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The Effect of Gerga Orange Peel Extract Nanoemulsion Gel on Wound Area in Rats

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Abstract
Introduction: *Gerga orange peel contains flavonoids, tannins, saponins, vitamin C, alkaloids, and essential oils that may support wound healing through antioxidant, anti-inflammatory, and antimicrobial activity.* ***Objective:*** *To determine the effect of Gerga orange peel extract nanoemulsion gel on wound area changes in male Wistar rats.* ***Method:*** *This post-test-only controlled laboratory experiment included 24 male Wistar rats divided into six groups: negative control, 0.1% gentamicin sulfate positive control, Gerga orange peel extract gel, and Gerga orange peel extract nanoemulsion gels at 2.5%, 5%, and 7.5%. Wound area was measured on days 1, 7, and 14 using ImageJ. Data were analyzed using Kruskal-Wallis and post hoc tests.* ***Result and Discussion:*** *All groups showed wound area reduction. The greatest median reduction from day 1 to day 7 occurred with 2.5% nanoemulsion (93.340 mm²). The greatest reductions from day 7 to day 14 and day 1 to day 14 occurred with extract gel (149.392 mm² and 175.985 mm²). A significant between-group difference was found only from day 7 to day 14 ($p = 0.010$).* ***Conclusions:*** *Gerga orange peel extract gel and Gerga orange peel extract nanoemulsion gel reduced wound area, but the most effective nanoemulsion concentration could not be established.*

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Introduction

A wound disrupts tissue continuity, compromises the protective barrier of the skin, and may increase fluid loss and susceptibility to infection. Repair occurs through overlapping stages of hemostasis, inflammation, proliferation, and remodeling. Disturbance of the transition between these stages can delay closure and contribute to abnormal scar formation (Almadani et al., 2021; Kolimi et al., 2022). Natural products remain relevant in wound-care research because plant-derived compounds may provide antioxidant, anti-inflammatory, and antimicrobial effects (Carvalho et al., 2021; Wida Ekaputri et al., 2024; Miftahurrahmah & Purwakanthi, 2021). Gerga orange peel (Citrus reticulata var. Gerga Kerinci) is a potentially useful plant by-product because it contains flavonoids, tannins, alkaloids, saponins, vitamin C, and essential oils. (Hafizah et al., 2025; Noviarni & Hartati, 2024; Yunita et al., 2022; Zulfan, N., 2022). These compounds may support wound repair by limiting oxidative stress, moderating excessive inflammation, restricting microbial growth, and facilitating new-tissue formation (Carvalho et al., 2021).

A nanoemulsion system was selected for topical delivery because its small droplets may enlarge the contact surface, promote more uniform distribution of active constituents, and improve tissue penetration (Donthi et al., 2023). However, these advantages are influenced by preparation stability, active-compound release, concentration, and gel-base characteristics. Therefore, the effectiveness of a nanoemulsion must be demonstrated through biological observation in a wound model.

In this study, wound healing was observed macroscopically through changes in wound area measured using ImageJ. Wound area was selected as the primary outcome because it provides a direct, objective, non-invasive, and repeatable measure of wound closure and can be assessed serially in the same animal without tissue collection. Digital image analysis provides a more objective and quantifiable estimate of wound size than visual inspection alone (Schroeder et al., 2021). However, inflammatory responses, antimicrobial activity, fibroblast activity, and collagen formation were not directly assessed; therefore, these mechanisms serve only as the biological rationale for the intervention and are not interpreted as outcomes of this study. This study aimed to determine the effect of Gerga orange peel extract nanoemulsion gel on wound area changes as a macroscopic indicator of wound healing in male Wistar rats (Miftahurrahmah et al., 2025).

Method

This study was a laboratory experiment using a post-test-only controlled group design. It was conducted at the Biomedical Laboratory, Faculty of Medicine and Health Sciences, Universitas Jambi, from June 2025 to June 2026. The study subjects were healthy, active male Wistar rats aged 2-3 months, weighing 150-250 g, without wounds or physical defects. Rats that became ill or died during the study were excluded. The sample size was calculated using the Federer formula, $(n - 1)(t - 1) >= 15$. With six treatment groups, the minimum requirement was four rats per group, resulting in a total of 24 rats. The subjects were allocated to six groups: an untreated negative control, a 0.1% gentamicin sulfate positive control, Gerga orange peel extract gel, and Gerga orange peel extract nanoemulsion gels at concentrations of 2.5%, 5%, and 7.5%.

Gerga orange peels were washed, separated from the fruit, cut into small pieces, dried at 45-50 °C, ground, and extracted with 70% ethanol by maceration. The concentrated extract was stored in a dark glass bottle. The nanoemulsion was prepared by dissolving

the extract in Tween 80 and distilled water, followed by homogenization at 10,000-15,000 rpm. The gel was prepared using a 3% hydroxypropyl methylcellulose base, after which the extract or nanoemulsion was incorporated until homogeneous. The rats were acclimatized for one week. The dorsal area was shaved, disinfected with 10% povidone-iodine, and anesthetized using lidocaine. A wound approximately 2 cm in diameter and 0.3 cm deep, or extending to the subcutaneous layer, was created on the back. Treatment was applied twice daily at 08:00 and 17:00 Western Indonesian Time for two weeks, using approximately 0.5 g of preparation per rat for each application.

Wound-area data were obtained from photographs taken on days 1, 7, and 14. The same camera, consistent lighting, a distance of approximately 20 cm, and a ruler for scale calibration were used. Wound area was measured using ImageJ and expressed in mm². Data were analyzed univariately using the mean or median (minimum-maximum) and bivariately using the Kruskal-Wallis test, followed by post hoc analysis when a statistically significant difference was identified. Ethical approval number: 1898/UN21.8/PT.01.04/2025.

Result and Discussion

1. Results

All groups showed a reduction in wound area from day 1 to day 14. The smallest mean wound area on day 14 was observed in the positive-control group, whereas the largest mean wound areas on days 1 and 7 were observed in the Gerga orange peel extract gel group. The mean wound-area data are presented in Table 1.

Table 1
Mean Wound Area by Treatment Group

Group	Day 1 (mm²)	Day 7 (mm²)	Day 14 (mm²)
Negative control	140.909	85.797	34.055
Positive control	155.286	119.875	17.072
Extract gel	270.264	223.393	63.901
Nanoemulsion 2.5%	184.785	98.076	31.803
Nanoemulsion 5%	158.448	89.139	44.878
Nanoemulsion 7.5%	193.762	103.658	35.818

Changes in wound area between observation periods varied among the groups. The median reductions in wound area are presented in Table 2.

Table 2
Median (Minimum-Maximum) Reduction in Wound Area Between Observation Days

Group	Days 1-7 (mm²)	Days 7-14 (mm²)	Days 1-14 (mm²)
Negative control	55.072 (34.572-75.733)	37.296 (30.182-102.194)	88.191 (73.107-177.927)
Positive control	33.609 (27.180-47.248)	105.393 (88.243-112.184)	137.092 (126.827-151.847)
Extract gel	26.593 (3.161-131.138)	149.392 (85.405-253.780)	175.985 (88.566-384.918)
Nanoemulsion 2.5%	93.340 (54.835-105.323)	59.128 (48.304-98.532)	151.280 (105.515-203.855)
Nanoemulsion 5%	68.961 (63.172-76.145)	39.053 (32.252-66.685)	107.805 (95.840-142.830)
Nanoemulsion 7.5%	89.471 (54.610-126.865)	61.758 (52.710-95.136)	151.228 (107.320-222.001)

The Kruskal-Wallis analysis of wound-area reduction showed that a statistically significant difference among groups occurred only during the day 7 to day 14 interval (Table 3).

Table 3
Kruskal-Wallis Test of Wound-Area Reduction

Observation interval	p-value	Interpretation
Days 1-7	0.060	Not significant
Days 7-14	0.010	Significant
Days 1-14	0.311	Not significant

Table 4
Post Hoc Analysis of Wound-Area Reduction from Day 7 to Day 14

Group comparison	Adjusted significance
Negative control vs. positive control	0.282
Negative control vs. extract gel	0.089
Negative control vs. nanoemulsion 2.5%	1.000
Negative control vs. nanoemulsion 5%	1.000
Negative control vs. nanoemulsion 7.5%	1.000
Positive control vs. extract gel	1.000
Positive control vs. nanoemulsion 2.5%	1.000
Positive control vs. nanoemulsion 5%	1.000
Positive control vs. nanoemulsion 7.5%	1.000
Extract gel vs. nanoemulsion 2.5%	0.861
Extract gel vs. nanoemulsion 5%	0.034*
Extract gel vs. nanoemulsion 7.5%	1.000
Nanoemulsion 2.5% vs. nanoemulsion 5%	1.000
Nanoemulsion 2.5% vs. nanoemulsion 7.5%	1.000
Nanoemulsion 5% vs. nanoemulsion 7.5%	1.000

*Note: *Statistically significant at $p < 0.05$.*

Based on the wound-area difference data, the greatest median reduction from day 1 to day 7 occurred in the 2.5% nanoemulsion group, at 93.340 mm². From day 7 to day 14 and from day 1 to day 14, the greatest median reductions occurred in the extract gel group, at 149.392 mm² and 175.985 mm², respectively.

The Kruskal-Wallis test showed a statistically significant difference among groups in wound-area reduction from day 7 to day 14 ($p = 0.010$). No significant differences were observed from day 1 to day 7 ($p = 0.060$) or from day 1 to day 14 ($p = 0.311$). Post hoc testing for the day 7 to day 14 interval identified a statistically significant difference only between the extract gel and 5% nanoemulsion groups ($p = 0.034$).

2. Discussion

Across the observation period, wound area decreased in every group. This pattern reflects endogenous repair mechanisms and may also reflect the effects of the applied formulations. Early closure is governed mainly by hemostasis and inflammation, followed by increasing contributions from granulation-tissue formation, angiogenesis, fibroblast activity, collagen synthesis, contraction, and re-epithelialization (Almadani et al., 2021; Kolimi et al., 2022).

The greatest median wound-area reduction from day 1 to day 7 was observed in the 2.5% nanoemulsion group. This finding suggests that a low nanoemulsion concentration may produce a favorable early response in wound contraction or closure. In theory, the small droplet size of a nanoemulsion increases the contact surface area and promotes more uniform distribution of active compounds over the wound (Donthi et al., 2023). Nevertheless, the subsequent observation interval did not demonstrate a consistent concentration-dependent increase in nanoemulsion effectiveness.

From day 7 to day 14 and from day 1 to day 14, the greatest median wound-area reductions were observed in the Gerga orange peel extract gel group. This result indicates that the conventional extract incorporated in a gel base was still able to support wound healing effectively. The significant difference identified only between the extract gel and 5% nanoemulsion groups suggests that a nanoemulsion at a particular concentration does not necessarily produce a better effect than a conventional extract. The effectiveness of topical preparations depends not only on the nanoemulsion system but also on stability, homogeneity, active-compound release, particle size, gel viscosity, and the biological response of each experimental animal (Donthi et al., 2023; Sevinç-Özakar et al., 2022).

The wound-healing potential of Gerga orange peel may be associated with its flavonoids, tannins, saponins, vitamin C, alkaloids, and essential oils (Noviarni & Hartati, 2024; Yunita et al., 2022). Flavonoids and phenolic compounds act as antioxidants and anti-inflammatory agents, whereas tannins, saponins, and essential oils may exert antimicrobial activity (Indah Ayudia et al., 2025; Wida Ekaputri Hz. et al., 2023; Tri Hardiningsih et al., 2025; Humaryanto et al., 2023). These compounds may help control excessive inflammation, reduce oxidative stress, decrease microbial contamination, and support fibroblast activity and collagen formation during the proliferative phase (Fairuz et al., 2023).

The present results are consistent with reports that citrus-peel preparations can support tissue repair. Studies involving Citrus aurantium and Citrus hystrix have described improved cutaneous healing, keratinocyte proliferation, and cell migration, supporting the biological plausibility of citrus-derived topical agents (Cherukuri et al., 2023; Moolsup et al., 2023). Gerga orange peel therefore has a biological basis for further

development as an active ingredient in topical preparations, although more complete formulation testing is required.

This study had several limitations. Observation was performed macroscopically by measuring wound area with ImageJ; therefore, microscopic processes such as inflammatory-cell counts, fibroblast activity, angiogenesis, re-epithelialization, and collagen deposition were not assessed. In addition, the number of animals per group was relatively small, initial wound areas varied, the physical characteristics of the nanoemulsion were not comprehensively evaluated, and follow-up was limited to 14 days. These limitations may have reduced the ability of the study to identify the most effective nanoemulsion concentration.

Conclusion

Gerga orange peel extract gel and Gerga orange peel extract nanoemulsion gel were associated with progressive wound-area reduction in male Wistar rats, and all groups showed smaller wound areas by day 14. However, the day-1 wound area varied among groups; therefore, the magnitude of absolute wound-area reduction should be interpreted cautiously and should not be used alone to determine comparative treatment effectiveness.

A statistically significant difference among groups was detected only during the day 7-to-day 14 interval, and post hoc analysis identified a difference between the extract-gel and 5% nanoemulsion groups. No significant between-group difference was found across the full day 1-to-day 14 interval. Therefore, the available findings do not establish which nanoemulsion concentration is the most effective. These conclusions are limited to macroscopic wound-area changes and do not demonstrate underlying inflammatory, antimicrobial, fibroblast, or collagen-related mechanisms.

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