	Diff	SE	df	t-ratio	p	Remark
Cream	Negative Control – Positive Control	2.238	1.006	30	2.224	0.083	Not significant
	Negative Control – 50% Concentration	3.470	1.006	30	3.448	0.005	Significant
	Positive Control – 50% Concentration	1.232	1.006	30	1.224	0.448	Not significant
Ointment	Negative Control – Positive Control	4.045	1.006	30	4.020	0.001	Significant
	Negative Control – 50% Concentration	4.989	1.006	30	4.957	< .001	Significant
	Positive Control – 50% Concentration	0.943	1.006	30	0.937	0.621	Not significant

Note: A significance level of $p < 0.05$ was used. Exact *p*-values are reported. Smaller *p*-values indicate stronger statistical significance.

IV. DISCUSSION

The results demonstrated that the methanolic leaf extract of *Citrus maxima* (Rutaceae) significantly enhanced wound healing activity, particularly in ointment formulation. This improved effect can be attributed to the presence of bioactive phytochemicals such as flavonoids, tannins, phenolics, alkaloids, and terpenoids, which were confirmed through phytochemical screening. These compounds are known for their anti-inflammatory, antimicrobial, and antioxidant properties, which collectively contribute to the different phases of wound repair. In particular, flavonoids and tannins play important roles in stimulating fibroblast proliferation, promoting angiogenesis, enhancing collagen synthesis, and accelerating epithelialization.

The ointment formulation outperformed the cream and control treatments, which may be due to the occlusive nature of ointments. This formulation provides prolonged skin contact and moisture retention, preventing desiccation and enhancing the local absorption of the extract. In contrast, the petroleum jelly base used in the negative control group served only as a protective barrier without active healing properties.

The study's findings are in agreement with previous research on Citrus species, which have demonstrated wound-healing potential due to similar phytochemical profiles. The results also support the ethnopharmacological use of *C. maxima* in traditional medicine for treating cuts, wounds, and skin ailments. Thus, the topical application of *C. maxima* extract, especially in ointment form, offers a promising, natural, and accessible alternative for wound care.

V. CONCLUSIONS

This study demonstrated that the 50% methanolic leaf extract of *Citrus maxima* (Rutaceae) exhibits significant wound-healing activity in an excision wound model. Phytochemical screening confirmed the presence of flavonoids, alkaloids, tannins, terpenoids, and phenolic compounds, which contribute to wound repair through antioxidant, anti-inflammatory, and antimicrobial mechanisms. Acute dermal toxicity assessment indicated that the extract is safe for topical application at doses up to 1000 mg/kg.

Topical formulations of the extract, prepared in cream and ointment bases, markedly enhanced wound contraction over a 14-day period. The ointment formulation achieved complete wound closure, while the cream reached 99.75% healing. Both showed statistically significant improvement compared to the negative control and comparable efficacy to the positive control (povidone-iodine). Area Under the Curve (AUC) analysis further supported the accelerated and sustained healing effect of the extract-based formulations.

These findings validate the traditional use of *C. maxima* leaves for wound treatment and support the use of its 50% methanolic extract, particularly in ointment form, as a safe, effective, and affordable natural alternative for dermal wound management.

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