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Red Ginger Extract Nanoemulgel: Antibacterial and Wound-Healing Efficacy Against Staphylococcus aureus in a Preclinical Mouse Model

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Wound infections by *Staphylococcus aureus* (*St. aureus*), exacerbated by biofilm formation and antibiotic resistance, are a major challenge in wound management. This study aims to test the efficacy of Red Ginger Extract Nanoemulgel (RGE Nanoemulgel) as a new topical agent in controlling bacterial infections and accelerating the healing of experimentally induced infected incision wounds in a preclinical mouse model. The study comprised in vitro antibacterial and anti-biofilm activity assays comparing RGE Nanoemulsion against Gentamicin, followed by in vivo efficacy testing in an animal model comparing RGE Nanoemulgel, RGE, and Mupirocin in terms of bacterial abundance (log CFU/gram) and wound contraction percentage. Results indicated that RGE Nanoemulsion demonstrated promising in vitro antimicrobial activity; at a concentration of 25%, antibacterial inhibition reached 75.11% (exceeding Gentamicin at 51.18%), and anti-biofilm inhibition reached 90.79% at 50% concentration. In vivo, RGE Nanoemulgel suppressed bacterial abundance to 3.96 log CFU/gram, comparable to Mupirocin, and achieved a wound contraction rate of 93.50% on Day 12, exceeding Mupirocin (63.16%). These preclinical findings suggest that RGE Nanoemulgel may represent a promising non-antibiotic topical candidate, combining antibacterial, anti-biofilm, and wound-supportive properties. Further toxicity, stability, and clinical validation studies are warranted before therapeutic claims can be established.

Keywords: Antibiofilm, Infected incisions, Red Ginger Extract Nanoemulgel, Wound contraction

INTRODUCTION

The skin is the largest organ in the body, making up approximately 12% to 16% of our total body weight and helping to protect the body from foreign microorganism (Fölster-Holst, 2022;Zaid et al., 2022). When this natural defense is damaged or compromised, microorganisms such as bacteria can easily invade and begin to multiply on its surface. One of the most common causes of these skin and soft tissue infections is the bacterium *Staphylococcus aureus* (Marzaman et al., 2023). The global incidence of S. aureus infections has risen substantially over recent decades, with nosocomial infection rates reported between 3% and 20% in various healthcare settings (Marzaman et al., 2023; Li et al., 2025).

Bacteria *S. aureus* has the ability to form biofilms, a protective structure that significantly increases its resistance, making it more resistant to conventional antibiotics and the body's immune system (Parastan et al., 2020). Wounds that are not treated promptly can develop into more serious infections, including cellulitis, abscesses, and even potentially life-threatening sepsis.

Conventional therapy for wound infections caused by *S. aureus* generally uses antibiotics such as penicillin, clindamycin, and vancomycin. However, the emergence of bacterial strains resistant to various antibiotics, including methicillin-resistant Staphylococcus aureus, has led to the development of new strains (MRSA), complicates treatment and increases morbidity and mortality rates due to this infection (Bhattacharya, 2014; Li et al., 2025). Therefore, new approaches that are more effective and have fewer side effects are urgently needed.

Natural product-based strategies have attracted considerable interest as potential alternatives to conventional antimicrobials. Among these, red ginger (Zingiber officinale var. rubrum) has demonstrated notable antibacterial and anti-biofilm properties, attributed to bioactive compounds including gingerol, shogaol, and zingiberene (Semwal et al., 2015; Wang et al., 2020). However, the direct therapeutic application of ginger extract is limited by its poor solubility, low bioavailability, and instability under physiological conditions. Nanoemulsion-based delivery systems have been explored to overcome these limitations by

enhancing solubility, penetration, and bioavailability of hydrophobic active compounds (Begum et al., 2024). While previous studies have investigated ginger-derived nanoformulations and essential oil nanoemulsions for antibacterial applications (Wang et al., 2020). Rahman et al., 2020), data on their efficacy in a combined antibacterial, anti-biofilm, and wound-healing model remain limited. The present study aimed to bridge this gap by evaluating the efficacy of RGE Nanoemulgel a nanoemulsion-based gel formulation of red ginger extract in a preclinical S. aureus-infected incision wound mouse model, encompassing both in vitro antimicrobial characterization and in vivo wound healing assessment.

METHODS

Red Ginger Extract Nanoemulsion (RGE Nanoemulsion)

Red ginger extract (RGE) was incorporated into the oil phase consisting of virgin coconut oil (VCO) and stirred using a magnetic stirrer at 500 rpm for 15 minutes. The mixture was then processed by probe sonication at 40 kHz with 40% amplitude for 10 minutes at room temperature (25°C) to reduce droplet size into the nanometer range. The water phase was prepared by mixing Tween 80 and glycerin and stirring at 500 rpm for 15 minutes. The water phase was then slowly added to the oil phase under continuous stirring at 500 rpm for 15 minutes. Aquabidest was added dropwise to the combined mixture and stirred until homogeneous. The rationale for selecting 0.25% RGE was based on preliminary dose-finding experiments demonstrating optimal antibacterial activity at this concentration without cytotoxic effects on the formulation matrix. Tween 80 at 35% was selected as a non-ionic surfactant with an established safety profile for topical use, while glycerin at 20% served as a co-surfactant to stabilize the nanoemulsion system. The composition is presented in Table 1.

Table 1. Composition of NRGE

Material	Utility	Concentration
RGE	Active Ingredients	0.25%
VCO	Oil Phase	5%
Tween 80	Surfactant	35%
Glycerin	Co-surfactant	20%
Aquadest	Solvent	Add 100 ml

Red Ginger Extract Nanoemulgel (RGE-Nanoemulgel) Blinding

Na-CMC powder was sprinkled over 20 ml of hot aquabidest in a mortar and left for 15 minutes until the Na-CMC swelled. The swollen Na-CMC was crushed with a stamper until the gel texture became homogeneous. Then, nipagin and nipasol were gradually added and crushed again until everything was evenly mixed. Next, the nanoemulsion (which has optimum antibiofilm activity) was mixed into the gel preparation and crushed. The remaining aquabidest was poured back into the gel preparation and crushed until homogeneous.

Antibacterial Activity Test of RGE Nanoemulsion against *S. aureus* Bacteria

Antibacterial activity was assessed against *S. aureus* using a turbidimetric microdilution method (Adha et al., 2025). *S. aureus* was cultured in TSB medium at 37°C until the logarithmic growth phase was reached, and the concentration was adjusted to 106 CFU/mL by absorbance measurement. Serial dilutions of RGE

Nanoemulsion (100%, 50%, 25%, 12.5%, 6.25%, 3.125%) were prepared from the stock solution (Table 1). A total of 100 µL of TSB medium (negative control), 50 µg/mL gentamicin sulfate solution (positive control), or respective RGE Nanoemulsion dilutions were added to the wells of a 96-well plate, followed by 100 µL of bacterial suspension. Plates were incubated at 37°C for 24 hours. Aliquots of 100 µL were withdrawn every 12 hours and absorbance measured at 595 nm using a microplate reader. All assays were performed in at least triplicate. The turbidimetric method measures optical density as a surrogate for bacterial growth; while this does not directly confirm cell viability, it provides a validated and widely used quantitative indicator of bacterial proliferation (Adha et al., 2025). Minimum inhibitory concentration (MIC) determination was performed based on the lowest concentration showing no visible turbidity. The antibacterial inhibition (%) was calculated as:

$$\text{Antibacterial level (\%)} = (ODk\text{-}0Ds)/ODk \times 100\% \quad (1)$$

where ODk indicates the absorbance value of the negative control well and ODs indicates the absorbance value of the positive control well/sample.

Inhibitory Activity of RGE Nanoemulsion on S. aureus Biofilm Formation in vitro

Anti-biofilm activity was assessed using a crystal violet staining assay (Choi et al., 2020; Haney et al., 2021). S. aureus suspension (106 CFU/mL, 100 µL) was added to wells of a 96-well microplate. RGE Nanoemulsion was tested at 0.5×MIC, 1×MIC, 2×MIC, and 4×MIC concentrations; gentamicin sulfate (50 µg/mL) served as the positive control, and RPMI medium served as the negative control. After incubation at 37°C for 24 hours, planktonic cells were removed and wells were washed twice with PBS. Fixation was performed with methanol for 15 minutes. Following methanol removal, 100 µL of 1% crystal violet solution was added to each well and incubated for 30 minutes; excess stain was aspirated and wells rinsed with PBS. Subsequently, 100 µL of 96% ethanol was added and incubated for 10 minutes with shaking at 100 rpm. Absorbance was measured at 595 nm using an ELISA reader. Assays were performed in biological triplicate with three technical replicates each. Results were normalized against the negative control. Biofilm inhibition (%) was calculated as:

Biofilm inhibition rate (%) = (ODkn – ODs)/ODkn x 100% (2)

where ODk indicates the absorbance value of the negative control and ODs indicates the absorbance value of the sample.

Infected Wound Modeling

The infected wound mouse model was adapted from the method described by Zhang et al., (2023). Male mice aged 8–10 weeks and weighing 150–200 g were used and were divided into negative control, positive control, and experimental groups (n = 6 per group, determined based on the Federer formula for animal experimentation). Animals were housed under standard laboratory conditions (temperature 22 ± 2°C, 12-hour light/dark cycle, ad libitum access to food and water). All procedures were conducted in accordance with ethical approval obtained from the Institutional Animal Care and Use Committee (IACUC), Universitas Wijaya Kusuma Surabaya (Approval No: 116/SLE/FK/UWKS/2025). Mice were anesthetized, dorsal fur was removed with a razor, and the surgical area was disinfected with 10% povidone iodine. A 2 cm full-thickness incision was made on each animal. A total of 100 µL of S. aureus suspension in PBS (1×107 CFU/mL) was applied to the wound and allowed to colonize for 24 hours to establish a bacterial infection model. The term 'chronic wound' is not applied, as the 12-day observation period does not fulfill established chronicity induction criteria.

Wound Healing Rate Analysis

Treatment was administered once daily, 24 hours after wound infection, to the negative control group (no treatment), positive control group (mupirocin gel), and the RGE Nanoemulgel treatment group, as well as the RGE group. Wound length was measured on Days 0, 4, 8, and 12 post-treatment using ImageJ software. A minimum of six animals per group was used, with wound measurements performed in triplicate per animal. Wound contraction rate (%) was calculated as:

Wound healing rate (%) = (S1-SN)/S1 x 100%,

where S1 is the wound length on the first day when the infected wound was formed and SN is the wound length on the nth day after treatment (Pletzer et al., 2016)

CFU test of bacteria on infected skin tissue

Three mice from each group were euthanized on days 0, 4, 8, and 12, and approximately 1 cm along the outer edge of the skin wound was excised and washed with cold saline. The tissue was weighed and ground in 1 mL of PBS buffer solution. A total of 1 mL of tissue homogenate was serially diluted, planted on MHA agar media, and incubated for 24 hours at 37oC. The number of colonies was calculated as the number of bacteria in the tissue expressed in CFU/gram.

Statistical Analysis

All measurements were performed in triplicate and reported as mean ± standard deviation (SD). Prior to analysis, normality was assessed using the Shapiro-Wilk test and homogeneity of variance by Levene's test. Statistical analysis was performed using one-way ANOVA followed by Dunnett's multiple comparison test using SPSS 22.0. The level of significance was set at $P < 0.05$. Effect sizes (eta squared, η^2) were calculated to assess the magnitude of group differences.

RESULTS AND DISCUSSION

Antibacterial Activity Test of RGE Nanoemulsion against *St. aureus* Bacteria

Antibacterial testing was performed using a microdilution method based on a turbidity factor that is linear with bacterial growth. Absorbance values are directly proportional to the test concentration, so higher concentrations increase antibacterial activity.

Based on the data in Figure 1, it shows that RGE Nanoemulsion has strong in vitro antibacterial efficacy against St. aureus. RGE Nanoemulsion concentrations of 25% (75.11%) and 50% (72.44%) showed higher inhibition percentages than the Positive Control Gentamicin (51.18%). Even at 12.5%, inhibition remained at 56.81%, still surpassing Gentamicin. These findings should be interpreted within the context of the turbidimetric method used, which reflects optical density reduction rather than direct bacterial viability. Nonetheless, these results provide quantitative evidence for the antibacterial potential of RGE Nanoemulsion.

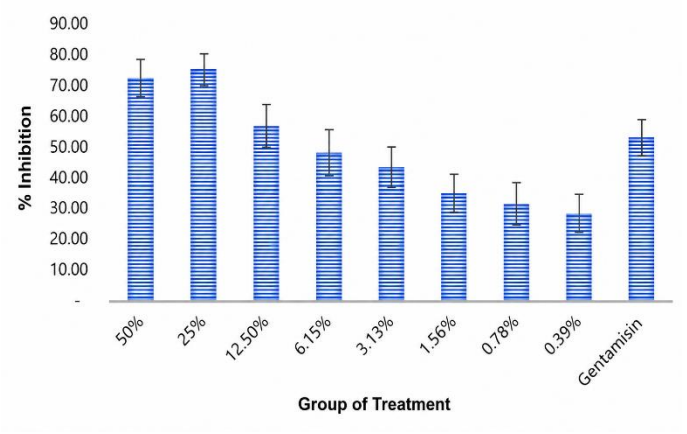

Figure 1. Antibacterial activity of REG nanomulsion against *s. aureus*. Eror bars represent mean ± SD (n = 3). Asterisks (*) indicate statistical significance vs. Gentamicin control (P < 0.05, ANOVA with Dunnett's post hoc test

These results are consistent with literature supporting the enhanced efficacy of nanoformulations. Budianto reported a zone of inhibition of approximately 14.5 mm using conventional red ginger ethanol extract at high concentrations, while the 75.11% inhibition achieved by RGE Nanoemulsion in the present study provides quantitative evidence for the added value of nanotechnology (Budianto et al., 2022; Rahman et al., 2020). Similarly demonstrated a significant reduction in MIC values of ginger nanoparticles compared to crude extract. Collectively, these findings support the hypothesis that nanoformulation enhances the antimicrobial efficacy of ginger, likely through improved solubility, increased surface area, and enhanced membrane penetration of active compounds.

Antibiofilm Activity Test of Red Ginger Extract Nanoemulsion against *St. aureus* Bacterial Biofilm

The results of the anti-biofilm activity test in Table 2 demonstrate the excellent efficacy of Red Ginger Extract Nanoemulsion (RGE Nanoemulsion) in inhibiting *St. aureus* biofilm formation. At the highest concentration of 50%, RGE Nanoemulsion achieved an impressive average biofilm inhibition percentage of 90.79%. Even at the lowest concentration tested, 6.15%, NRGE still maintained an inhibition rate of 60.98%. These figures drastically exceed the performance of the Positive Control Gentamicin, which was only able to inhibit biofilm by 44.52%. This finding is crucial, as biofilm is a polymeric matrix that protects bacteria and is known to be a major cause of wound chronicity and antibiotic therapy failure.

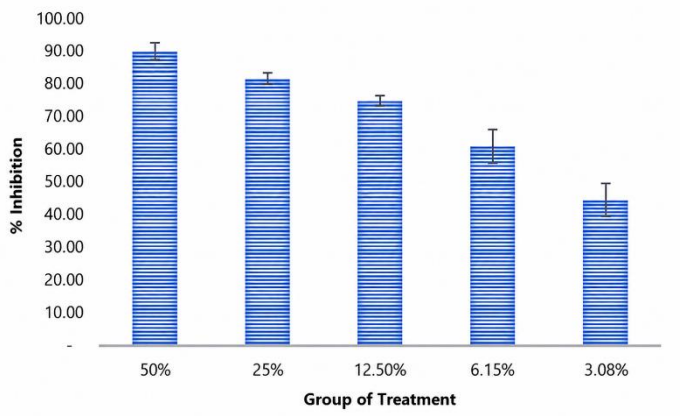

Figure 2. Anti-biofilm activity of RGE Nanoemulsion against *S. aureus* biofilm. Error bars represent mean ± SD (n = 3 biological replicates, 3 technical replicates each). Results normalized against negative control.

The results of this study are consistent with several previous studies that used herbal essential oils as antibiophilic agents. Cui et al. (2020) reported that S. aureus biofilms were successfully inhibited by cardamom essential oil (Cui et al., 2020). Cinnamon essential oil and tea tree essential oil can damage *E. coli and S. aureus* biofilms (Millezi et al., 2016). In addition, Chen et al (2021) showed that borage essential oil was able to inhibit the metabolic activity of Shigella flexneri (Chen et al., 2021). Clove essential oil also has the ability to disrupt biofilms by disrupting intercellular communication.

The mechanisms underlying the antibacterial and anti-biofilm activity of RGE Nanoemulsion may involve disruption of the bacterial cell membrane, leading to increased permeability and leakage of intracellular components including nucleic acids and proteins. These mechanistic hypotheses are proposed based on prior literature (Zhang et al., 2023) and were not directly measured in the present study; future mechanistic investigations are warranted to confirm these pathways. Zingiberene, a major sesquiterpene in red ginger, has been suggested to intercalate DNA and inhibit transcription in *S. aureus*, with downstream impairment of ATP production, EPS synthesis, and quorum sensing (QS) disruption. These combined actions may account for the dual antibacterial and anti-biofilm efficacy observed.

The effect of Nanoemulgel RGE on bacterial growth in infected incision wounds

The results showed that Nanoemulgel RGE achieved the greatest reduction in bacterial counts in infected incision wounds, with an average log CFU/gram of 3.96 on Day 12. This result is equivalent to the Positive Control Gentamicin (3.92). This indicates that NRGE has the potential as a therapeutic candidate due to its ability to efficiently control St. aureus colonies, which is an important step in treating infected wounds. In addition, the reduction in bacterial counts in the RGE Nanoemulgel group also exceeded that of the regular Red Ginger Extract

(RGE) group (a reduction of 2.82). This indicates that the nanoemulsification process is essential to transform in vitro activity into high efficacy in vivo. This superiority is due to increased penetration and bioavailability of ginger's active compounds into complex infected wound tissue.

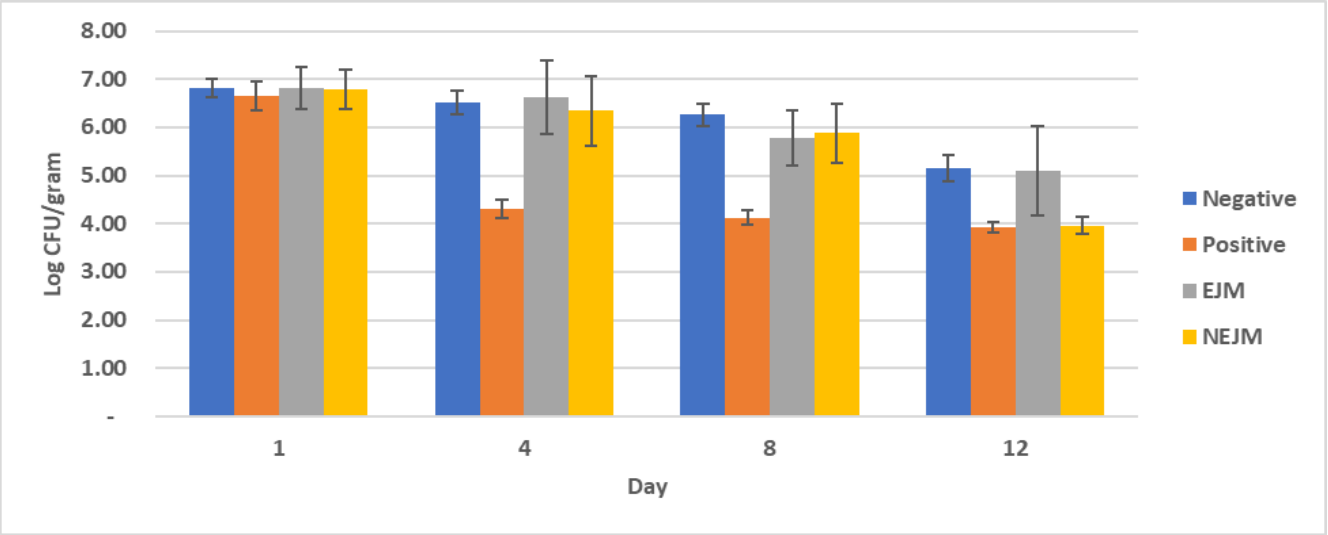

Figure 3. Bacterial load (log CFU/gram) in infected incision wounds over time. Error bars represent mean ± SD. Asterisks (*) indicate P < 0.05 vs. negative control (ANOVA with Dunnett's post hoc test).

The high activity of NRGE in reducing the number of bacteria in infected wounds is closely related to its activity as an anti-biofilm. Ginger essential oil has been shown to have the ability to disrupt biofilms by interfering with the bacteria's Quorum Sensing mechanism (Zhang et al., 2023). By inhibiting biofilm formation, RGE Nanoemulgel more easily clears the source of infection in wounds. In the context of AMR, NRGE's success aligns with recent studies seeking non-antibiotic solutions, positioning RGE Nanoemulgel as a natural therapeutic agent capable of competing with standard antibiotics without increasing the selection pressure that drives resistance.

These results are broadly consistent with findings by Zul et al. (2025), who demonstrated that bromelain, another plant-derived topical agent, reduced S. aureus infection in a murine wound model. RGE Nanoemulgel offers a potential dual benefit antibacterial activity comparable to Mupirocin alongside the anti-inflammatory properties of ginger phytochemicals (gingerol, shogaol) which may synergistically support the wound healing microenvironment (Semwal et al., 2015). This dual capability merits further investigation, particularly through histopathological and molecular analyses.

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The Effect of Nanoemulgel RGE on Healing of Infected Cut Wounds

The results of the study in Figure 4 show that NRGE achieved the highest efficacy in healing infected incision wounds, evidenced by a very high contraction percentage of 93.50% on Day 12. This achievement is equivalent to the RGE group and far exceeds the Gentamicin Positive Control which only achieved a contraction of 63.16%. The almost complete wound closure (remaining wound length of 0.13 cm) in the NRGE group indicates that this formulation is able to facilitate the proliferation and remodeling phases efficiently, which is the ultimate goal of wound management.

The high wound healing rate in the RGE Nanoemulgel group was related to the role of ginger extract in supporting tissue regeneration. This is consistent with previous research using ginger rhizome as an agent in wound healing ((Lestari, 2020; Rema Zonia, 2023; Sadikim et al., 2018)). Ginger extract has a significant effect in enhancing wound healing due to its bioactive compounds, such as gingerol and shogaol, which

are known to have anti-inflammatory and antioxidant activities. These properties help create a conducive wound environment, supporting accelerated wound healing. This includes facilitating cell proliferation and collagen synthesis, which are key components in tissue repair and closure (Bhosale et al., 2023). The higher wound healing rates of RGE Nanoemulgel and RGE compared to Gentamicin indicate that ginger phytochemical compounds provide healing benefits that conventional antibiotics do not.

The results of this study align with the current direction of wound infection therapy development, namely using nano-based delivery systems. Another study showed that the Essential Oil Nanoemulsion Hydrogel formulation was designed for dual purposes: as an anti-biofilm agent and to improve the efficacy of wound infection treatment (Cai et al., 2023). The nanoemulsion form has excellent delivery for topical agents intended for infected wounds. Thus, RGE Nanoemulgel could serve as a formulation that combines antimicrobial and regenerative benefits.

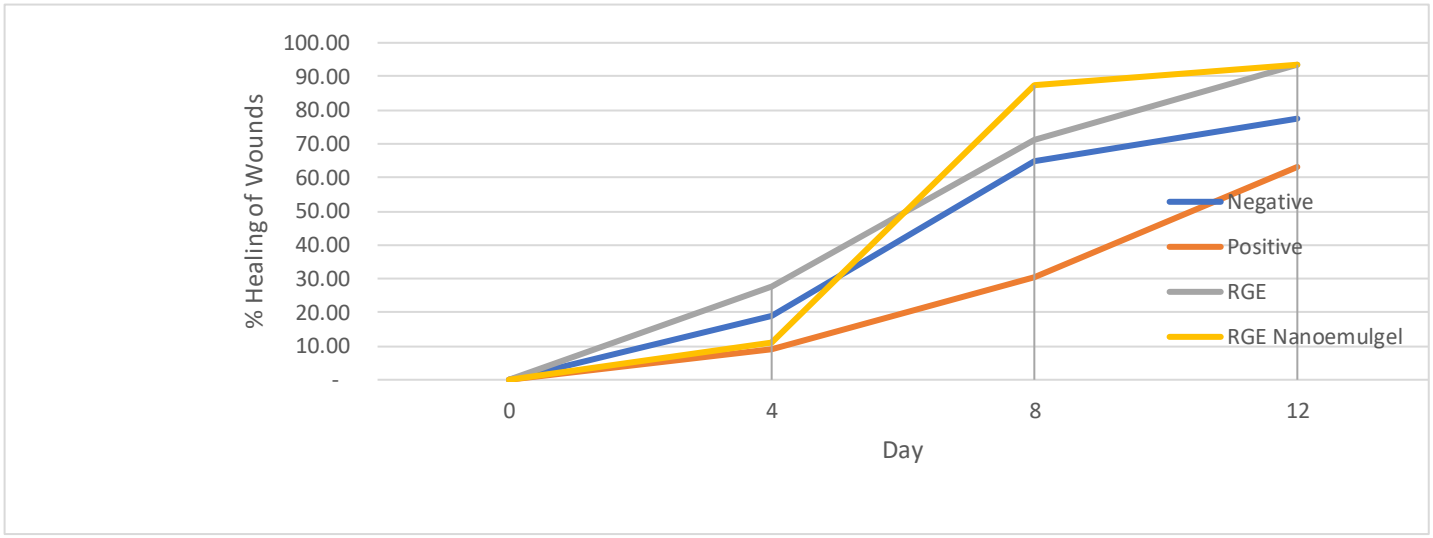

Figure 4. Wound contraction rate (%) over 12 days. Error bars represent mean ± SD. Groups: Negative control (no treatment), Mupirocin (positive control), RGE, and RGE Nanoemulgel. Statistical annotations: P < 0.05.

Notably, wound contraction rates were nearly identical between RGE Nanoemulgel and RGE groups (both ~93.50%). This finding suggests that nanoformulation primarily enhanced antibacterial efficacy rather than wound regeneration per se, and that the regenerative benefit is principally attributable to ginger phytochemicals themselves, irrespective of formulation type. Future studies incorporating histopathological analysis and collagen density quantification would be valuable to clarify this distinction.

The present findings align with the broader trajectory of nano-based wound infection therapies.Cai et al. (2023) demonstrated that essential oil nanoemulsion hydrogels serve dual functions as anti-biofilm agents and wound healing promoters. RGE Nanoemulgel represents a convergent formulation strategy addressing both objectives, warranting further development.

This study has several important limitations that should be acknowledged. First, physicochemical characterization of the nanoemulsion, including particle size distribution, polydispersity index (PDI), zeta potential, and stability evaluation, was not performed and should be included in future work to validate the nanoformulation. Second, in vitro MIC and MBC values according to CLSI/EUCAST standards were not formally reported. Third, no toxicity or irritation testing was conducted, and the safety profile of the formulation requires evaluation. Fourth, histopathological analysis of wound tissue was not performed, which limits assessment of healing quality at the tissue level. Fifth, the observation period of 12 days is relatively short, and the wound model does not fulfill established criteria for chronic wound induction. Finally, the sample size was limited and may reduce the statistical power of the in vivo findings.

CONCLUSIONS

This study demonstrates that Red Ginger Extract Nanoemulgel (RGE Nanoemulgel) exhibits promising preclinical potential as a topical agent for the management of *S. aureus* infected incision wounds in a mouse model. In vitro, RGE Nanoemulsion displayed higher antibacterial inhibition (75.11% at 25%) compared to Gentamicin (51.18%) and strong anti-biofilm efficacy (90.79% inhibition at 50%). In vivo, RGE Nanoemulgel suppressed bacterial load to levels comparable to Mupirocin and achieved a wound contraction rate of 93.50% on Day 12. These findings are preliminary and require confirmation through physicochemical characterization, toxicity studies, histopathological assessment, and clinical validation prior to establishing definitive therapeutic claims. Nonetheless, RGE Nanoemulgel represents a potentially effective non-antibiotic topical candidate meriting further investigation.

SUGGESTION

Based on the results of this study, it is recommended to conduct molecular analysis (e.g., gene expression) to validate the anti-biofilm mechanism and identify pathways that promote tissue regeneration, complemented by histopathological analysis of wounds to measure the quality of healing. As a final implementation, it is recommended to initiate clinical trials (phase I/II) in patients with wound infections (especially resistant ones) and explore the optimization of delivery systems, such as hydrogel formulations, to maximize the topical efficacy of Nanoemulgel RGE.

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ETHICAL APPROVAL AND INFORMED CONSENT

This study was conducted in accordance with the ethical principles for the use of laboratory animals and was approved by the Institutional Animal Care and Use Committee (IACUC) of Universitas Wijaya Kusuma Surabaya (Approval No: 116/SLE/FK/UWKS/2025). All experimental procedures involving animals were carried out in compliance with institutional and national guidelines for the care and use of laboratory animals. As this study involved animal subjects only and not human participants, individual informed consent was not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest

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