SCIENTIFIC CULTURE

www.sci-cult.com

10.5281/zenodo.122120261012

*SCIENTIFIC CULTURE, Vol. 12, No. 2.1, (2026), pp. 13450-13456*

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# Efficacy of Karo Traditional Oil as a Traditional Herbal Medicine from Indonesia in Accelerating Acute Wound Healing Significantly through EGF Stimulation

**Nova Primadina[1], Achmad Bashori[2], Mangestuti Agil[3], David S Perdanakusuma[4]**

*[1]Medical Faculty Lecturer of Muhammadiyah Surabaya University*

*[2]Pharmacology Lecturer of Pharmacology Department of Airlangga University Surabaya*

*[3]Pharmacy lecturer of Pharmacy Faculty Airlangga University Surabaya*

*[4]Plastic Surgery Department of Airlangga University - dr.Soetomo Hospital Surabaya – Indonesia*

***Received: 01/03/2026***
***Accepted: 26/04/2026***

*Corresponding author:*

## ABSTRACT

***Introduction/Objective:*** *Traditional herbal medicines have long been used for wound care, yet scientific validation remains limited. This study aimed to evaluate the wound healing efficacy of Karo Traditional Oil, an Indonesian herbal formulation, and its effect on epidermal growth factor (EGF) production in a murine acute wound model.*

***Methods:*** *Thirty-six male mice were randomly allocated into six groups (n=6 per group): negative controls (moist wound therapy), positive controls (carrier oil), and treatment groups (Karo Traditional Oil). A 2.5 cm full-thickness excisional wound was created on the dorsal skin. Treatments were applied topically daily. Wound closure was measured using Visitract and ImageJ software on days 3 and 7. Re-epithelialization thickness was assessed histologically, and EGF levels were quantified on days 1, 3, and 7. Data were analysed using one-way ANOVA with post-hoc tests.*

***Results:*** *The Karo Traditional Oil group demonstrated significantly faster wound closure on day 3 (59.20 ± 29.03%) and day 7 (81.05 ± 21.96%) compared to negative and positive controls ($p=0.001$ and $p=0.002$, respectively). Re-epithelialization thickness was also markedly increased on day 3 (0.67 ± 0.79 cm) and day 7 (2.05 ± 0.94 cm) ($p=0.001$ and $p=0.019$). EGF levels were significantly elevated in the treatment group on day 3 (4.33 ± 1.93) and day 7 (5.83 ± 2.51) ($p=0.004$ and $p=0.016$).*

***Conclusion:*** *Karo Traditional Oil significantly accelerates acute wound healing by enhancing re-epithelialization and stimulating EGF production. These findings provide scientific support for the traditional use of Karo Traditional Oil and suggest its potential as a natural wound-healing agent. Further mechanistic and clinical studies are recommended*

***KEYWORDS:*** *Karo Traditional Oil, wound healing, re-epithelialization, epidermal growth factor, traditional herbal medicine, Indonesian Herbal Medicine*

## INTRODUCTION

Wound healing represents a highly orchestrated physiological response aimed at restoring the structural and functional integrity of damaged skin. This multifaceted process encompasses four overlapping phases—hemostasis, inflammation, proliferation, and tissue remodelling—driven by complex interactions among cells, extracellular matrix components, and signalling molecules. Across diverse cultures, traditional herbal medicines have served as accessible and culturally resonant options for wound management for centuries. Indonesia, with its exceptional biodiversity and deep ethnomedicinal heritage, hosts numerous indigenous formulations still widely used in community-based care. Among these, Karo Traditional Oil, derived from traditional knowledge of the Karo ethnic community in North Sumatra, has been empirically applied for wound treatment. Nevertheless, despite its longstanding use, comprehensive scientific evidence supporting its therapeutic efficacy and molecular mechanisms remains limited (1).

The burden of wounds constitutes a major global health and economic challenge. Recent data indicate that the global wound care market was valued at approximately USD 22–25 billion in 2025, with projections reaching USD 35–44 billion by 2033–2035. In the United States, chronic wounds affect about 10.5 million Medicare beneficiaries, incurring annual costs of roughly USD 22.5 billion for Medicare alone, while worldwide expenditure reached USD 148.65 billion in 2022. Rising rates of diabetes, obesity, aging populations, and antimicrobial resistance have further increased the prevalence of both acute and hard-to-heal wounds. Although acute wounds are generally expected to resolve efficiently, many transition into chronic states due to infection, poor vascular supply, or suboptimal care, resulting in prolonged healing times and higher complication rates (2).

Failure to address impaired wound healing leads to substantial clinical, economic, and psychosocial repercussions, including extended hospital stays, recurrent infections, sepsis, potential limb amputation, and diminished quality of life. These issues disproportionately burden healthcare systems in low- and middle-income countries such as Indonesia, where advanced wound therapies may be inaccessible or unaffordable. From a biological perspective, delayed healing is frequently associated with disrupted signaling of critical growth factors. Epidermal Growth Factor (EGF), in particular, is essential for re-epithelialization, as it promotes keratinocyte proliferation, migration, and differentiation via the EGFR pathway and downstream cascades such as MAPK/ERK. Insufficient EGF activity impairs these regenerative processes, contributing to stalled wound closure. Herbal agents capable of modulating inflammation and naturally upregulating endogenous EGF therefore represent a valuable link between traditional knowledge and contemporary molecular wound biology (3) .

The present study holds significant importance by providing preclinical evidence of the wound-healing efficacy of Karo Traditional Oil in an acute excisional wound model, specifically by stimulating EGF production. By rigorously validating this traditional Indonesian herbal medicine, the research not only substantiates longstanding ethnomedicinal practices but also enriches the field of ethnopharmacology. Such efforts are vital to harness natural resources, develop affordable therapeutic alternatives, and address unmet needs in wound management across varied clinical and socioeconomic contexts (4).

## MATERIALS AND METHOD

### 2.1. Animal Model and Experimental Design

This study utilised a randomised post-test-only control group design. Fifty-four male Wistar rats (150 ± 30 g) were obtained from the Integrated Research and Testing Laboratory, Universitas Gadjah Mada, Yogyakarta. The animals were acclimatised for at least two weeks in the animal facility maintained at 23°C, housed individually with ad libitum access to food pellets and water, and randomly assigned to nine experimental groups.

### 2.2. Anaesthesia and Wound Creation

Rats were anaesthetised via intramuscular injection of 0.3 ml/100 g body weight of a cocktail containing ketamine (2 ml), xylazine (1.25 ml), acepromazine (0.33 ml), and NaCl (6.41 ml). A standardised 2.5 × 2.5 cm full-thickness square excisional wound was created on the dorsal thoracic region of each rat. The negative control group received moist NaCl gauze dressings, the positive control group was treated with gauze impregnated with the carrier oil of Karo Traditional Oil, and the treatment group received gauze impregnated with Karo Traditional Oil. Observations were conducted on days 1, 3, and 7 post-wounding. All procedures were performed by the same researcher to minimise inter-operator variability.

### 2.3. Wound Area Measurement

Digital photographs of the wounds were taken immediately after creation and on days 3 and 7 using a Canon DSLR M50 camera with standardised settings, distance, and animal positioning. Wound areas were analysed using ImageJ software. The percentage of re-epithelialization was calculated using the formula:

% Re-epithelialization = 100 – [(wound area on day 3 or 7 / initial wound area on day 1) × 100].

### 2.4. Histological and Immunohistochemical Evaluation

On day 7, all animals were euthanised humanely. Wound tissues, including surrounding margins, were excised, fixed in 10% buffered formalin for 24 hours, processed through graded ethanol dehydration, cleared in xylene, and embedded in paraffin. Sections were stained with hematoxylin and eosin (H&E) and examined under light microscopy (×40 to ×100

magnification) by two blinded pathologists. Epidermal Growth Factor (EGF) expression was assessed via immunohistochemical staining.

### 2.5. Statistical Analysis

Data were expressed as mean ± standard deviation (SD). Normality and homogeneity were tested before analysis. One-way ANOVA followed by post-hoc multiple comparisons was performed using SPSS version 23. Statistical significance was considered at $p < 0.05$.

## RESULT

**Table 1**. Wound size (%) analysis of the studied group in the third and seventh days of the study

| Groups | Third day<br>Mean ± SD | Seventh day<br>Mean ± SD |
|---|---|---|
| Negative Control Group | -11.61 ± 24.29 [a] | 12,67 ± 16,57 [a] |
| Positive Control Group | 8.05 ± 8.08 [a] | 25,41 ± 20,07 [a] |
| Treatment Group | 59.20 ± 29.03 [b] | 81,05 ± 21,96 [b] |
| p value | 0,001 * | 0,002 * |

Data are given as percentages, * statistically significant difference between a and b

SD = standart deviation

The present study demonstrated that topical application of Karo Traditional Oil significantly accelerated acute wound healing in a full-thickness excisional wound model in male Wistar rats. Wound closure, re-epithelialization thickness, and epidermal growth factor (EGF) levels were markedly improved in the treatment group compared to both negative and positive controls.

As shown in Table 1, on day 3 post-wounding, the treatment group achieved a mean re-epithelialization rate of 59.20 ± 29.03%, significantly higher than the negative control group (−11.61 ± 24.29%) and the positive control group (8.05 ± 8.08%), with $p = 0.001$. By day 7, wound closure in the Karo Traditional Oil group reached 81.05 ± 21.96%, compared to 12.67 ± 16.57% in the negative control and 25.41 ± 20.07% in the positive control ($p = 0.002$). These findings were visually supported by standardized photographic documentation (Figure 1), which clearly illustrated faster wound contraction and better tissue regeneration in the treatment group.

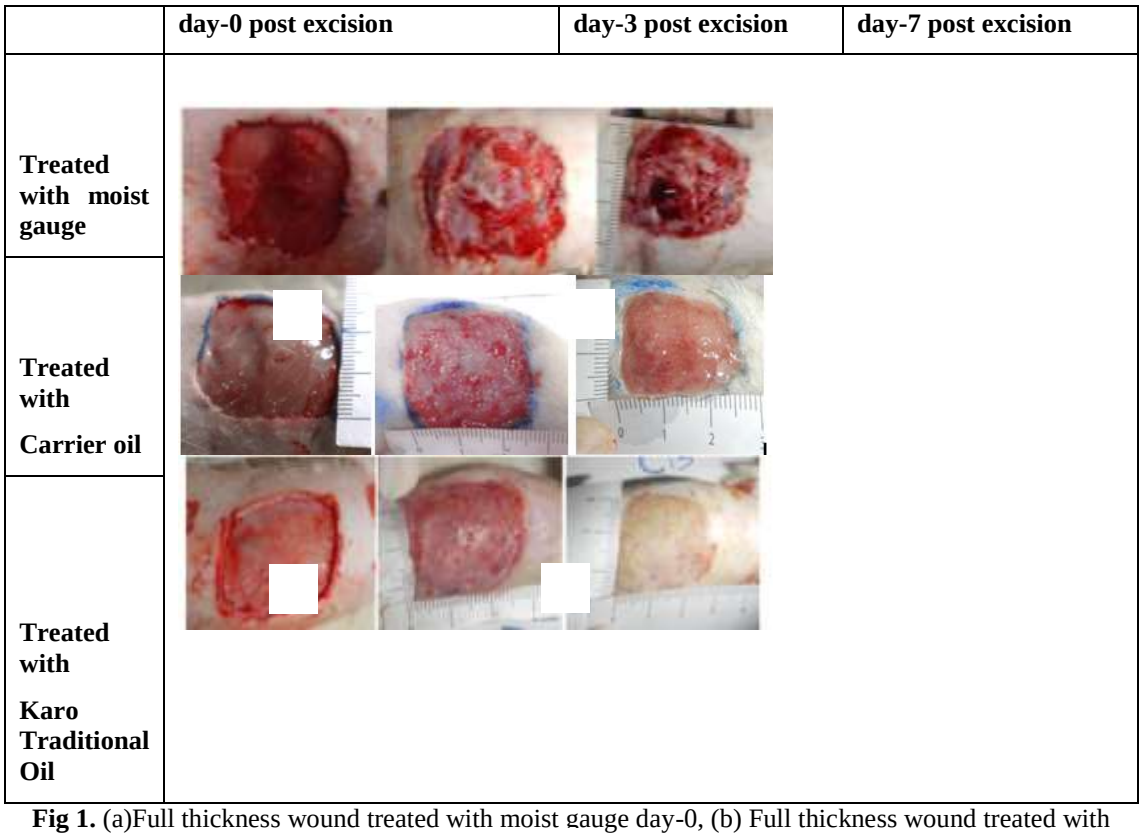


**Fig 1.** (a)Full thickness wound treated with moist gauge day-0, (b) Full thickness wound treated with moist gauge day-3, (c) Full th ess wound treated with moist gauge day-7, (d) Full thickness wound treated with carrie day-0, (e) Full thickness wound treated with carrier oil day-3, (f) Full thickness

wound treated with carrier oil day-7, (g) Full thickness wound treated with Karo Traditional Oil day-0, (h) Full thickness wound treated with Karo Traditional Oil day-3, (i) Full thickness wound treated with Karo Traditional Oil day-7.

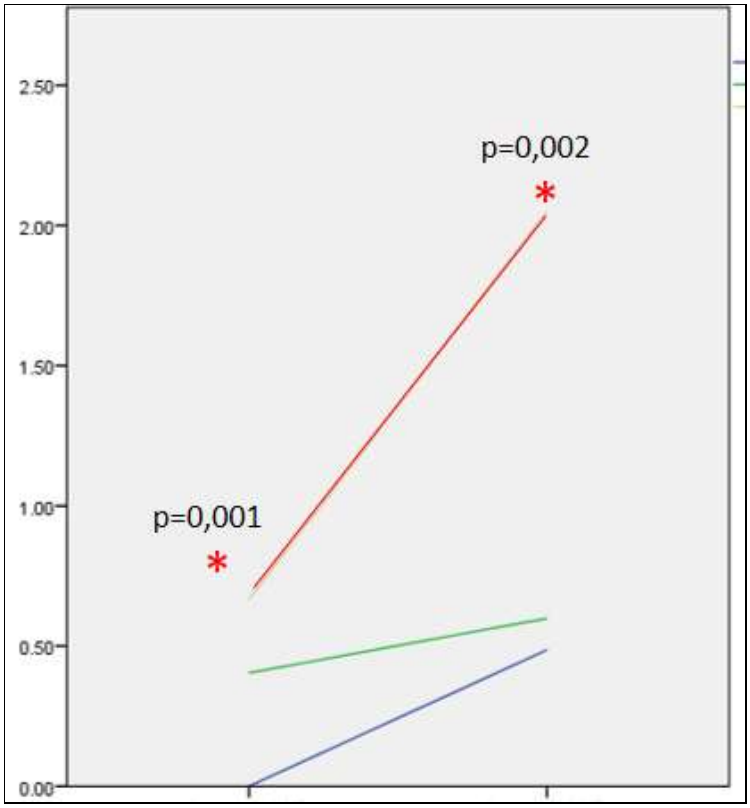


**Fig 2.** (a)Full thickness wound treated with moist gauge day-0, (b) Full thickness wound treated with moist gauge day-3, (c) Full thickness wound treated with moist gauge day-7,

Histological assessment further confirmed these macroscopic improvements. As presented in Table 2, re-epithelialization thickness on day 7 was significantly greater in the treatment group (2.05 ± 0.94 µm) compared to both control groups (p = 0.019). On day 3, although a trend toward improvement was observed, the difference did not reach statistical significance (p = 0.155).

**Table 2. Re-epithelialization thickness analysis of the studied group in the third and seventh days of the study**

| **Groups** | **Third day**<br>**Mean ± SD (*n*)** | **Seventh day**<br>**Mean ± SD (*n*)** |
|---|---|---|
| **Negative Control Group** | 0,00 ± 0,00 (6) | 0,49 ± 0,78 [a] (6) |
| **Positive Control Group** | 0,40 ± 0,64 (6) | 0,60 ± 0,69 [a] (6) |
| **Treatment Group** | 0,67 ± 0,79 (6) | 2,05 ± 0,94 [b] (6) |
| **p value** | 0,155 | 0,019 * |

Data are given as percentages, * statistically significant difference between a and b

Immunohistochemical analysis revealed that Karo Traditional Oil significantly upregulated EGF expression. As detailed in Table 3, EGF levels in the treatment group were higher on day 3 (4.33 ± 1.93) and day 7 (5.83 ± 2.51) compared to controls (p = 0.004 and p = 0.016, respectively). This elevation in EGF corresponded temporally with accelerated keratinocyte migration and re-epithelialization

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**Table 3. EGF cytokine level count analysis of the studied group in the first, third and seventh days of the study**

| Groups | First day Mean ± SD (*n*) | Third day Mean ± SD (*n*) | Seventh day Mean ± SD (*n*) |
|---|---|---|---|
| **Negative Control Group** | 4,53 ± 1,90 (6) | 2,53 ± 0,62 [a] (6) | 3,27 ± 1,96 [a] (6) |
| **Positive Control Group** | 4,27 ± 1,40 (6) | 3,70 ± 2,29 [a] (6) | 3,17 ± 0,46 [a] (6) |
| **Treatment Group** | 6,30 ± 1,65 (6) | 4,33 ± 1,93 [b] (6) | 5,83 ± 2,51[b] (6) |
| **p value** | 0,103 | 0,004* | 0,016* |

* statistically significant difference between a and b

These results align with the established role of EGF in wound healing biology. EGF is known to stimulate keratinocyte proliferation and migration through the MAPK/ERK signaling pathway, thereby accelerating re-epithelialization. The findings are consistent with previous studies on herbal formulations rich in monoterpenes such as α-pinene, which have been reported to modulate inflammatory responses and promote growth factor secretion. However, the magnitude of improvement observed in this study appears more pronounced than in several earlier reports using single-plant extracts, suggesting possible synergistic effects among the multiple bioactive compounds in Karo Traditional Oil.

Several contextual factors may have influenced the outcomes, including standardized wound creation by a single researcher (minimizing technical variability), controlled laboratory environment, and the specific phytochemical profile of the oil (dominated by 74.47% α-pinene). Limitations include the short observation period and the use of healthy young rats, which may not fully represent healing dynamics in comorbid or aged subjects.

This study contributes to the growing body of evidence supporting the scientific validation of Indonesian traditional medicines. By demonstrating that Karo Traditional Oil accelerates wound healing through EGF stimulation, the findings strengthen the bridge between ethnomedicine and modern pharmacology, offering promising implications for the development of accessible, natural wound care products in clinical and community settings.

## DISCUSSION

The present study provides compelling preclinical evidence that Karo Traditional Oil, a traditional herbal formulation from the Karo ethnic community in North Sumatra, Indonesia, significantly accelerates acute wound healing in a full-thickness excisional wound model. Topical application of Karo Traditional Oil resulted in markedly faster wound closure, enhanced re-epithelialization thickness, and elevated epidermal growth factor (EGF) levels compared to both negative (moist therapy) and positive (carrier oil) controls. These findings not only validate the longstanding ethnomedicinal use of Karo Oil but also elucidate a plausible molecular mechanism involving EGF stimulation, offering a valuable contribution to the field of ethnopharmacology and integrative wound care.

On the third and seventh days post-wounding, the treatment group exhibited re-epithelialization rates of 59.20% and 81.05%, respectively—substantially higher than the control groups. This accelerated closure aligns with histological observations showing significantly greater re-epithelialization thickness by day 7. Immunohistochemical analysis further revealed elevated EGF expression in the treatment group from day 3 onward, suggesting that the oil's bioactive constituents actively upregulate this critical growth factor. These results are consistent with the well-established role of EGF in wound biology. EGF promotes keratinocyte proliferation, migration, and differentiation primarily through activation of the EGFR/MAPK/ERK signaling pathway, processes essential for successful re-epithelialization. Recent studies continue to affirm that timely and sufficient EGF signaling is a rate-limiting step in efficient wound repair, particularly in the proliferative phase.

The phytochemical profile of Karo Traditional Oil, dominated by 74.47% α-pinene along with other monoterpenes such as δ-3-carene, sabinene, limonene, and camphene, likely underpins its therapeutic efficacy. α-Pinene and related monoterpenes possess documented anti-inflammatory, antioxidant, and pro-regenerative properties. A 2024 study on α-terpineol (a related monoterpene) preconditioned mesenchymal stem cells demonstrated enhanced wound healing through increased expression of EGF, VEGF, FGF, and TGF-β, coupled with reduced inflammatory cytokines. Similar mechanisms may operate here, whereby α-pinene-rich Karo Oil modulates macrophage polarization toward a pro-healing M2 phenotype, thereby stimulating growth factor secretion while simultaneously exerting direct effects on keratinocytes. This dual action—indirect via immune modulation and direct on epithelial cells—may explain the superior healing outcomes observed compared to carrier oil alone.

Recent global estimates underscore the urgency of identifying effective, accessible wound therapies. In 2022, worldwide wound care expenditure reached approximately USD 148.65 billion, with chronic wounds affecting millions amid rising diabetes, aging populations, and antimicrobial resistance. In Indonesia, where traditional medicines remain the first line of care for many communities, scientifically validating indigenous formulations such as Karo Oil addresses both cultural relevance and health equity. Unlike many single-herb extracts studied previously, Karo Oil's multi-component nature suggests potential synergistic effects, a phenomenon increasingly recognized in contemporary ethnopharmacological research on complex herbal preparations.

When compared with prior investigations, the current findings show more pronounced effects than those reported for several other Indonesian herbal oils or essential oils. While earlier work on Karo Oil documented anti-inflammatory activity through TNF-α suppression and IL-10 stimulation, the present study extends these observations by linking the formulation directly to EGF upregulation and measurable histological improvements. This positions Karo Oil as a promising candidate that may outperform some conventional herbal agents in acute wound settings. Nevertheless, differences in experimental models, extraction methods, and observation periods across studies warrant cautious interpretation. The use of healthy young rats in this study likely facilitated robust healing responses; outcomes in diabetic or aged models might differ, reflecting real-world clinical complexities.

Several factors may have influenced the observed results. Methodological strengths, including standardized wound creation by a single operator, blinded histopathological evaluation, and consistent environmental conditions, minimized bias. However, the short 7-day observation period, while sufficient for acute wound assessment, limits insights into long-term remodeling and scar quality. The animal model, although standard, does not fully replicate human skin physiology or the polymicrobial environment often encountered in clinical wounds. Additionally, while the phytochemical composition was referenced from prior analyses, batch-to-batch variability in traditionally prepared oils remains a potential source of inconsistency that requires standardization for future translational efforts.

From a theoretical perspective, these results enrich the understanding of how traditional multi-herbal formulations interact with modern wound healing pathways. They support the concept of "ethno-molecular bridging," whereby indigenous knowledge systems are interrogated through contemporary biomedical lenses to uncover actionable mechanisms. Practically, Karo Traditional Oil offers several advantages: low cost, cultural acceptability, and relative ease of production using locally available plants. In resource-limited settings across Indonesia and similar biodiverse regions, such validated traditional medicines could serve as sustainable adjuncts or alternatives to expensive advanced wound dressings.

Despite promising results, several limitations should be acknowledged. The study focused exclusively on acute wounds; chronic wound models would provide greater clinical relevance. Safety profiling, including potential cytotoxicity, allergenicity, and long-term effects, requires further investigation. Moreover, while EGF elevation was clearly demonstrated, downstream signaling pathways (e.g., detailed MAPK/ERK phosphorylation) were not fully elucidated and merit additional molecular studies.

Future research should prioritize several directions: (1) standardization and quality control of Karo Traditional Oil production; (2) evaluation in diabetic, infected, or ischemic wound models; (3) human clinical trials to assess efficacy, safety, and patient-reported outcomes; and (4) formulation development, such as incorporation into advanced delivery systems (hydrogels or nanoemulsions) to enhance stability and bioavailability. Mechanistic studies employing transcriptomics or proteomics could further map the molecular networks modulated by the oil's constituents.

## CONCLUSSION

In conclusion, this study demonstrates that Karo Traditional Oil significantly accelerates acute wound healing through enhanced re-epithelialization and EGF stimulation. By providing robust scientific validation of an indigenous Indonesian herbal medicine, the findings contribute meaningfully to ethnopharmacology, support evidence-based integration of traditional knowledge into healthcare, and highlight the untapped potential of biodiversity-rich nations in addressing global wound care challenges. As the burden of wounds continues to rise worldwide, such culturally grounded, affordable therapeutic options represent not only scientific progress but also a step toward more equitable and sustainable healthcare solutions

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