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Preparation of gel using *Commelina benghalensis* - zinc oxide nanoparticles and its wound pathogen control

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ABSTRACT:

The rise of antibiotic-resistant infections has spurred interest in alternative antimicrobial therapies. *Commelina benghalensis,* rich in potassium, iron, calcium, ascorbic acid, and beta carotene, has long been used in traditional medicine. Combined with zinc oxide (ZnO) nanoparticles, known for their antibacterial properties, it presents a promising approach for advanced wound healing treatments. In this study, a gel formulation containing ZnO nanoparticles and *Commelina benghalensis* extract was developed and evaluated against common wound pathogens, including Pseudomonas aeruginosa and Staphylococcus aureus.Characterization using Fourier-transform infrared spectroscopy (FTIR), dynamic light scattering (DLS), and scanning electron microscopy (SEM) confirmed the gel's composition and structure. The gel exhibited significant antibacterial activity, effectively inhibiting the growth of S. aureus and P. aeruginosa. Its controlled release properties allowed sustained antibiotic delivery for up to 96 hours, enhancing therapeutic efficacy. Furthermore, the addition of hydroxyethyl cellulose improved rheological properties, facilitating faster wound healing in animal models. Overall, the synergistic antibacterial action of *Commelina benghalensis* and ZnO nanoparticles, coupled with enhanced rheological characteristics and controlled drug release, highlights the gel's potential for managing wound infections and promoting healing.

Keywords:*Commelina benghalensis*, zinc oxide nanoparticles, antibacterial activity, wound healing, controlled release, gel formulation

1 INTRODUCTION

The increasing danger of infections resistant to antibiotics has sparked extensive research into alternative antimicrobial medications. *Commelina benghalensis leaves* are a good source of beta-carotene, ascorbic acid, calcium, iron, and potassium(Orni, Shetu et al. 2018, Ambika, Manojkumar et al. 2019).*Commelina benghalensis has* been investigated for its potential in vitro roles as an antidiarrheal, anthelmintic, antibacterial, anxiolytic, antioxidant, antitumor, anticancer, and thrombolytic agent(Ezeabara, Chukwu et al. 2019).Zinc oxide nanoparticles' antibacterial properties combined with the unique properties of *Commelina benghalensis* present a promising avenue for the development of improved wound healing therapies.*Commelina benghalensis supposedly* treats fever, headaches, leprosy, jaundice, and discomfort. It has been used to treat insanity, psychosis, thrush in the mouth, and epilepsy. *Commelina benghalensis has* been used traditionally to cure a variety of ailments, including burns, leprosy, sore throats, pain, and inflammations. It has also been used as a laxative, emollient, and demulcent. In order to encourage the production of urine and treat diseases linked to water retention and urinary tract infections, *Commelina benghalensis is* also used as a diuretic(Kansagara and Pandya 2019, Tayyeb, Priya et al. 2024).*Commelina benghalensis* has long been used to cure a variety of conditions, such as burns, leprosy, sore throats, pain, and inflammations(Chockalingam, Sasanka et al. 2020, Roshan, Jothipriya et al. 2020). It is also a laxative, emollient, and demulcent. *Commelina benghalensis leaves* are a good source of potassium, iron, calcium, ascorbic acid and Beta carotene(Sundaram, Bupesh et al. 2022). The characteristics of the gel made with *Commelina benghalensis* -Zinc oxide nanoparticles help suppress wound pathogens. *Commelina benghalensis (*CBE) treatment enhanced LPO and substantially reversed the changes in antioxidant profiles(Kokilavani, Suriyakalaa et al. 2014, Marunganathan, Kumar et al. 2024). In reaction to acidic medium, the gel can sustain and regulate the release of the antibiotic vancomycin for up to 96 hours. Additionally, it demonstrates strong and effective antibacterial action against two common wound infections, *S. aureus and P. aeruginosa*.Polysaccharides, such hydroxyethyl cellulose, improve the gel's microstructure and rheological characteristics(Nasim, Rajeshkumar et al. 2021, Sivakumar, Geetha et al. 2021). This leads to the production of irregularly shaped particles that come together to form aggregates. When applied to burn wounds, the gel of ZnO nanoparticles treated with hydroxyethyl cellulose has a strong regenerative impact and promotes faster healing than control groups. The rate at which animals treated with zinc oxide nanoparticle gel cure burn wounds is 16.23% higher than that of animals treated with hydroxyethyl cellulose gel(Devi 2016, Balaji, Bhuvaneswari et al. 2022).

Commelina benghalensis and zinc oxide nanoparticles were also used to make a gel that has excellent thermal stability and strong antibacterial activity against a variety of diseases, making it appropriate for biological applications. Overall, the gel made with zinc oxide nanoparticles from *Commelina benghalensis shows* encouraging results and healing qualities for the treatment of wound pathogens(Sabnam, Sangeetha et al. 2024).By using nanocomposite hydrogels, formulations' antibacterial qualities are increased, adverse effects are decreased, and drug delivery effectiveness is improved(Devi 2016, Nasim, Kumar et al. 2020). In comparison to alternative wound pathogen controls, zinc oxide nanoparticles infused into gel improved bacterial clearance, increased tissue creation, and promoted epidermal regeneration, according to antimicrobial tests.Zinc oxide nanoparticles added to the hydrogels have excellent antibacterial properties against various infections, which makes them appropriate for use in wound healing applications(El-Hamid and El Bous 2019, Ravikumar, Marunganathan et al. 2024, Rizwana, Sangeetha et al. 2024).Because of the hydrogels' regulated release characteristics, medications or antibacterial agents can be delivered consistently and precisely.Analyzing the hydrogels' structure, morphology, gel fraction, swelling level, antibacterial qualities, biocompatibility, and biodegradation are all part of their characterization. Commelina, which has in vitro antioxidants and antibacterial properties, is incorporated into the gel to significantly restrict the growth of food pathogenic bacteria and scavenge free radicals.Zinc oxide nanoparticles are combined with various polymers, including polyvinyl alcohol, chitosan, carboxymethylcellulose, and hyaluronic acid, to optimize the hydrogel formulations.In vitro investigations on drug release kinetics, antibacterial activity, and cell viability, as well as in vivo research on wound healing in animal models, are used to evaluate the hydrogels. In addition to natural *Commelina benghalensis* extracts, nanotechnology added to the gel produces significant results such as antibacterial capabilities from zinc oxide nanoparticles(Girija and Ganesh 2022, Chhabria, Prathap et al. 2024).

The results indicate that these hydrogels based on zinc oxide nanoparticles have the potential to be effective drug delivery systems and medicines with improved antibacterial properties and controlled release capabilities(Hasan, Hossain et al. 2009, Jain, Selvi et al. 2021). They can also be used as wound pathogen healing systems.Through an examination of the synthesis process, characterization, and evaluation of the gel's efficacy in managing wound infections and overcoming biological barriers, this work illuminates a possible breakthrough in wound care.Numerous characterization approaches are employed in order to assess the properties of the gel formulation. The size and shape of nanoparticles can be determined using morphological analysis techniques such as scanning electron microscopy (SEM). By evaluating the particle size distribution and zeta potential using dynamic light scattering (DLS), the stability and colloidal behavior of the gel are made clear. Additionally, by detecting interactions between components, Fourier-transform infrared spectroscopy, or FTIR, is utilized to confirm that nanoparticles have been successfully absorbed into the gel matrix(Ponmanickam, Gowsalya et al. 2022, Bharathi, Babu et al. 2024). The bacterial strains that commonly cause wound infections were shown to be susceptible to the gel matrix compositions' effectiveness.Due to the combination of zinc oxide nanoparticles and *Commelina benghalensis extract*, the gel formulation exhibits strong antibacterial qualities. By rupturing bacterial membranes and producing reactive oxygen species (ROS), zinc oxide nanoparticles demonstrate intrinsic antibacterial action by causing bacterial cell death. The bioactive components of *Commelina benghalensis extract*, such as flavonoids and alkaloids, which have antibacterial and anti-inflammatory qualities, enhance this action. By successfully blocking the growth of common wound pathogens including Staphylococcus aureus and Pseudomonas aeruginosa, these ingredients work together to lower the risk of infection and promote wound healing.Creating a gel with *Commelina benghalensis* -Zinc Oxide nanoparticles is a potentially effective method for managing wounds.Commercialized goods with nanoparticles manufactured from zinc oxide are reported to be more efficient in lowering colon cancer cell proliferation by utilizing the antibacterial capabilities of both natural extracts and nanomaterials.It provides a successful way to manage wound infections and encourage wound healing. Its clinical effectiveness and safety profile need to be further investigated in order to prepare the path for its incorporation into standard wound care procedures(Rajeshkumar and Lakshmi 2021).

2 Materials and methods

2.1 Preparation of Test Samples

The *Commelina benghalensis* -mediated zinc oxide nanoparticles were synthesized through a method involving the presence of *Commelina benghalensis* extract during the nanoparticle formation process. Initially, zinc oxide nanoparticles were prepared by a suitable chemical or physical method, and then *Commelina benghalensis* extract was introduced into the reaction mixture. This extract likely acted as a capping and stabilizing agent, influencing the size, shape, and stability of the nanoparticles. Dynamic light scattering (DLS) was employed to determine the hydrodynamic size distribution of the nanoparticles in solution, providing insights into their colloidal stability(Khatun, Rahman et al. 2020, Verma, Prathap et al. 2024). Additionally, scanning electron microscopy (SEM) was utilized to observe the morphology and confirm the size range of the nanoparticles, ensuring uniformity and reproducibility of the synthesis process. These characterization techniques collectively verified the formation of *Commelina benghalensis* -mediated zinc oxide nanoparticles with the desired properties for subsequent antimicrobial testing and biomedical applications(Okoko , Anbarasu, Vinitha et al. 2024).

2.2 Microbial Cultures

Before conducting the time-kill assay, bacterial cultures of Pseudomonas aeruginosa, Escherichia coli, and Staphylococcus aureus were subjected to a pre-incubation phase in Mueller Hinton broth devoid of antimicrobial agents(Rajeshkumar and Lakshmi 2021, Umapathy, Pan et al. 2024). This pre-incubation step, lasting 4 hours at 37°C, aimed to synchronize the growth of the bacterial cultures to the early-to-mid log phase. By allowing the bacteria to reach a consistent growth stage before initiating the assay, variations in initial bacterial densities were minimized, ensuring more accurate and comparable results across different treatment groups. The pre-incubation process also facilitated uniform metabolic activity and physiological state among the bacterial populations, optimizing the conditions for subsequent exposure to *Commelina benghalensis* -mediated zinc oxide nanoparticles and the assessment of their bactericidal effects using the time-kill curve assay(Ezung, Singh et al. 2023).

2.3 Time-Kill Curve Assay

In the time-kill curve assay, each well of a 96-well ELISA plate was prepared with 90 µL of pre-heated antimicrobial-free Mueller Hinton broth. To initiate the assay, 30 µL of the pre-incubated bacterial inoculum, adjusted to 0.5 McFarland standard in sterile phosphate-buffered saline, was added to each well, resulting in a final volume of 120 µL per well. *Commelina benghalensis* -mediated zinc oxide nanoparticles were introduced into designated wells at concentrations ranging from 25 to 125 µg/mL, while an untreated control containing only bacterial inoculum and broth served as a comparison. Additionally, a standard antibiotic control, such as Bacteria-Amoxyrite or Fungi-Flucanazole, was included at a clinically relevant concentration. The ELISA plate was then incubated at 37°C, and samples were collected at regular intervals (0, 2, 4, 6, 8, and 24 hours) for analysis. This setup allowed for the assessment of the concentration-dependent bactericidal effects of *Commelina benghalensis* -mediated zinc oxide nanoparticles against Pseudomonas aeruginosa, Escherichia coli, and Staphylococcus aureus over specified time points, providing crucial insights into their potential as antimicrobial agents for wound care applications(Ahamed, Farzana et al. 2019, Giridharan, Chinnaiah et al. 2024).

2.5 Statistical Analysis

The data from the time-kill curve assay were subjected to statistical analysis to evaluate the concentration-dependent bactericidal effects of *Commelina benghalensis* -mediated zinc oxide nanoparticles compared to the untreated control and standard antibiotic(Sangeetha, Taniya et al. 2024). Statistical tests such as analysis of variance (ANOVA) or t-tests were likely employed, depending on the experimental design and distribution of the data. These tests allowed for comparisons between different nanoparticle concentrations and time points, determining significant differences in bacterial growth inhibition. The results were interpreted to assess the effectiveness of the nanoparticles in reducing bacterial viability over time, quantified as log10 colony-forming units (CFU) per milliliter. The statistical analysis provided robust evidence of the nanoparticles' antimicrobial activity, highlighting their potential as a therapeutic agent against Pseudomonas aeruginosa, Escherichia coli, and Staphylococcus aureus infections in wound care management(Rajeshkumar, Lakshmi et al. 2021).

3. Results

Fig 1 . preparation of plant extract

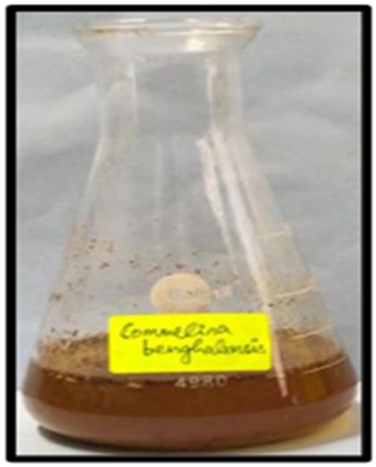

Fig 2. *Commelina benghalensis* mixed with distilled water

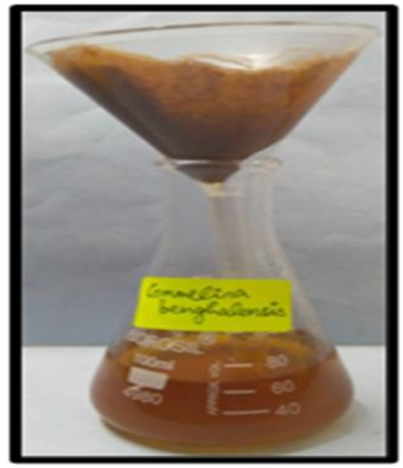

Fig 3. Commelina benghalensis solution filtered using Whatman filter paper no. 1

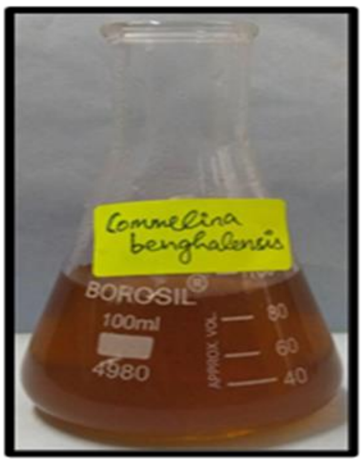

Fig 4. Filtered solution of plant extract

Fig 5. Zinc sulphate solution

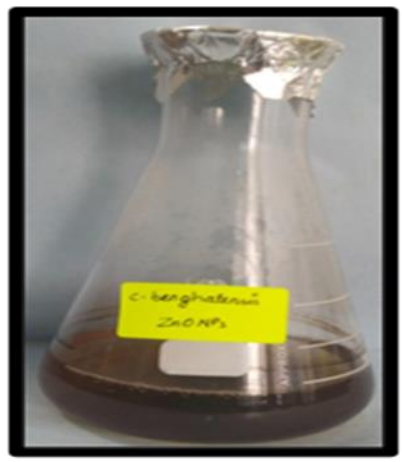

Fig.6 : Commelina benghalensis mixed with ZnO nanoparticles

Table 1 presents the zone of inhibition values (in mm) corresponding to different concentrations (25, 50, and 100 µg/mL) of *Commelina benghalensis* -mediated zinc oxide nanoparticles against various bacterial strains, including Pseudomonas sp., E. coli, S. aureus, and E. faecalis. Each cell in the table denotes the diameter of the clear zone around the nanoparticle-containing discs on agar plates, indicating the extent of bacterial growth inhibition.For Pseudomonas sp., E. coli, and S. aureus, the zone of inhibition generally increases with higher nanoparticle concentrations, suggesting a concentration-dependent antibacterial effect(Tiwari, Lahkar et al. 2013, Johnson, Shanmugam et al. 2022). Specifically, E. coli and S. aureus show larger zones of inhibition at higher concentrations (50 and 100 µg/mL), indicating stronger antimicrobial activity against these pathogens compared to lower concentrations (25 µg/mL). In contrast, E. faecalis exhibits consistent zone sizes across all nanoparticle concentrations tested, suggesting a uniform response to the antimicrobial properties of the nanoparticles.The standard values listed in the table, all showing a zone of inhibition of 9 mm, likely represent control samples where no nanoparticles were added, ensuring a baseline for comparison. These results provide quantitative data on the efficacy of *Commelina benghalensis* -mediated zinc oxide nanoparticles against common bacterial pathogens, highlighting their potential as antimicrobial agents in biomedical applications, particularly in wound healing and infection control(Kumar, Kumar et al. 2023, Anbarasu, Vinitha et al. 2024).

Microbes	**25**	**50**	**100**	**Standard**
Pseudomonas sp.	**9**	**9**	**9**	**9**
E. coli	**9**	**10**	**12**	**9**
S. aureus	**9**	**9**	**12**	**9**
E. faecalis	**9**	**9**	**9**	**9**

Table 1. Table 1 represents the Numerical value of Zone of inhibition (in mm) with respective concentrations of 25, 50 and 100 micrograms per milliliter

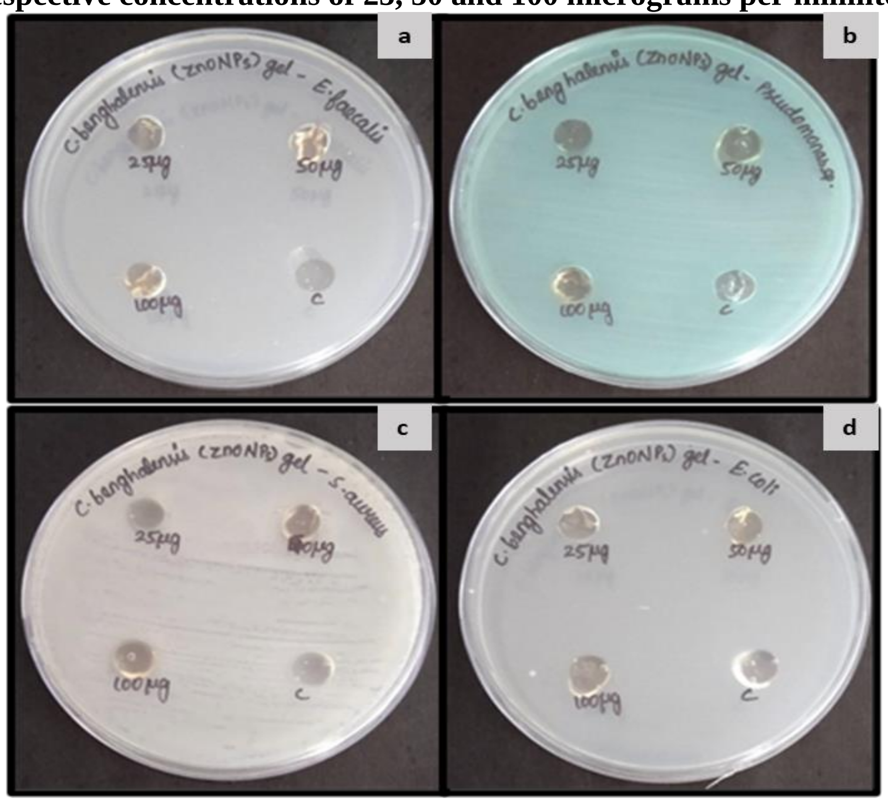

Fig 7. Bactericidal effects of *Commelina benghalensis* -mediated zinc oxide nanoparticles againsta.*Enterococcus faecalis* b. *Pseudomonas aeruginosa* c. *Staphylococcus aureus* d. *Escherichia coli*

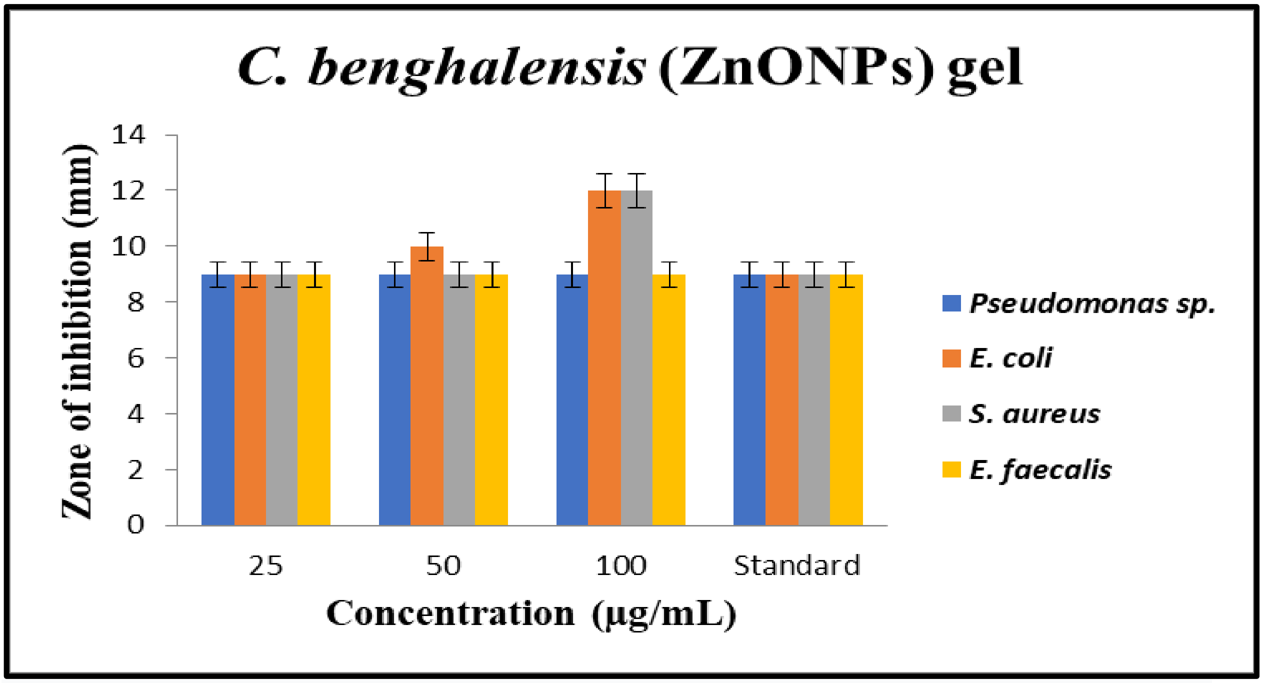

Fig 8. the graph indicates that the standard control is represented by a constant value of 9 mm for zone of inhibition, serving as a baseline comparison against the varying concentrations (25, 50, and 100 µg/mL) of *Commelina benghalensis* (ZnONPs) gel tested against Pseudomonas sp., E. coli, S. aureus, and E. faecalis(Batool, Aziz et al. 2020).

Represents antibacterial activity of C. benghalensis (ZnONPs) gel against 4 bacterial species:Pseudomonas sp., E. coli, S. aureus, and E. faecalis measured by the zone of inhibition (in mm) at concentrations of 25, 50, and 100 µg/mL, compared to standard. At 25 µg/mL, all bacteria exhibit an inhibition zone of approximately 8.5 mm. At 50 µg/mL, the zone of inhibition increases to approximately 9 mm for all bacteria. At 100 µg/mL, Pseudomonas sp. and E. faecalis have an inhibition zone of approximately 10 mm, S. aureus shows around 11 mm, and E. coli exhibits the highest inhibition zone at 12 mm. The standard consistently shows an inhibition zone of approximately 10 mm for all bacteria. The data indicates that the antibacterial activity of the gel increases with concentration, achieving the highest effectiveness at 100 µg/mL, particularly against E. coli with a 12 mm zone of inhibition. The gel's effectiveness at 100 µg/mL is comparable to the standard, especially for Pseudomonas sp. and E. faecalis. E. coli is the most susceptible to the gel at higher concentrations(Divyadharsini, UmaMaheswari et al. 2022, Khalid, Martin et al. 2024).

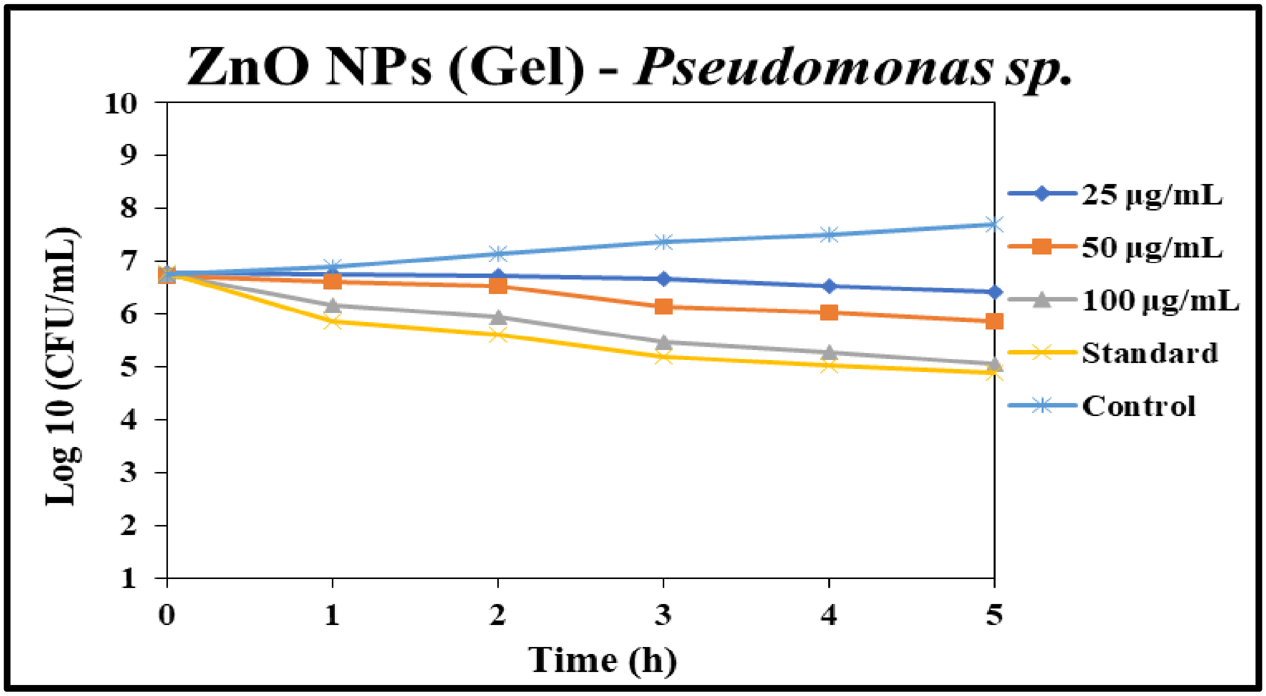

Fig 9. the graph indicates that the X-axis represents time in hours, while the Y-axis represents the colony forming units per milliliter (CFU/mL) of Pseudomonas, demonstrating the time-dependent bactericidal effect of ZnO NPs gel.

The antibacterial activity of ZnO NPs gel against Pseudomonas sp. is concentration-dependent, with higher concentrations (100 µg/mL) showing a more significant reduction in bacterial count compared to 25 and 50 µg/mL. At 100 µg/mL, the gel's effectiveness is almost comparable to the standard, indicating strong antibacterial properties(Raj, Martin et al. 2024). In contrast, the control group exhibits an increase in bacterial count over time, highlighting the proliferative capability of Pseudomonas sp. without treatment. Additionally, the antibacterial activity is time-dependent, with longer exposure leading to a more significant reduction in bacterial count. The most substantial reduction is observed within the first 2 hours, after which the bacterial count stabilizes, especially at higher concentrations.Implications for Wound Treatment:ZnO NPs gel, particularly at higher concentrations, could be a potent antibacterial agent for treating wounds infected with Pseudomonas sp.The gel's effectiveness in reducing bacterial load can help prevent infections and promote faster wound healing(Manickam, Giridharan et al. 2022, Sneha Sree, Selvi et al. 2024).

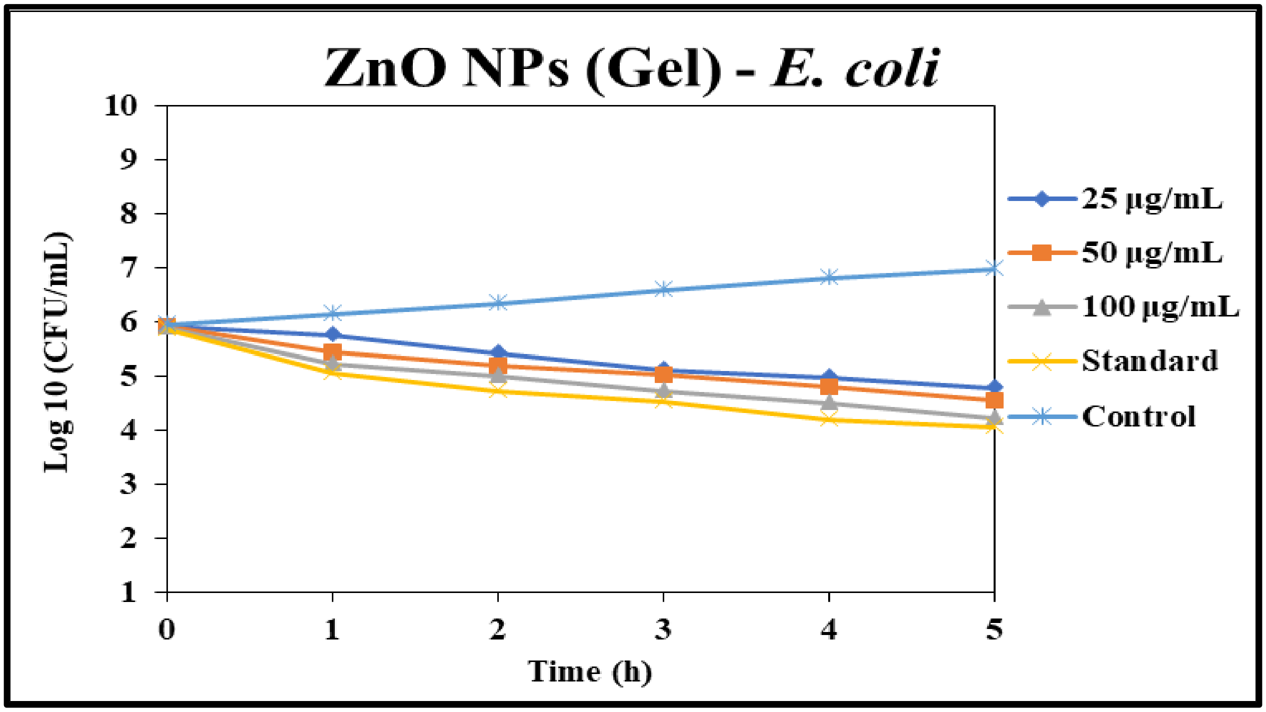

Fig 10. the graph specifies that the X-axis represents time in hours, while the Y-axis depicts the colony forming units per milliliter (CFU/mL) of E. coli, illustrating the temporal dynamics of the bactericidal activity exerted by ZnO NPs gel.

The effect of different concentrations of ZnO nanoparticles (ZnO NPs) gel on E. coli shows that higher concentrations (50 µg/mL and 100 µg/mL) are more effective in reducing bacterial counts over time compared to lower concentrations (25 µg/mL). At 25 µg/mL, the bacterial count remains relatively stable around 6 log CFU/mL, while at 50 µg/mL, it decreases to around 4.5 log CFU/mL, and at 100 µg/mL, it drops to around 4 log CFU/mL by the 5th hour. The standard treatment also reduces the count to about 4 log CFU/mL. This indicates a dose-dependent antibacterial effect of ZnO NPs, with higher concentrations leading to more significant reductions in E. coli growth.

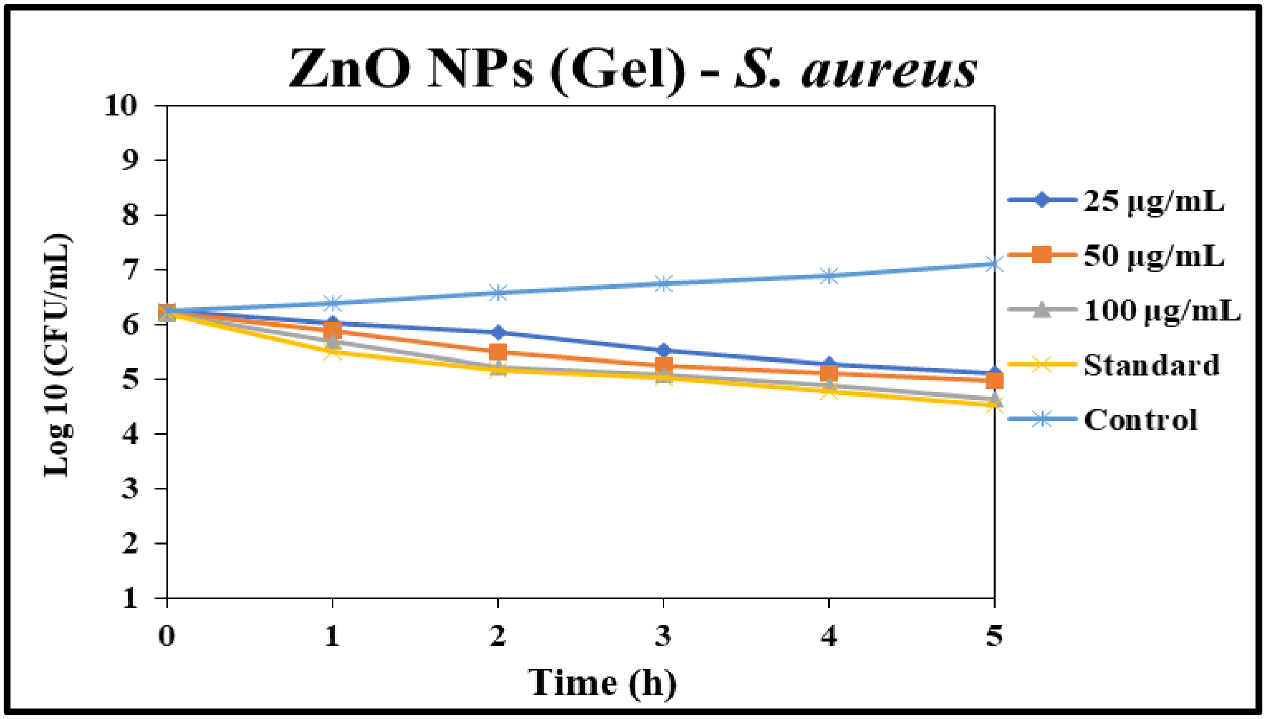

Figure 11. The graph clarifies that the X-axis denotes time in hours, while the Y-axis measures the colony forming units per milliliter (CFU/mL) of Staphylococcus aureus, illustrating the time-dependent impact of ZnO NPs gel on bacterial viability.

The antibacterial activity of ZnO nanoparticles (ZnO NPs) in gel form against Staphylococcus aureus over a 5-hour period. Initially, all groups, including the control, start with a bacterial count of approximately 6 Log 10 CFU/mL. As time progresses, the control group shows an increase in bacterial count, reaching nearly 7 Log 10 CFU/mL by the end of 5 hours. In contrast, groups treated with ZnO NPs demonstrate a decrease in bacterial counts over time. The highest concentration of ZnO NPs (100 µg/mL) exhibits the most significant antibacterial effect, reducing bacterial counts to around 5 Log 10 CFU/mL by the end of the period. The 50 µg/mL and 25 µg/mL concentrations also show reductions, although less pronounced compared to the 100 µg/mL group. A standard treatment shows antibacterial efficacy similar to the 100 µg/mL ZnO NP group. Therefore, the graph clearly indicates a dose-dependent relationship, where higher concentrations of ZnO NPs are more effective in reducing S. aureus bacterial counts over the experimental timeframe(Kamath, Nasim et al. 2022).

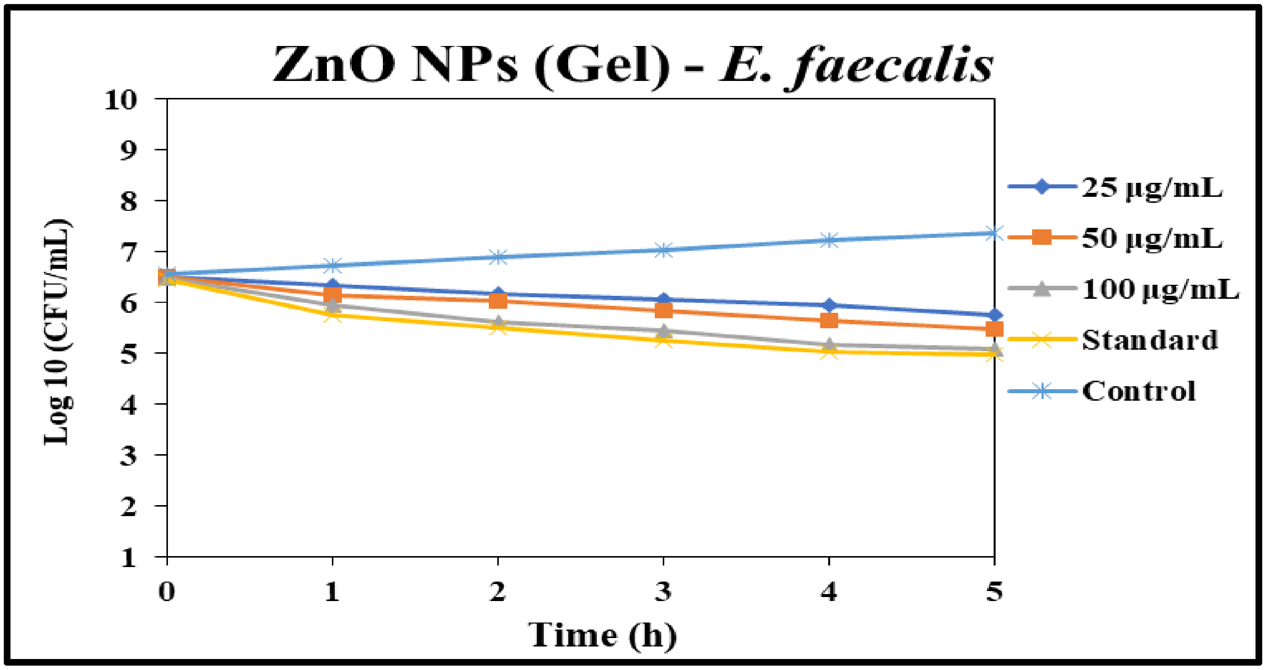

Fig 12. The graph indicates that the X-axis represents time in hours, while the Y-axis displays the colony forming units per milliliter (CFU/mL) of Enterococcus faecalis, demonstrating the temporal changes in bacterial viability under the influence of ZnO NPs gel.

The graph depicts the antibacterial efficacy of ZnO nanoparticles (ZnO NPs) in gel form against Enterococcus faecalis (E. faecalis) over a 5-hour period. Initially, all groups start with a bacterial count of approximately 6 Log 10 CFU/mL at 0 hours. By the end of the observation period, the control group shows an increase in bacterial count to about 7 Log 10 CFU/mL. In contrast, the groups treated with ZnO NPs at concentrations of 25 µg/mL, 50 µg/mL, and 100 µg/mL, as well as the standard treatment, exhibit decreasing bacterial counts over time. The highest concentration of ZnO NPs (100 µg/mL) demonstrates the most significant antibacterial activity, reducing the bacterial count to approximately 5 Log 10 CFU/mL by the 5-hour mark, comparable to the standard treatment(Nasim, Jabin et al. 2022).

4 Discussion

The study explores the synergistic potential of *Commelina benghalensis* -mediated zinc oxide nanoparticles (ZnO NPs) in developing an advanced gel formulation for wound healing applications. *Commelina benghalensis,* rich in bioactive compounds like beta-carotene, ascorbic acid, and minerals such as calcium and potassium, has been traditionally used for its therapeutic properties ranging from antibacterial and antioxidant to anti-inflammatory effects. When combined with ZnO NPs, known for their intrinsic antibacterial properties through membrane disruption and reactive oxygen species (ROS) generation, the gel formulation exhibited significant antimicrobial efficacy against common wound pathogens like Staphylococcus aureus and Pseudomonas aeruginosa.The gel's effectiveness was demonstrated through various experiments, including zone of inhibition assays and time-kill kinetic analyses, revealing concentration-dependent bactericidal effects over time(Vaishnavi and Rajasekar 2021, Deepika, Ramamurthy et al. 2022). Hydrogel formulations enhanced with zinc oxide nanoparticles and *Commelina benghalensis* extract showed improved thermal stability, controlled release capabilities, and enhanced rheological properties due to additives like hydroxyethyl cellulose. These enhancements are critical for maintaining sustained antimicrobial activity and promoting accelerated wound healing, as evidenced by improved tissue regeneration rates observed in animal models compared to controls.Characterization techniques such as scanning electron microscopy (SEM), dynamic light scattering (DLS), and Fourier-transform infrared spectroscopy (FTIR) confirmed the successful integration of nanoparticles into the gel matrix and elucidated their structural and chemical interactions. This comprehensive approach underscores the potential of nanotechnology-enhanced hydrogels in overcoming biological barriers and optimizing therapeutic outcomes in wound care management(Gandhi, Gurunathan et al. 2021).

5 Conclusion

In conclusion, the gel formulation incorporating *Commelina benghalensis* -mediated zinc oxide nanoparticles represent a promising advancement in wound healing therapies. The synergistic antibacterial properties of ZnO NPs and bioactive compounds from *Commelina benghalensis* offer effective control of wound pathogens, facilitating faster recovery and reduced infection risks. The enhanced gel properties, including controlled drug release and improved rheology, underscore its potential for clinical applications in treating various wound types. Further research and clinical trials are warranted to fully assess its safety, efficacy, and potential integration into standard wound care protocols.

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