

Evaluation of the Wound-Healing Activity of Hydroalcoholic Extracts of the Seeds and Leaves of *Mirabilis jalapa* L. (Nyctaginaceae) on Experimental Burns in Rabbits

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ABSTRACT

Introduction

Mirabilis jalapa L. is a medicinal plant traditionally used in wound treatment across various global regions. While its wound-healing potential has primarily been documented using leaf extracts, the therapeutic efficacy of seed extracts on burn wound models remains uninvestigated.

Purpose

This study aimed to evaluate and compare the burn wound-healing properties of hydroalcoholic extracts derived from *M. jalapa* leaves and seeds in rabbits.

Methods

Leaf and seed powders were macerated in 70% v/v ethanol. The resulting dry extracts were formulated into petrolatum-based ointments at 10% w/w concentration. Four standardized thermal burn wounds (2 cm diameter) were induced on the dorsum of eight adult rabbits (*Oryctolagus cuniculus*, 1.5–2.5 kg), allowing each animal to serve as its own internal control across four concurrent treatment sites: Group A (leaf extract ointment, 10%), Group B (seed extract ointment, 10%), Group C (negative control: white petrolatum jelly), and Group D (positive control: 1% silver sulfadiazine; Flammazine®). Topical applications were performed daily for 14 days.

Results

Extraction yields were 20.10% for leaves and 14.70% for seeds. Qualitative phytochemical screening revealed the presence of alkaloids, coumarins, flavonoids, phenolic compounds, polysaccharides, saponins, steroids, and tannins in both extracts, with terpenoids present exclusively in the leaves. By Day 14, complete wound closure (100.00% ± 0.00%) was achieved in Groups A, B, and D, demonstrating regular, smooth re-epithelialized tissue and dense hair regrowth. In contrast, the negative control group (Group C) achieved a significantly lower wound contraction rate (83.38% ± 0.15%, $p < 0.001$), with persistent eschar formation. One-way ANOVA and Tukey's HSD post-hoc analysis confirmed that both leaf and seed extracts significantly accelerated wound contraction relative to the negative control ($p < 0.05$).

Conclusion

Hydroalcoholic extracts of *M. jalapa* leaves and seeds accelerate wound contraction and tissue repair in experimental burns, validating the plant's traditional ethnomedicinal use and demonstrating its potential as an effective natural wound-healing agent.

INTRODUCTION

Wound healing is a dynamic, complex physiological process requiring orchestrated events across four overlapping phases: hemostasis, inflammation, proliferation, and tissue remodeling (Eming et al., 2014). Thermal burn injuries severely disrupt these physiological processes, causing extensive tissue necrosis, vascular damage, persistent oxidative stress, and increased susceptibility to systemic infections (Nielson et al., 2017; Wang et al., 2018). Burn injuries represent a major global health challenge, disproportionately affecting populations in low- and middle-income countries where access to specialized burn care facilities is limited (World Health Organization [WHO], 2023).

Standard medical management of burn wounds relies heavily on topical antimicrobial agents such as silver sulfadiazine (1% cream). However, prolonged application of synthetic topical agents can lead to adverse clinical outcomes, including delayed re-epithelialization, cutaneous toxicity, emergence of resistant pathogens, and high financial cost (Di Domenico et al., 2020). Consequently, there is growing scientific interest in discovering novel, accessible, phytotherapeutic alternatives with high tissue-regenerative and antimicrobial capabilities (WHO, n.d.).

Mirabilis jalapa L. (Nyctaginaceae), commonly known as the four o'clock flower, is an ethnomedicinally important plant widely used in tropical and subtropical regions to treat cutaneous lesions, abscesses, and inflammation (Liya et al., 2021; Saha et al., 2020). Previous pharmacological evaluations have investigated the wound-healing activities of its leaves and roots. Puspasari and Saputri (2018) reported that aqueous leaf extracts promoted excision wound closure in rat models, while Gogoi et al. (2014) demonstrated that terpenoid-rich fractions of *M. jalapa* tuberous roots facilitated wound healing in rats. Furthermore, recent *in vitro* studies highlighted significant antioxidant and biological activities in seed extracts (Atrooz et al., 2024).

Despite these findings, comparative evaluations examining the therapeutic efficacy of *M. jalapa* seed extracts against leaf extracts on thermal burn models remain lacking. Therefore, this study evaluated and

compared the *in vivo* burn wound-healing potential of hydroalcoholic leaf and seed extracts of *M. jalapa* in a standardized rabbit model, providing scientific validation for its traditional therapeutic application.

METHODS

Plant Collection and Identification

Fresh leaves and mature seeds of *M. jalapa* were harvested in March 2025 and July 2025, respectively, in Kinshasa, Democratic Republic of the Congo (coordinates: 5°S 23' 26.8" S, 15°E 18' 56.9" E). Botanical verification and authentication were performed by comparison with voucher specimen P04973971 deposited at the Herbarium of the National Museum of Natural History in Paris (MNHN, n.d.).

Figure 1:
Fresh leaves (A) and mature seeds (B) of *Mirabilis jalapa* L.



Animal Care and Ethics Statement

Eight adult European rabbits (*Oryctolagus cuniculus*, Leporidae), comprising male and female specimens weighing between 1.5 and 2.5 kg, were obtained from the animal facility of the Faculty of Sciences and Technologies, University of Kinshasa. Animals were housed individually in standard ventilated polypropylene cages under controlled environmental conditions (25 ± 2°C, 12-h light/dark cycle) with *ad libitum* access to standard laboratory chow and fresh water. All experimental procedures received formal ethical approval from the Ethics Committee of the School of Public Health, University of Kinshasa (Approval No. ESP/CE/41B/2026).

Preparation of Hydroalcoholic Extracts

Harvested leaves and seeds were shade-dried at room temperature (25--28°C) for 14 days and ground into coarse powders using an electric mill (Müller & Heindl, 2006). Powder samples (200 g each) were subjected to static maceration in 2000 mL of hydroalcoholic solvent (ethanol/water, 70:30 v/v) for 72 hours at ambient temperature with continuous mechanical stirring at 400 rpm (Azwanida, 2015; WHO, 2011).

Extract suspensions were filtered through a Büchner funnel fitted with Whatman No. 2 filter paper. The filtrates were concentrated under reduced pressure using a rotary evaporator and subsequently dried in a convection oven at 40°C. Yield percentages were calculated using Equation 1 (Azwanida, 2015):

Yield (%) = [Mass of dry extract (g) ÷ Mass of initial dry plant powder (g)] × 100

Qualitative Phytochemical Screening

Qualitative chemical profiling was performed on the crude dry extracts to identify secondary metabolite classes using standard colorimetric and precipitation protocols described by Evans and Evans (2009).

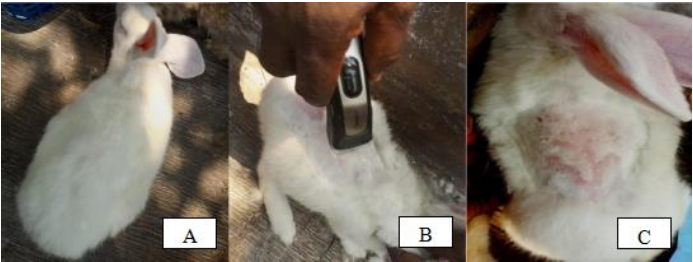
Formulation of Topical Ointments

Topical ointments (10% w/w) were prepared under aseptic conditions. Briefly, 6.5 g of dried leaf or seed extract was incorporated into 58.5 g of white petrolatum (Vaseline®) previously melted at 40°C. The mixture was stirred continuously with a stainless-steel spatula until a uniform, homogeneous ointment was obtained (Zuikina et al., 2018). The 10% w/w concentration was selected based on optimization studies by Kasmadi et al. (2021), which established superior wound contraction rates at 10% compared to 5% and 7.5% formulations.

Induction of Experimental Burn Wounds

Standardized thermal burn injuries were created using modified methods described by Walker and Mason (1968) and Galiano et al. (2004). Twenty-four hours prior to burn induction, the thoracolumbar dorsal region of each rabbit was closely shaved using an electric clipper, finished with a safety razor, and disinfected with 70% ethanol.

Figure 2: Dorsal preparation of rabbit prior to (A), during (B), and after (C) shaving



General anesthesia was induced via intramuscular injection of ketamine (50 mg/kg), combined with local subcutaneous ring block infiltration of lidocaine (4 mg/kg) around the operative field (Table 1).

Table 1: Anesthetic Regimen Applied During Burn Wound Induction

Anesthetic Agent	Concentration	Route of Administration	Administered Dose
Ketamine	50 mg/mL	Intramuscular	50 mg/kg
Lidocaine	20 mg/mL	Circular subcutaneous infiltration	4 mg/kg

Immobilized animals were placed in prone position. Four thermal burn wounds were induced on the shaved dorsal skin of each rabbit using a solid aluminum cylinder (2 cm diameter, 100 g) preheated in boiling water (100°C) for 5 minutes. The heated metallic stamp was applied perpendicularly to the skin surface for 20 seconds under its own weight without additional manual pressure, creating 32 thermal wounds across 8 rabbits (Anis et al., 2022).

Figure 3: Dorsal view of a rabbit showing four standardized circular burn wounds 24 hours post-induction (Day 1)

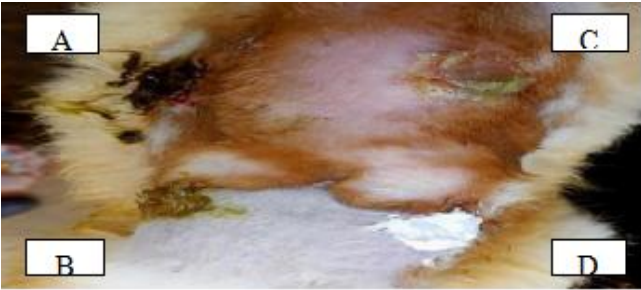


The experimental design utilized an intra-individual control model: four treatment protocols were applied simultaneously to the four distinct burn sites on each animal (Table 2; Figure 4). Daily topical treatments (0.5 g) were administered continuously for 14 days.

Table 2: Distribution of Experimental Treatment Groups across Burn Wounds (n = 32)

Group	Treatment Protocol	Applied Product	Assigned Sites (n)
A	10% Leaf Extract Ointment	Hydroalcoholic leaf extract in white petrolatum	8
B	10% Seed Extract Ointment	Hydroalcoholic seed extract in white petrolatum	8
C	Negative Control	Plain white petrolatum jelly (Vaseline®)	8
D	Positive Control	1% Silver sulfadiazine cream (Flammazine®)	8

Figure 4:
Daily application of treatment formulations across dorsal wound sites (A, B, C, D)



Evaluation of Wound Healing and Morphometric Parameters

Horizontal (D_h) and vertical (D_v) wound diameters were recorded every 2 days using a digital caliper calibrated in millimeters. Surface areas (S) were calculated using the elliptical surface formula (Equation 2; Galiano et al., 2004):

$$CR (\%) = [(S_i - S_t) / S_i] \times 100$$

where S_i is the initial wound surface area at Day 1, and S_t is the wound surface area at observation Day t .

Qualitative parameters including eschar formation, tissue granulation, margin erythema, hair re-growth, and animal body weight changes were documented throughout the 14-day study period.

Statistical Analysis

Data normality was confirmed using the Shapiro-Wilk test. Differences in wound surface area and contraction rates across treatment groups and temporal intervals were analyzed using one-way Analysis of Variance (ANOVA). Post-hoc pairwise comparisons were conducted using Tukey’s Honestly Significant Difference (HSD) test. Quantitative data are presented as Mean $\pm$ Standard Deviation (SD). Statistical significance was thresholded at $p < 0.05$. Data analyses were executed using RStudio (version 3.6.3; R Foundation for Statistical Computing) and Microsoft Excel 2013.

Table 5:
Baseline Wound Surface Area (cm^2) at Day 1 Across Animals ($n = 8$)

Treatment Site	Rabbit 1	Rabbit 2	Rabbit 3	Rabbit 4	Rabbit 5	Rabbit 6	Rabbit 7	Rabbit 8	Mean $\pm$ SD
A (Leaves)	3.140	3.297	2.983	3.297	3.140	3.140	3.297	2.983	3.160 $\pm$ 0.131
B (Seeds)	3.140	3.140	2.983	3.140	3.297	3.140	3.140	3.297	3.160 $\pm$ 0.101
C (Control)	3.297	3.140	3.297	2.983	2.983	3.140	2.983	3.297	3.140 $\pm$ 0.145
D (Flammazine®)	3.140	3.297	3.140	3.140	3.140	2.983	3.140	3.297	3.160 $\pm$ 0.101

Macroscopic Observation of Wound Healing

- i. Days 1–6: Mild perilesional edema was observed in all groups. Thick, dark orange-brown crusts/eschars covered the wound beds.
- ii. Days 6–8: Eschars remained firmly adherent across all groups. Group A displayed hyperemic, pinkish marginal re-epithelialization rings.

RESULTS

Extraction Yields

Maceration of 200 g powdered plant material yielded 40.20 g (20.10%) of leaf extract and 29.50 g (14.75%) of seed extract (Table 3).

Table 3:
Extraction Yields of *Mirabilis jalapa* Leaves and Seeds

Plant Part	Powder Mass (g)	Extract Dry Mass (g)	Extraction Yield (%)
Leaves	200.00	40.20	20.10
Seeds	200.00	29.50	14.75

Phytochemical Composition

Qualitative phytochemical screening confirmed the presence of alkaloids, coumarins, flavonoids, phenolic compounds, polysaccharides, saponins, steroids, and tannins in both leaf and seed extracts. Terpenoids were detected exclusively in leaf extracts (Table 4).

Table 4:
Phytochemical Constituents of *Mirabilis jalapa* Hydroalcoholic Extracts

Secondary Metabolite Class	Leaf Extract	Seed Extract
Alkaloids	+	+
Coumarins	+	+
Flavonoids	+	+
Phenolic compounds	+	+
Polysaccharides	+	+
Quinones	-	-
Saponins	+	+
Steroids	+	+
Tannins	+	+
Terpenoids	+	-

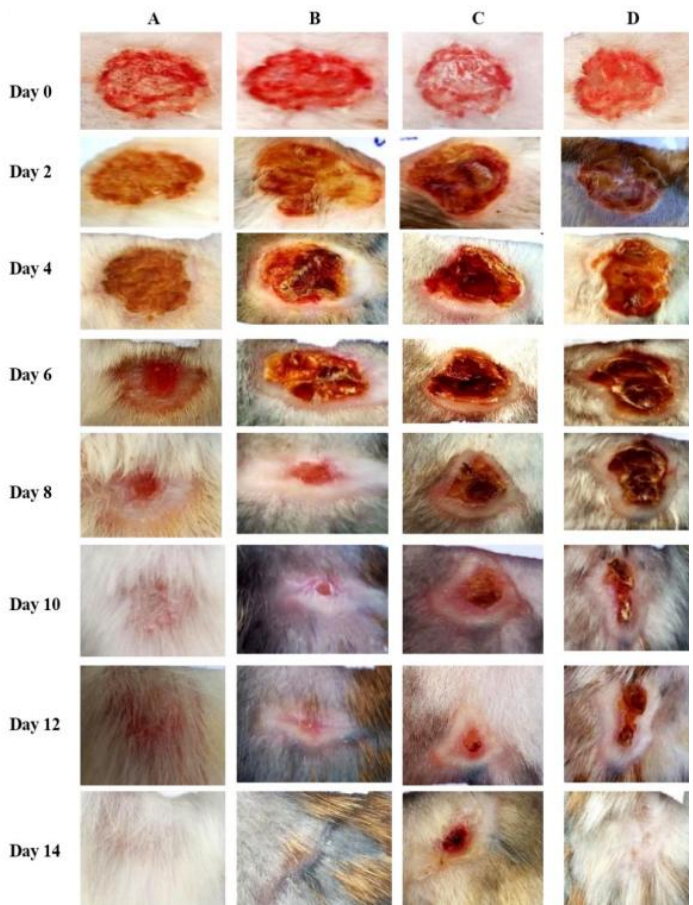
Note. (+) indicates presence; (-) indicates absence.

Baseline Burn Surface Assessment

On Day 1 post-burn induction, open necrotic lesions were formed across all treatment sites. The mean baseline surface area across all 32 wounds was $3.155 \pm 0.115 \text{ cm}^2$ (Table 5). The calculated coefficient of variation (CV) was 3.65% ($CV = [SD/Mean] \times 100$), confirming uniformity and reproducibility of burn induction.

- iii. **Days 8-10:** Partial eschar detachment occurred in Groups A, B, and D, revealing vibrant pink granulation tissue. Group D exhibited localized central contraction points. In contrast, Group C wounds maintained partially attached brownish-yellow crusts with persistent marginal inflammation.
- iv. **Day 12:** Complete re-epithelialization and closure were observed in Group A (100% contraction).
- v. **Day 14:** Full macroscopic closure, smooth epidermal restoration, and prominent hair regrowth were achieved in Groups A, B, and D. Group C maintained persistent central crusts and incomplete epidermal continuity (Figure 5).

Figure 5: Serial macroscopic appearance of burn wounds across experimental groups from Day 0 to Day 14.



Quantitative Wound Area Reduction

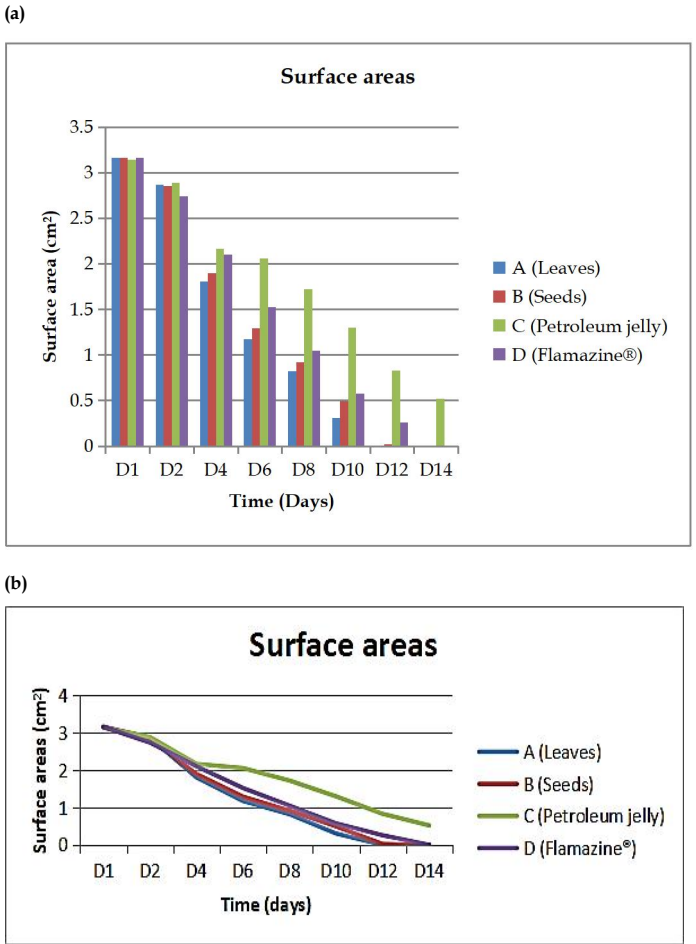
The mean wound surface area decreased progressively across all groups over time (Table 6). Group A (leaves)

demonstrated the fastest surface area reduction, reaching 0.000 cm² on Day 12. Group B (seeds) and Group D (Flamazine®) achieved complete closure by Day 14, whereas Group C retained a mean unhealed wound area of 0.522 ± 0.149 cm².

Table 6: Temporal Changes in Mean Wound Surface Area (cm²) Across Experimental Groups

Timeline	Group A (Leaves)	Group B (Seeds)	Group C (Negative Control)	Group D (Flamazine®)
Day 1	3.160 ± 0.131	3.160 ± 0.101	3.140 ± 0.145	3.160 ± 0.101
Day 2	2.871 ± 0.105	2.852 ± 0.125	2.888 ± 0.079	2.742 ± 0.109
Day 4	1.809 ± 0.088	1.900 ± 0.123	2.168 ± 0.133	2.104 ± 0.058
Day 6	1.177 ± 0.051	1.291 ± 0.140	2.058 ± 0.117	1.523 ± 0.070
Day 8	0.824 ± 0.042	0.919 ± 0.088	1.724 ± 0.151	1.050 ± 0.102
Day 10	0.307 ± 0.052	0.494 ± 0.079	1.300 ± 0.181	0.575 ± 0.043
Day 12	0.000 ± 0.000	0.024 ± 0.021	0.833 ± 0.125	0.258 ± 0.035
Day 14	0.000 ± 0.000	0.000 ± 0.000	0.522 ± 0.149	0.000 ± 0.000

Figure 6 (a) and (b): Progressive reduction in wound surface area (cm²) over 14 experimental days

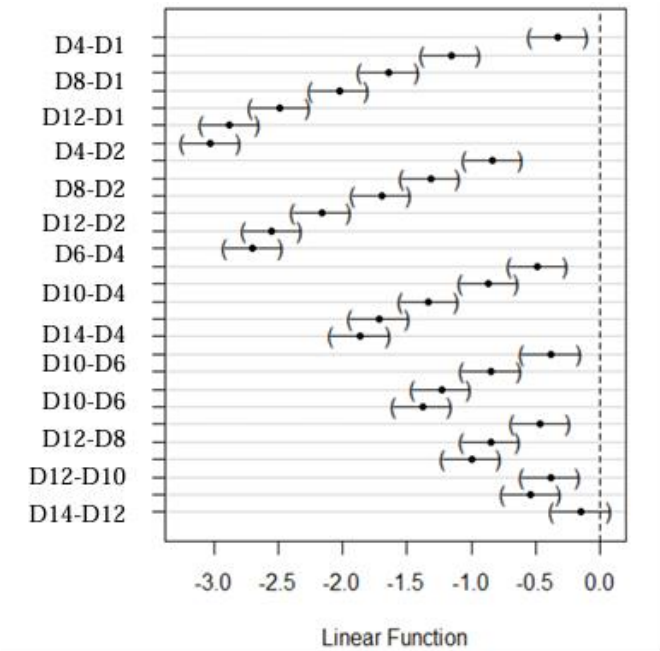


One-way ANOVA demonstrated significant time-dependent ($F = 500.6$, $p < 0.001$; [Table 7](#)) and treatment-dependent ($F = 3.451$, $p = 0.0172$; [Table 8](#)) differences in wound surface reduction. Tukey's HSD post-hoc test confirmed that wound closure was statistically equivalent between Day 12 and Day 14 ($p = 0.43$; [Figure 7](#)), while both plant extract formulations showed significant efficacy compared to petrolatum jelly ($p < 0.05$; [Figure 8](#)).

Table 7:
One-Way ANOVA Summary for Wound Surface Area Reduction Across Days

Source of Variation	Degrees of Freedom (df)	Sum of Squares (SS)	Mean Square (MS)	F-statistic	p-value
Day	7	285.96	40.85	500.60	< 0.001
Residuals	248	20.24	0.08	—	—

Figure 7:
Multiple comparisons of mean differences in wound area across post-burn days (95% family-wise confidence level)



Evaluation of Wound Contraction Rates

By Day 14, Groups A, B, and D achieved $100.00\% \pm 0.00\%$ wound contraction. In contrast, Group C achieved $83.38\% \pm 0.15\%$ contraction ([Table 9](#); [Figure 9](#)).

Table 9:
Wound Contraction Rates (%) Across Treatment Groups Over 14 Days

Timeline	Group A (Leaves)	Group B (Seeds)	Group C (Negative Control)	Group D (Flammazine®)
Day 1	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Day 2	9.130 ± 0.088	10.901 ± 0.125	8.656 ± 0.079	12.640 ± 0.109
Day 4	42.733 ± 0.088	39.845 ± 0.123	30.969 ± 0.133	33.416 ± 0.058
Day 6	62.733 ± 0.051	59.130 ± 0.140	34.468 ± 0.117	51.801 ± 0.070
Day 8	73.913 ± 0.042	70.901 ± 0.088	45.094 ± 0.151	66.770 ± 0.102
Day 10	90.279 ± 0.052	84.348 ± 0.079	59.000 ± 0.181	81.801 ± 0.043
Day 12	100.000 ± 0.000	99.193 ± 0.021	73.468 ± 0.125	91.832 ± 0.035
Day 14	100.000 ± 0.000	100.000 ± 0.000	83.375 ± 0.149	100.000 ± 0.000

Table 8:
One-Way ANOVA Summary for Wound Area Reduction Across Treatment Groups

Source of Variation	Degrees of Freedom (df)	Sum of Squares (SS)	Mean Square (MS)	F-statistic	p-value
Treatment	3	12.08	4.028	3.451	0.0172
Residuals	252	294.11	1.167	—	—

Figure 8:
Multiple comparisons of mean differences in wound area across treatment groups (95% family-wise confidence level)

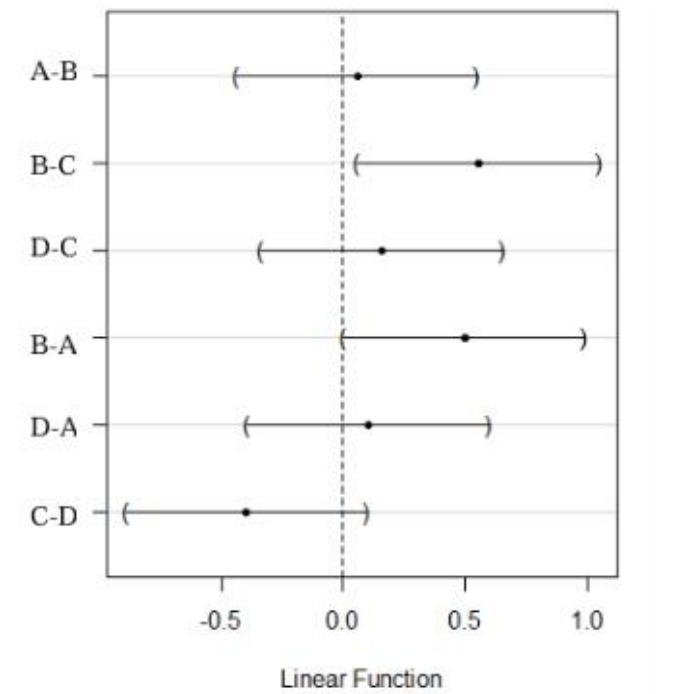


Figure 9:
Percentage wound contraction curves across treatment groups over 14 days

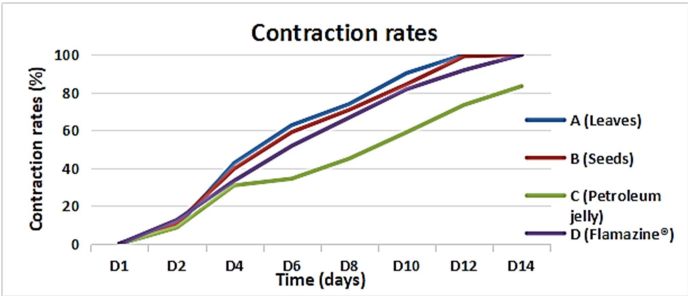
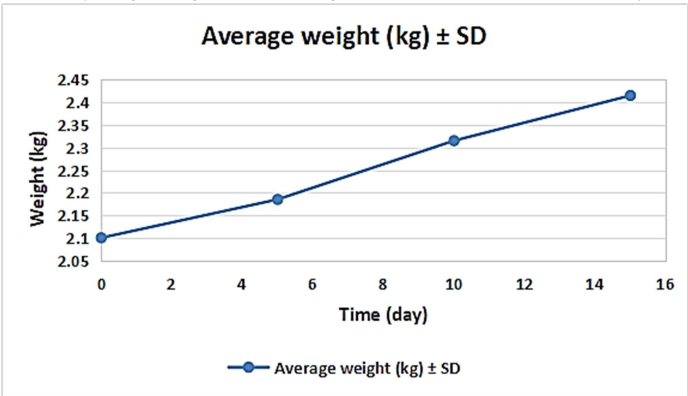


Table 10:
Body Weight Progression (kg) of Experimental Rabbits (n = 8)

Timeline	Rabbit 1	Rabbit 2	Rabbit 3	Rabbit 4	Rabbit 5	Rabbit 6	Rabbit 7	Rabbit 8	Mean ± SD
Day 0	1.995	2.175	1.874	2.013	2.286	1.967	2.126	2.364	2.100 ± 0.170
Day 5	2.172	2.245	1.953	2.092	2.355	2.037	2.196	2.428	2.185 ± 0.160
Day 10	2.340	2.395	2.110	2.205	2.476	2.164	2.311	2.519	2.315 ± 0.140
Day 15	2.406	2.474	2.187	2.333	2.593	2.287	2.437	2.603	2.415 ± 0.140

Figure 10.:
Mean body weight progression (kg) of experimental rabbits over 15 days



DISCUSSION

Hydroalcoholic extraction yielded a higher mass fraction from leaves (20.10%) than from seeds (14.75%), reflecting a greater density of polar secondary metabolites in leaf tissues. The 70% ethanol mixture effectively extracts both polar and moderately polar bioactive components, including polyphenols, flavonoids, and saponins (Azwanida, 2015; Evans & Evans, 2009).

Phytochemical profiling revealed rich pools of secondary metabolites in both extracts. Flavonoids and phenolic compounds attenuate local inflammatory responses, scavenge reactive oxygen species (ROS), and promote capillary angiogenesis and fibroblast migration (Rani et al., 2016). Saponins promote keratinocyte proliferation and extracellular matrix accumulation (Lee et al., 2020), while tannins function as astringents, precipitating tissue

General Animal Health and Body Weight Progression

No systemic toxicity, behavioral alterations, anorexia, or mortality was observed in any animal. All rabbits gained weight steadily throughout the study period, increasing from a mean weight of 2.100 ± 0.17 kg on Day 0 to 2.415 ± 0.14 kg on Day 15 (Table 10; Figure 10).

proteins to construct a protective barrier that reduces exudate loss and prevents bacterial colonization (Scalbert, 1991). Polysaccharides enhance fibroblast cell division and collagen cross-linking (Chithra et al., 1998). Terpenoids, identified exclusively in leaf extracts, possess potent anti-inflammatory and antioxidant activities that enhance dermal tissue repair (Barreto et al., 2014; Scherer et al., 2019). The presence of terpenoids in leaf extracts may account for the accelerated wound closure observed in Group A relative to Group B. These phytochemical findings align with reports by Al-Snafi et al. (2021) and Li et al. (2009).

Experimental burn induction demonstrated high technical precision, as evidenced by baseline surface areas (3.155 ± 0.115 cm²) closely approximating the theoretical 3.14 cm² stamp area, with a coefficient of variation below 5% (CV = 3.65%) (Galiano et al., 2004; Zar, 2010).

During early inflammatory phases (Days 1–6), edema and thick eschars formed across all wounds. By Day 6, Group A achieved 62.73% contraction (1.177 cm²) and Group B achieved 59.13% contraction (1.291 cm²), outperforming both Flammazine® (51.80%, 1.523 cm²) and petrolatum jelly (34.47%, 2.058 cm²). Rapid attenuation of early inflammation by phytoconstituents appears to accelerate transition into the proliferative phase (Singh et al., 2010). Petrolatum jelly serves primarily as an occlusive barrier, lacking intrinsic regenerative activity (Winter, 1962). While silver sulfadiazine acts as an effective topical

antimicrobial, it can exert transient cytotoxic effects on regenerating keratinocytes and fibroblasts, slightly delaying early contraction (Di Domenico et al., 2020).

During the proliferative and remodeling phases (Days 6–14), granulation tissue formation and re-epithelialization progressed rapidly in extract-treated groups (Cañedo-Dorantes & Cañedo-Ayala, 2019; Spielman et al., 2023). Group A reached total wound closure by Day 12, whereas Groups B and D reached full closure on Day 14. Wounds treated with *M. jalapa* formulations regenerated smooth, pliable skin with restoration of dense hair follicles, indicating functional dermal repair supported by hair follicle stem cell activation (Ito et al., 2005). Conversely, Group C retained unhealed ulcerations (0.522 cm²) with persistent central eschars on Day 14.

Topical administration of 10% w/w leaf and seed ointments was well tolerated locally and systemically. Continued body weight gain across all subjects confirmed the safety of these formulations under the tested conditions. Overall macroscopic therapeutic efficacy followed the hierarchy: Leaves > Seeds ≈ Flammazine® > Petrolatum Jelly.

CONCLUSION

This study demonstrates that topical hydroalcoholic extracts from both leaves and seeds of *Mirabilis jalapa* L. significantly accelerate wound contraction and tissue restoration in experimental thermal burns. Leaf extract formulations promoted complete wound closure by Day 12, whereas seed extracts and silver sulfadiazine achieved full healing by Day 14. These findings validate the ethnomedicinal use of *M. jalapa* for wound care and highlight the potential of seed extracts as effective therapeutic agents. Future investigations should focus on histopathological quantification, biochemical evaluation of collagen and inflammatory cytokines, dose-response optimization, and bio-guided isolation of active constituent compounds.

Strengths and Limitations

Study Strengths

- i. First comparative evaluation of *Mirabilis jalapa* seed extract against leaf extract in a burn wound model.

- ii. Rigorous intra-individual control design, reducing inter-animal physiological variability.
- iii. Standardized formulation dosing (10% w/w) and standardized thermal burn induction (CV < 5%).
- iv. Continuous longitudinal evaluation of morphometric and clinical parameters.

Study Limitations

- a. Lack of histological analysis (e.g., H&E, Masson's trichrome) and biochemical assays (e.g., hydroxyproline, hydroxyproline-to-collagen ratio, inflammatory cytokine profiling).
- b. Single dose concentration (10% w/w), precluding evaluation of dose-response relationships.
- c. Lack of long-term stability testing for topical ointment formulations.

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Authors' Contributions

- i. Conceptualization & Study Design: JNL, DSL
- ii. Experimental Execution: DSL, MBM, RMN, AMK
- iii. Data Analysis & Visualization: DSL, JNL, MMN
- iv. Manuscript Draft & Editing: DSL, JNL, MMN, GMB, NRN
- v. Supervision & Project Administration: JNL, MMN

Data Availability Statement: The data that support the findings of this study are available from the corresponding author, [LJN], upon reasonable request.

Ethical Approval: Experimental protocol approved by the Ethics Committee of the School of Public Health, University of Kinshasa (Approval No. ESP/CE/41B/2026).

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REFERENCES

- Al-Snafi, A. E., Talab, T. A., Jabbar, W. M., & Alqahtani, A. M.** (2021). Chemical constituents and pharmacological activities of *Mirabilis jalapa*: A review. *International Journal of Biological and Pharmaceutical Sciences Archive*, 1(2), 34–45. <https://doi.org/10.30574/ijbpsa.2021.1.2.0303>
- Anis, A., Sharshar, A., El Hanbally, S., & Shehata, A. A.** (2022). Histopathological evaluation of the healing process of standardized skin burns in rabbits: Assessment of a natural product with honey and essential oils. *Journal of Clinical Medicine*, 11(21), Article 6417. <https://doi.org/10.3390/jcm11216411>
- Atrooz, O. M., Alhmoud, J. F., Farah, H. S., Al-Tarawneh, F. M., & Sohemat, A. A.** (2024). In vitro assessment of biological and cytotoxic activity of methanol seed extract of Jordanian *Mirabilis jalapa* L. *Tropical Journal of Natural Product Research*, 8(2), 6087–6092. <https://doi.org/10.26538/tjnpr/v8i2.5>
- Azwanida, N. N.** (2015). A review on the extraction methods use in medicinal plants, principle, strength and limitation. *Medicinal & Aromatic Plants*, 4(3), Article 1000196. <https://doi.org/10.4172/2167-0412.1000196>
- Barreto, R. S. S., Albuquerque-Júnior, R. L. C., Araújo, A. A. S., Almeida, J. R. G. S., Santos, M. R. V., Barreto, A. S., DeSantana, J. M., Siqueira-Lima, P. S., Quintans, J. S. S., & Quintans-Júnior, L. J.** (2014). A systematic review of the wound-healing effects of monoterpenes and iridoid derivatives. *Molecules*, 19(1), 846–862. <https://doi.org/10.3390/molecules19010846>
- Cañedo-Dorantes, L., & Cañedo-Ayala, M.** (2019). Skin acute wound healing: A comprehensive review. *International Journal of Inflammation*, 2019, Article 3706315. <https://doi.org/10.1155/2019/3706315>
- Chithra, P., Sajithlal, G. B., & Chandrakasan, G.** (1998). Influence of *Aloe vera* on collagen characteristics in healing dermal wounds in rats. *Molecular and Cellular Biochemistry*, 181(1–2), 71–76. <https://doi.org/10.1023/a:1006813510959>
- Di Domenico, E. G., De Angelis, B., Cavallo, I., Sivori, F., Orlandi, F., Morais D’Autilio, M. F. L., Trento, E., & Toma, L.** (2020). Silver sulfadiazine eradicates antibiotic-tolerant *Staphylococcus aureus* and *Pseudomonas aeruginosa* biofilms in patients with infected diabetic foot ulcers. *Journal of Clinical Medicine*, 9(12), Article 3807. <https://doi.org/10.3390/jcm9123807>
- Eming, S. A., Martin, P., & Tomic-Canic, M.** (2014). Wound repair and regeneration: Mechanisms, signaling, and translation. *Science Translational Medicine*, 6(265), Article 265sr6. <https://doi.org/10.1126/scitranslmed.3009337>
- Evans, W. C., & Evans, D.** (2009). General methods associated with the phytochemical investigation of herbal products. In *Trease and Evans’ pharmacognosy* (16th ed., pp. 135–147). W.B. Saunders. <https://doi.org/10.1016/B978-0-7020-2933-2.00017-4>
- Galiano, R. D., Michaels, J., Dobryansky, M., Levine, J. P., & Gurtner, G. C.** (2004). Quantitative and reproducible murine model of excisional wound healing. *Wound Repair and Regeneration*, 12(4), 485–492. <https://doi.org/10.1111/j.1067-1927.2004.12404.x>
- Gogoi, J., Nakhuru, K. S., Chattopadhyay, P., Rai, A. K., & Veer, V.** (2014). Hypertrophic scar formation on application of terpenoid fraction of tuberous root of *Mirabilis jalapa* L. on excision wound model in Wistar albino rats. *International Scholarly Research Notices*, 2014, Article 583730. <https://doi.org/10.1155/2014/583730>
- Ito, M., Liu, Y., Yang, Z., Nguyen, J., Liang, F., Morris, R. J., & Cotsarelis, G.** (2005). Stem cells in the hair follicle bulge contribute to wound repair but not to homeostasis of the epidermis. *Nature Medicine*, 11(12), 1351–1354. <https://doi.org/10.1038/nm1328>
- Kasmadi, F. S., Prasetya, G., Ramadhani, N., Aji, N. P., & Samudra, A. G.** (2021). Uji efektivitas ekstrak daun bunga pukul empat (*Mirabilis jalapa* L.) sebagai obat luka bakar pada kelinci putih jantan. *Jurnal Ilmiah Pharmacy*, 8(1), 10–18. <https://doi.org/10.52161/jiphar.v8i1.202>
- Lee, Y., Choi, H. K., N’deh, K. P. U., Choi, Y.-J., Fan, M., Kim, E.-K., Chung, K.-H., & An, J. H.** (2020). Inhibitory effect of *Centella asiatica* extract on DNCB-induced atopic dermatitis in HaCaT cells and BALB/c mice. *Nutrients*, 12(2), Article 411. <https://doi.org/10.3390/nu12020411>
- Li, L.-Z., Tuerxunayi, & Abdulla, A.** (2009). Preliminary analysis of effective components in flowers and leaves of *Mirabilis jalapa*. *Food Science*, 30(4), 233–235. <https://doi.org/10.7506/spkx1002-6630-200904051>
- Liya, F. I., Yasmin, M. F., Chowdhury, N. S., Charu, T. K., & Fatema, I. B.** (2021). *Mirabilis jalapa*: A review of ethno and pharmacological activities. *Advancement in Medicinal Plant Research*, 9(1), 1–10.
- Martin, P., & Nunan, R.** (2015). Cellular and molecular mechanisms of repair in acute and chronic wound healing. *British Journal of Dermatology*, 173(2), 370–378. <https://doi.org/10.1111/bjd.13954>

- Müller, J., & Heindl, A. (2006). Drying of medicinal plants. In R. J. Bogers, L. E. Craker, & D. Lange (Eds.), *Medicinal and aromatic plants* (pp. 237–252). Springer. https://doi.org/10.1007/1-4020-5449-1_17
- Muséum national d'Histoire naturelle (MNHN). (n.d.). Specimen P04973971, Herbar P (Paris). Retrieved October 5, 2026, from <https://science.mnhn.fr/institution/mnhn/collectio n/p/item/p04973971>
- Nielson, C. B., Duethman, N. C., Howard, J. M., Moncure, M., & Wood, J. G. (2017). Burns: Pathophysiology of systemic complications and current management. *Journal of Burn Care & Research*, 38(1), e469–e481. <https://doi.org/10.1097/BCR.0000000000000355>
- Porter, C., Herndon, D. N., Børsheim, E., Chao, T., Reidy, P. T., Borack, M. S., Rasmussen, B. B., Chondronikola, M., Saraf, M. K., & Sidossis, L. S. (2016). The metabolic stress response to burn trauma: Current understanding and therapies. *The Lancet*, 388(10052), 1417–1426. [https://doi.org/10.1016/S0140-6736\(16\)31469-6](https://doi.org/10.1016/S0140-6736(16)31469-6)
- Puspasari, P., & Saputri, F. C. (2018). Effect of the water extract of the four o'clock herb (*Mirabilis jalapa* L.) on the healing of open wounds in rats. *International Journal of Applied Pharmaceutics*, 10(Special Issue 1), 155–158. <https://doi.org/10.22159/ijap.2018.v10s1.33>
- Rani, S., Amanjot, G., Surya, P. G., Kanwar, K., & Kaur, S. (2016). Wound healing potential of medicinal plants with their screening models: A comprehensive review. *Journal of Drug Delivery and Therapeutics*, 6(1), 56–66. <https://doi.org/10.22270/jddt.v6i1.1184>
- Rowan, M. P., Cancio, L. C., Elster, E. A., & Burmeister, D. M. (2015). Burn wound healing and treatment. *Critical Care*, 19, Article 243. <https://doi.org/10.1186/s13054-015-0961-2>
- Saha, S., Deb, J., & Deb, N. K. (2020). Review on *Mirabilis jalapa* L. (Nyctaginaceae): A medicinal plant. *International Journal of Herbal Medicine*, 8(2), 14–18.
- Scalbert, A. (1991). Antimicrobial properties of tannins. *Phytochemistry*, 30(12), 3875–3883. [https://doi.org/10.1016/0031-9422\(91\)83426-L](https://doi.org/10.1016/0031-9422(91)83426-L)
- Scherer, M. M. C., Marques, F. M., Figueira, M. M., Peisino, M. C. O., Schmitt, E. F. P., Kondratyuk, T. P., Endringer, D. C., Scherer, R., & Fronza, M. (2019). Wound healing activity of terpinolene and α -phellandrene by attenuating inflammation and oxidative stress in vitro. *Journal of Tissue Viability*, 28(2), 94–99. <https://doi.org/10.1016/j.jtv.2019.02.003>
- Singh, M., Kumar, V., Singh, I., Gauttam, V., & Kalia, A. N. (2010). Anti-inflammatory activity of aqueous extract of *Mirabilis jalapa* Linn. leaves. *Pharmacognosy Research*, 2(6), 364–367. <https://doi.org/10.4103/0974-8490.75456>
- Spielman, A. F., Griffin, M. F., Parker, J., Cotterell, A. C., Wan, D. C., & Longaker, M. T. (2023). Beyond the scar: A basic science review of wound remodeling. *Advances in Wound Care*, 12(2), 57–67. <https://doi.org/10.1089/wound.2022.00499>
- Walker, H. L., & Mason, A. D. (2000). A standard animal burn. *Journal of Trauma*, 8(6), 1049–1051. <https://doi.org/10.1097/00005373-196811000-00006>
- Wang, Y., Beekman, J., Hew, J., Jackson, S., Issler-Fisher, A. C., Parungao, R., Lajevardi, S. S., Li, Z., & Maitz, P. K. M. (2018). Burn injury: Challenges and advances in burn wound healing, infection, pain and scarring. *Advanced Drug Delivery Reviews*, 123, 3–17. <https://doi.org/10.1016/j.addr.2017.09.018>
- Winter, G. D. (1962). Formation of the scab and the rate of epithelization of superficial wounds in the skin of the young domestic pig. *Nature*, 193(4812), 293–294. <https://doi.org/10.1038/193293a0>
- World Health Organization. (2011). *Quality control methods for medicinal plant materials*. World Health Organization. <https://iris.who.int/handle/10665/44479>
- World Health Organization. (2023, October 13). Burns. <https://www.who.int/news-room/fact-sheets/detail/burns>
- World Health Organization. (n.d.). *Traditional, complementary and integrative medicine*. Retrieved October 5, 2026, from <https://www.who.int/health-topics/traditional-complementary-and-integrative-medicine>
- Zar, J. H. (2010). *Biostatistical analysis* (5th ed.). Prentice Hall.
- Zuikina, E., Polovko, N., Silayva, L., & Kovalova, T. (2018). Biopharmaceutical choice justification of basis for dermatological ointment. *Ukrainian Biopharmaceutical Journal*, 2018(3), 190–195. <https://doi.org/10.24959/ubphj.18.190>