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CHARACTERIZATION OF WOUND HEALING GEL THROUGH EUPHORBIA NIVULIA MEDIATED IRON OXIDE NANOPARTICLE AND CONFIRMATION AGAINST WOUND PATHOGENS

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ABSTRACT:
The wound healing process is a very complicated biological procedure which comprises several events, such as hemostasis, inflammation, proliferation, and tissue remodelling. Microbial infections frequently interrupt this process and can cause chronic wounds and complications. Thus, it is important to develop efficient wound healing materials with antibacterial activity. A Euphorbia nivulia–mediated iron oxide (Fe_2O_3) nanoparticles containing wound healing gel was developed and characterized for physicochemical properties and antimicrobial activity against the prominent wound pathogens in this study. Iron oxide nanoparticles were synthesized using extracts of fresh leaves of Euphorbia nivulia via the green synthesis method that utilized plant phytochemicals as reducing and stabilizing agents. To maximize their potential for topical therapy, the resultant nanoparticles were then incorporated into a gel formulation. To ensure the stability of formulations and effectiveness for topical application, the formulation was characterized by visual observation, UV–visible spectroscopy, pH measurement, spreadability, and viscosity analysis. Formation of the Fe_2O_3 nanoparticles was confirmed by UV–visible spectroscopic studies via a characteristic absorption peak. The nanoparticle gel was tested against Candida albicans, Pseudomonas species, Actinobacter species, Enterococcus faecium, and Escherichia coli by the agar well diffusion method. Results showed concentration-dependent antimicrobial activity against all tested isolates, with maximum inhibition observed against Candida albicans. Time–kill curve analysis showed prolonged antimicrobial activity over a four-hour time period. In summary, it can be concluded that an iron oxide nanoparticle gel mediated by the Euphorbia nivulia plant may have potential as an effective formulation in wound healing and infection control, we targeted under SDG 3; Good Health Well-being, 14; Life on Land.

KEYWORDS: Antimicrobial activity, Iron oxide nanoparticles, Proliferation, Wound healing gel, Wound pathogens

INTRODUCTION
Haemostasis, inflammation, proliferation, and tissue remodelling are some of the stages in the intricate biological process of wound repair. When combined, the actions restore injured tissues to their former shape and functionality [1]. However, after receiving therapy for healed wounds, problems such as diabetic ulcers, burns, and chronic wounds may arise [2]. Making prompt and sufficient healing an ongoing, crucial health concern. The great majority of persistent injuries have microbial infections, which slow down the healing process by preventing tissue regeneration and prolonging inflammation. In the field of biomedical, the need for antibacterial wound healing systems has emerged as a major research topic [3]. Nanotechnology is now known as a promising subject for developing improved therapeutic materials for wound care. Nanoparticles have unique physicochemical properties such as high surface area, better reactivity, and more bioavailability, making them a promising option for medical approaches [4]. Experts have explored numerous forms of nanoparticles, and iron oxide nanoparticles have gained considerable interest because of their ability to adhere to magnetic properties and their ability to drive tissue healing [5]. They also show antibacterial activity against a number of pathogenic microorganisms, giving these nanoparticles viable options for use in wound healing [6]. The utilisation of plant extracts for the green manufacturing of nanoparticles has gained tremendous interest as an ecologically sustainable and

economically efficient substitute to typical chemical procedures [7]. This environmentally friendly approach eliminates the use of hazardous chemicals, while phytochemicals with therapeutic activity act as reducing and stabilising agents. Furthermore, these phytochemicals not only aid in nanoparticle synthesis but also increase their function in the body [8, 31]. Bioactive compounds found in medicinal plants, such as secondary metabolites (flavonoids, phenolics, alkaloids, and terpenoids), play an important role in the production of nanoparticles and their positive impacts [9]. Euphorbia nivulia is a medicinal plant that grows in tropical and subtropical regions. It was originally used in medical treatments to heal wounds, skin infections, and other inflammatory conditions [10]. The plant's pharmacological effects, which include antibacterial, antioxidant, anti-inflammatory, and wound healing activity, are established. Euphorbia nivulia includes bioactive chemicals, which could be employed as a test candidate for the green manufacture of nanoparticles with improved potential for treatment [11,32]. The nanoparticles of iron oxide prepared with the bio-reductive nature of Euphorbia nivulia extract can maintain one hand, the biological properties that come from the extraction of the plant, and on the other hand, imbibe functional advantages associated with nanomaterials. The combined impacts of phytochemicals and metallic oxide nanoparticles can boost antibacterial activity and restore wound healing capabilities [12].

Topical gels have been widely utilised in wound treatment because of their ease of administration, patient compliance, and ability to establish humid conditions at the wound's location [13]. The gel formulation can function as an efficient carrier system for nanoparticles, providing regulated release of the active substances and a greater reach into the local tissues [14]. Moreover, gels have a soothing effect and alleviate irritation of the wound area while ensuring better contact between the therapeutic agent and the surface of the wound. The physicochemical properties of NP-based wound-healing gels must be evaluated to ensure their stability and effectiveness [15]. These techniques aid in examining the interactions of plant phytochemicals and metal ions with nanoparticles throughout their formation process [16]. In addition, prepared gel should be tested for antimicrobial activity against the prevalent wound pathogens, including Staphylococcus aureus, Pseudomonas aeruginosa, and Escherichia coli [17]. These microorganisms are commonly known to cause infections in wounds, delaying the healing process. The efficacy of the nanoparticle-based gel to prevent or control surgical wound infection must be evaluated [18].

Hence, the current study is designed for the preparation and characterization of Euphorbia nivulia-mediated iron oxide nanoparticles-based wound healing gel as well as its evaluation against wound-associated pathogens [19,33]. The combination of plant-derived nanotechnology with topical gel formulations can be a new, effective way to develop novel, advanced wound care therapeutics with enhanced healing and antimicrobial activity [20].

MATERIALS AND METHODS

Collection and preparation of Euphorbia nivulia:

The leaves of Euphorbia nivulia were procured and used as such after binding them in shade at room temperature to remove further impurities with distilled water. Dried leaves were pulverized using a mechanical grinder. To release the bioactive compounds, 1 g of the powdered plant material was dissolved in 100 mL of distilled water and heated at 60–70 °C for a duration of about 15–20 min. This extract was then filtered with Whatman No. 1 filter paper and stored at 4 °C until used for nanoparticle synthesis.

Iron oxide nanoparticles by means of green synthesis:

Iron oxide nanoparticles were synthesized using a green synthesis approach mediated by Euphorbia nivulila extract. A precursor solution was prepared by dissolving an appropriate concentration of iron salt in distilled water. The filtered plant extract was slowly added to the precursor solution under continuous magnetic stirring at room temperature. The resultant mixture was agitated constantly for 24 hours, and a visible colour change occurred, indicating the formation of iron oxide nanoparticles. Centrifugation at 8000 rpm for 10 minutes separated the synthesised nanoparticles. The resultant pellet was cleaned with distilled water to remove residual impurities and dried for further characterisation and processing [21].

Preparation of iron oxide nanoparticle-based wound healing gel:

The wound healing gel was created using an appropriate gel base, to maintain uniformity, the dried iron oxide nanoparticles were transferred to the gel base while remaining continuously stirred. Preliminary research enabled the adjustment of nanoparticle concentration in the gel. The finished product was kept in sealed containers at room temperature for further investigation.

Physicochemical characterization of the gel formulation:

pH determination

A calibrated digital pH meter was used to measure the pH of the generated gel, to ensure skin compatibility, just a tiny bit of gel was exfoliated in distilled water, and the pH was determined at ambient temperatures [22].

Evaluation of antimicrobial activity:

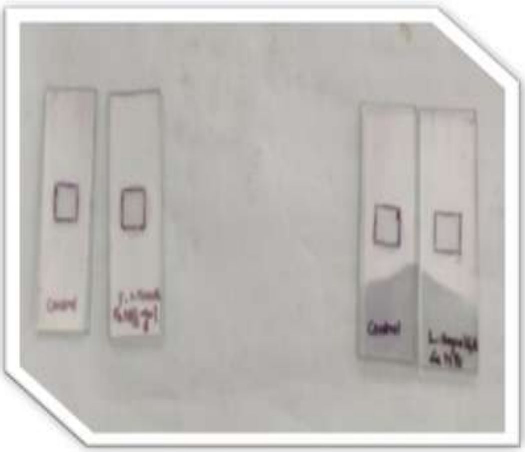
The antibacterial activity of an iron oxide nanoparticle-based gel was determined using the agar well diffusion method. Common wound pathogens that were tested were Candida albicans, Pseudomonas species, Actinobacter species, Enterococcus faecium, and Escherichia coli. Sterile nutrient agar plates were made and inoculated with standardised microbial suspensions. A sterile cork borer was used to create wells in the agar, and varied amounts of the nanoparticle gel were poured into each one. Incubation was carried out at ideal temperatures for 24 hours. Inhibition zones were measured in millimetres, and dose-dependent antibacterial activity was assessed. Positive controls used normal antibacterial agents, while negative controls used the gel base without nanoparticles [23].

Time-Kill Curve Assay:

To test the bactericidal activity of the herbal mixture, a time-kill kinetics experiment was utilised in conjunction with iron oxide nanoparticle tooth varnish. Iron oxide nanoparticles were used at different doses, including MIC (minimum inhibitory concentration), against test microorganisms such as C. albicans, Pseudomonas, Actinobacter, E. faecium, and E. coli. A sample was collected at regular intervals (0, 2, 4, 8, 12, and 24 hours), serially diluted, and plated on Mueller-Hinton agar. Plates were then incubated at 37 degrees Celsius for 24 hours, and viable colonies were counted to determine colony-forming units (CFU/mL) [24].

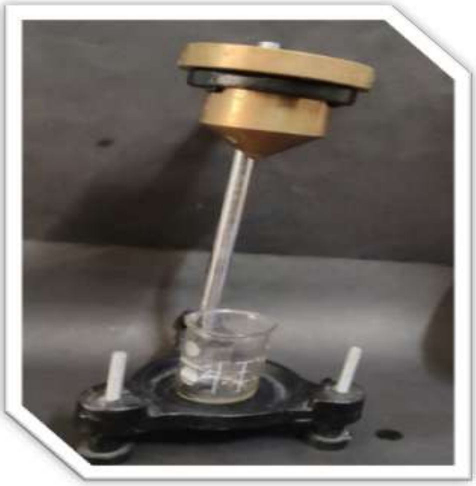
Spreadability

The conventional glass slide method was used to assess the gel's spread ability 2 glass slides were placed between a standard volume of gel, and the top slide was given a determined weight. Spread ability, or the ease of applying gel to the skin's surface, and the time it took to separate slides were also measured.



Viscosity:

A Brookfield viscometer was used to measure the gel formulation's viscosity at room temperature. To evaluate the formula's rheological behaviour, the test was run at different rotating speeds.



RESULTS

Visual observation

UV-visible spectroscopic analysis of Fe_2O_3 nanoparticles produced by Euphorbia nivulia

Based on UV–visible spectroscopic characterization, Euphorbia nivulia extract-mediated iron oxide nanoparticles were confirmed as shown in the provided spectra (Figure 1). The absorption spectrum showed a typical peak in the visible region corresponding to what has been reported for green-synthesized iron oxide nanoparticles [34], confirming metallic ion reduction into nanoparticles. The electronic transitions between iron oxide nanoparticles lead to the formation of this absorption band, suggesting the reduction of iron ions by phytochemicals derived from plants. In the present study, flavonoids, phenolics, and other reducing agents present in Euphorbia nivulia have most likely aided both the formation and stabilization of nanoparticles to prevent agglomeration while also imparting biological functionality. The UV–visible profile observed indicates the formation of Fe_2O_3 nanoparticles appropriate for biological assessment.

Figure 1: A schematic illustration of the green production of iron oxide nanoparticles using E. nivulia latex extract shows how the plant extract is processed, combined with ferric nitrate solution, filtered, incubated, and a nanoparticle suspension is created.

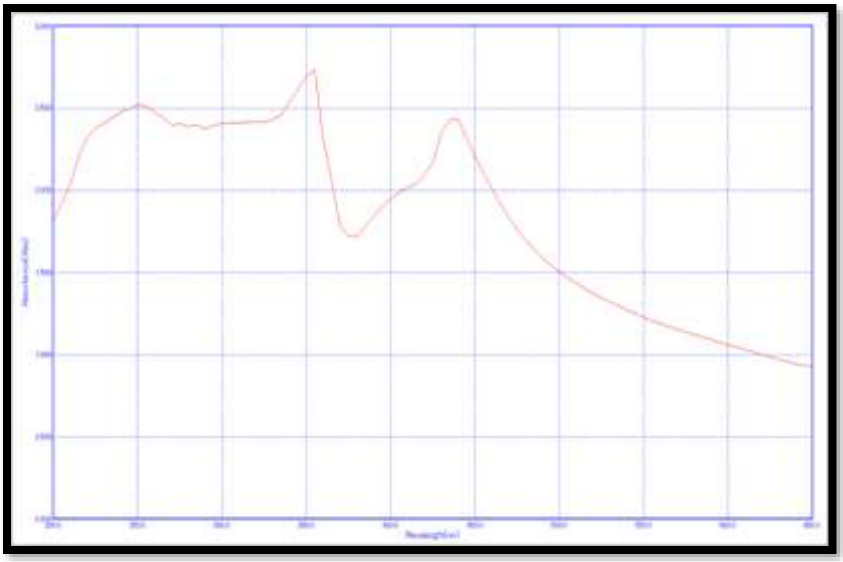


Figure 2: UV-visible absorption spectrum showing the synthesised nanoparticles' optical absorbance between 200 and 650 nm in wavelength.

Antimicrobial activity

The antimicrobial activity of the Euphorbia nivulia–mediated Fe_2O_3 nanoparticle gel was assessed using agar well diffusion against common wound pathogens Candida albicans, Pseudomonas species, Actinobacter species, Enterococcus faecium, and Escherichia coli (Figure 2). Analyses showed clear and measurable zones of inhibition surrounding wells that received the nanoparticle gel, while the control gel reagent with no nanoparticles produced little to no inhibitory effect. This confirms that the antibacterial activity encountered was due to the Fe_2O_3 nanoparticles and associated phytochemicals rather than the gel base itself. Quantitative assessment of antimicrobial effect revealed a significant dose-dependent increase in zone of inhibition for all tested microorganisms at 25, 50, and 100 µg/mL nanoparticle concentrations. The strains of Candida albicans were the most susceptible to the tested concentrations, demonstrating inhibition zones from 11 to about 14–15 mm when treated with concentrations between 25 and 100 µg/mL. The high antimycotic activity can be assigned to increased interaction of iron oxide NPs with the cell membrane and the generation of oxidative stress in fungal cells. Pseudomonas species and Enterococcus faecium showed inhibition "zones" of around 10–13 mm in increasing concentrations, indicating good antibacterial activity. The Actinobacter species exhibited moderate activity with inhibition zone diameters from 9 mm at low concentrations to around 12 mm at the concentration of 100 µg/mL. Notably, Escherichia coli showed lower sensitivity than other strains at 25 µg/mL (≈8 mm), but the inhibition increased to about 12 mm (inhibition) at 100 µg/mL. Such differences in susceptibility are likely a reflection of cell wall architecture and membrane permeability differences between gram-positive bacteria, gram-negative bacteria, and fungi.

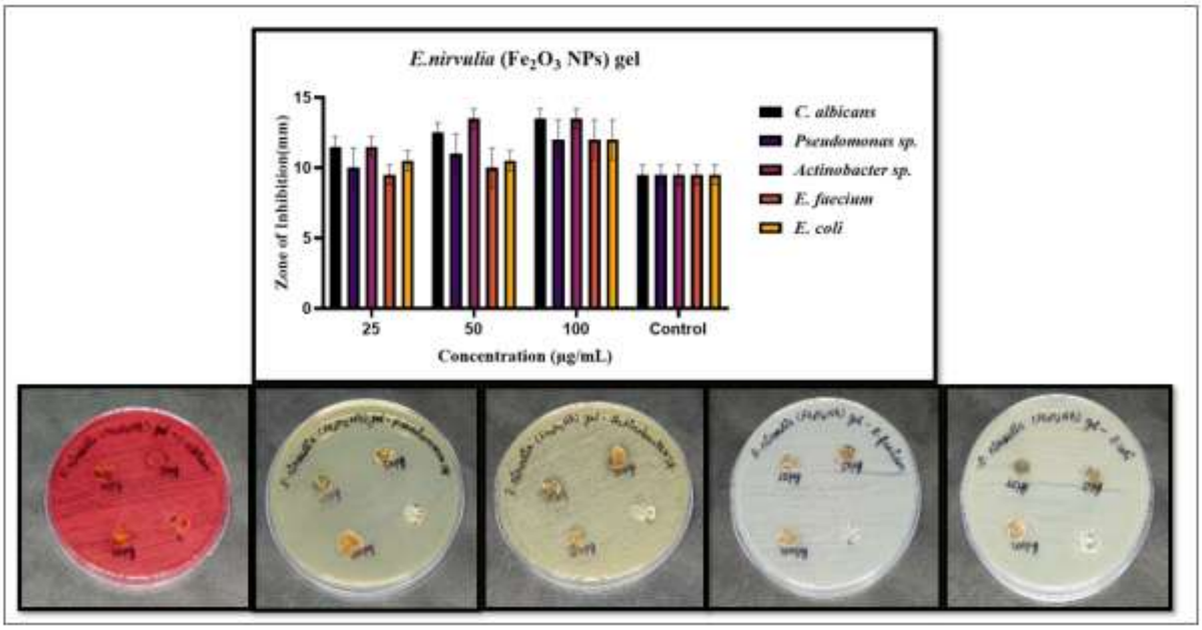


Figure 3: Agar well diffusion assay showing the antimicrobial activity of E. nivulia–mediated Fe_2O_3 nanoparticle gel against different pathogenic microorganisms at varying concentrations.

Time kill curve assay

A time–kill curve assay was conducted to assess the antimicrobial kinetics activity of the Fe_2O_3 nanoparticle gel over four hours against selected wound pathogens. While a gradual decline in microbial load was observed over time for the control group in all organisms, the standard antimicrobial showed a marked decrease in viable cells. The killing kinetics of the Fe_2O_3 nanoparticle gel were dose-dependent. The highest dosage (100 µg/mL) resulted in the greatest reduction in log CFU/mL. Candida albicans microbial load steadily dropped, but greater nanoparticle concentrations resulted in a considerable reduction by the fourth hour. Pseudomonas species and Escherichia coli both showed modest but constant declines in viable counts, demonstrating the antibacterial activity. Though the killing kinetics of Enterococcus faecium and Actinobacter species were slower, a definite decrease trend was observed across time and concentration. These results suggest that the nanoparticle gel offers long-term antimicrobial activity as opposed to fast, short-lived inhibition, which is beneficial for wound management applications.

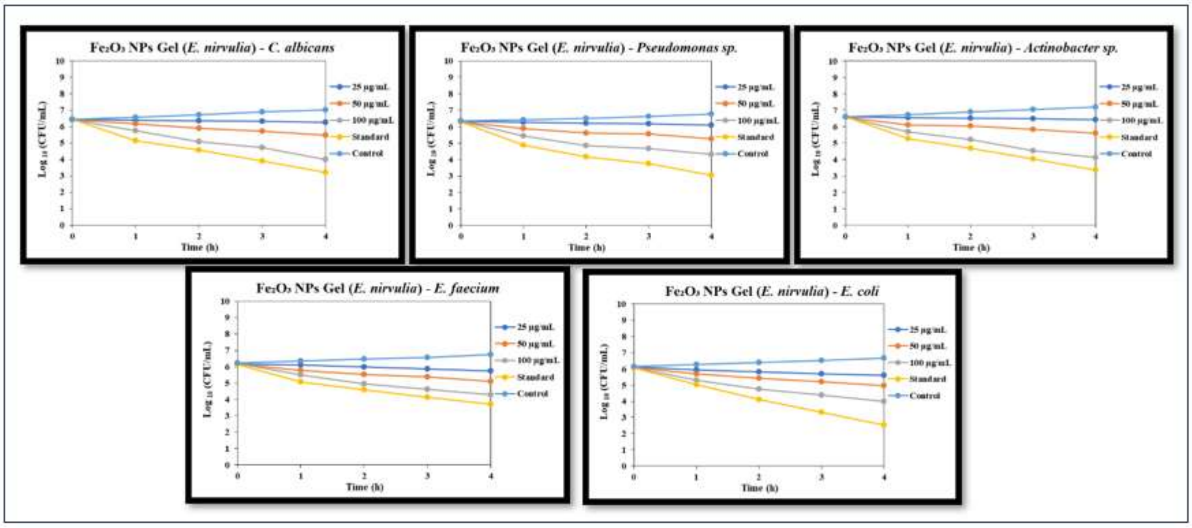


Figure 4: Time-dependent antimicrobial activity of E. nivulia–mediated Fe_2O_3 nanoparticle gel against different microbial strains measured by changes in log CFU over time.

DISCUSSION

The current study shows that the topical gel formulation of Euphorbia nivulia–mediated iron oxide nanoparticles possesses potent antimicrobial activity against a wide range of wound pathogens. The use of medicinal plant extracts for green synthesis has been regarded as an eco-friendly and bio-safe method that imparts nanoparticles with added functionality while reducing the toxicity associated with chemical synthesis approaches [25]. The UV–visible spectroscopic results verify that the Fe_2O_3 nanoparticles have been synthesized successfully, in agreement with the previous report of a plant-assisted synthetic approach on iron oxide nanoparticles. Phytochemicals present in Euphorbia nivulia were responsible for acting as reducing and capping agents, facilitating stability and biological activity of the nanoparticle. These surface-

functionalized nanoparticles were demonstrated to have improved antimicrobial activity owing to the synergistic effect between the metal oxides and plant-derived components [26].
We observed a concentration-dependent antimicrobial activity in the agar well diffusion assay, similar to other previous studies that depicted dose-dependent inhibition by metal oxide nanoparticles [27]. Given this, Candida albicans' increased sensitivity may imply that fungal cell membranes are more susceptible to oxidative stress and membrane damage caused by iron oxide NPs. Different bacterial species have different antimicrobial susceptibilities due to differences in their cell wall composition, permeability of membranes, and natural defences [28-29].
The Fe2O3 nanoparticle gel's long-term antibacterial effectiveness is supported by a time-kill kinetic analysis. The nanoparticle formulation showed persistent bacterial reduction over time, in contrast to conventional antimicrobials that might exhibit quick but transient efficacy [29-31]. In wound care settings, where prolonged exposure to bacteria can result in delayed healing and biofilm formation, this is very helpful. Iron oxide nanoparticles showed reduced microbial adhesion and anti-biofilm activity, both of which are beneficial for accelerating wound healing [32-33]. The gel's antimicrobial properties, spreadability, and viscosity highlight its potential use in topical wound applications. The main characteristics of the gel formulations longer adhesion to the wound surface, mechanical characteristics that allow controlled release of active agents, and improved patient compliance have a major impact on healing [34].
These results are beneficial, although this is only an in vitro analysis, ascertain the formulation's clinical perspective, more in vivo wound healing studies, cytotoxicity analyses, and prolonged stability assessments are needed. The present results, however, necessitate more research into its potential as a stand-alone or supplemental antibacterial wound care gel in the future.

CONCLUSION

In summary, Euphorbia nivulia leaf extract was used to create iron oxide (Fe_2O_3) nanoparticles, which were then added to a topical solution for wound healing. A relatively recent technique for creating nanoparticles that can be economical and ecologically benign is plant-mediated biosynthesis. This method eliminates the need for expensive and dangerous reducing agents. The phytochemical components present in each plant are largely responsible for the biological activity of different nanobiomaterials. In this study, we sought to demonstrate the synthesis of bioactive IONPs from biologically synthesised raw materials via plant extract reduction. The created nanoparticle gel demonstrated excellent physicochemical features, such as acceptable pH, good spreadability, and appropriate viscosity for topical skin application. The gel coated with Fe_2O_3 nanoparticles inhibited Candida albicans, Pseudomonas species, Actinobacter species, Enterococcus faecium, and Escherichia coli, according to antimicrobial profiling. The antibacterial action was concentration-dependent, with the highest inhibition at 100 µg/mL. Candida albicans was the microorganism with the highest sensitivity to the nanoparticle gel. Additionally, time-kill curve analysis demonstrated prolonged antibacterial activity for four hours, indicating that the nanoparticle formulation may eventually lower microbial load. Because it offers long-lasting defence against microbial colonisation, such prolonged antimicrobial activity is particularly beneficial in wound care. In summary, the results indicate that the Euphorbia nivulia–mediated iron oxide nanoparticle gel offers an excellent therapeutic formulation for wound healing as it provides dual advantages: combating infections and promoting healing.

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Ethics approval and consent to participate

All the data was available from public databases, and there is no need for ethics approval and consent.

Credit authorship contribution Statement:

AU: Writing-Review & editing, Writing Original Draft, PV & VR: Data curation, Visualisation, Drew the figures and pictures. SJ : Validation and editing with review of the manuscript. RS: Designed protocols for all the experiments, supervised, proofread, analysed the data, and revised the reviewer's comments, investigation, and Conceptualization. All authors reviewed and discussed the results, provided comments on the manuscript, and approved its final published version.

Consent for publication

Not applicable.

Conflict of interest

The authors state no conflict of interest

Declaration of competing interest

The authors declare that they have no conflict of interest.

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