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Original Article

Microbiology Section

Green-mediated Silver-Carbon-Chitosan Nanocomposite Wound Healing Gel using *Musa paradisiaca*: An In-vitro Study against Wound-assosciated Pathogens

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ABSTRACT

Introduction: The rise of antibiotic-resistant wound infections highlights the need for alternative antimicrobial agents. Green synthesis using plant extracts provides an eco-friendly approach, and *Musa paradisiaca* leaves contain phytochemicals that aid nanoparticle synthesis and stabilisation.

Aim: To synthesise a green-mediated silver–carbon–chitosan nanocomposite (C-AgNCs) using aqueous leaf extract of *Musa paradisiaca* leaf extract and evaluate its physicochemical characteristics, antimicrobial, antibiofilm, and time-kill activities against selected wound-associated pathogens.

Materials and Methods: This in-vitro experimental study was conducted at the Department of Microbiology, Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical Sciences, Chennai, Tamil Nadu, India, over a period of three months from January 2026 to March 2026. C-AgNCs were synthesised using a green method, aqueous leaf extract. Nanoparticles were confirmed by visible colour change and UV-visible spectroscopy, showing a Surface Plasmon Resonance (SPR) peak at 440 nm, indicating spherical morphology. Antimicrobial activity was assessed using the agar well diffusion method against *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Enterococcus faecium*, *Escherichia coli*, and *Staphylococcus aureus*. Time-kill kinetics was evaluated through optical density measurements over four hours. Antibiofilm activity was analysed at varying concentrations.

Results: Synthesised C-AgNCs exhibited significant antimicrobial activity, with a maximum inhibition zone of 18 mm against *Pseudomonas* sp. at 100 µL. Time-kill assays demonstrated dose- and time-dependent bactericidal effects, particularly against *E. coli* and *S. aureus*, with substantial reduction in viable cells within 2-4 hours. Antibiofilm showed concentration-dependent inhibition, with *E. coli* being highly sensitive.

Conclusion: Green-synthesised C-AgNCs demonstrate strong antimicrobial and antibiofilm properties and show promising potential as a sustained-release therapeutic agent for managing chronic wound infections.

Keywords: Anti-bacterial agents, Biofilms, Chitosan

INTRODUCTION

Nanotechnology has become an important area in biomedical research because nanoparticles possess unique physical and chemical properties, making them highly useful for a wide range of therapeutic applications [1]. Among different types of nanoparticles, silver nanoparticles (AgNPs) have been widely studied for their potent antimicrobial activity, notable anti-inflammatory effects, and ability to support wound healing. The green synthesis of AgNPs using plant-derived extracts is considered a sustainable and cost-effective method, offering improved biocompatibility and reducing reliance on toxic chemical substances [2].

Infections in chronic wounds caused by drug-resistant microorganisms are still a significant problem in modern healthcare. Silver nanoparticles (AgNPs) show strong, broad-spectrum antimicrobial properties by affecting the integrity of microbial cell membranes and inhibiting key intracellular proteins and enzymes [3]. Because of these characteristics, silver nanoparticles (AgNPs) are being more widely used in wound dressings, hydrogels, and topical products to improve wound care and healing outcomes. Moreover, they have modest immune responses and economic cytotoxicity. They are extensively investigated for molecular detection, medication delivery, and medical imaging. They also serve therapeutic purposes, such as in the creation of surgical meshes and the replacement of synthetic joints. Both caring for injuries and using remedies to promote wound repair [4]. The growth of microbes resistant to several antibiotics, combined with increased pressure to decrease health-care costs, has fuelled efforts to discover novel antimicrobial medicines that are both effective and cost-effective. As a result, there has been a renewed emphasis on silver-based antiseptics, which have broad-spectrum efficacy and a significantly lower risk of developing microbial resistance than standard antibiotics [5].

They are used in a variety of biological, pharmacological, and physical domains due to their potent anti-inflammatory and antibacterial properties. For example, an ointment or cream containing AgNPs for burns and wounds to prevent microbial infection. Wound healing is a complex process and takes a really long time. In order to shorten the process, scientists have formulated many nanotechnological techniques to achieve greater heights [6]. Vital properties of an optimal wound healing gel would be collagen production, remodelling to strengthen it, rejuvenating the elastic property of the skin, phagocytosis, chemotaxis and angiogenesis [7]. According to recent research, previously published, Polymerised Methacrylate (PMMA) containing one weight per cent graphene-AgNPs exhibited effective antibacterial properties against Streptococcus mutans and other infections of the oral cavity, as well as low toxicity to cell membranes [8].

Carbon is everywhere in this world: the air we breathe, the food we eat, etc. Medicinal plants formulated with carbon act as a homeostatic agent, which, in fact, is a good property when it comes to wound healing. A type of Carbon Nanoparticles (CNP)

called Carbon dots, when infused with heteroatoms, can increase their catalytic properties and help enhance their healing ability in skin regeneration [9]. A natural biocompatible polysaccharide called Chitosan is highly degradable and has antimicrobial properties. When biochemically engineered, it helps in skin regeneration, wound healing and controls pathogen growth. Chitosan formulated with Polyvinyl Alcohol (PVA) hydrogel is helpful in wound dressing and incorporated with nanoparticles like silver helps in reforming its antimicrobial properties and makes it a stable wound dressing substance [10]. CNPs and chitosan are also showing strong potential in biomedical use. Carbon-based nanomaterials improve antimicrobial activity and tissue regeneration, while chitosan, a biodegradable polysaccharide, is well known for its antimicrobial effects and ability to promote wound healing [11]. Adding nanoparticles to chitosan-based hydrogels enhances their stability and increases their effectiveness in wound healing applications.

Some natural substances that have the ability to cure wounds have procollagen production, antibacterial, antioxidant, and anti-inflammatory properties. *Musa paradisiaca* possesses antioxidant, antimicrobial, and anti-inflammatory properties and has been traditionally used for wound treatment. A combination of silver nanoparticles and banana extract has a high antimicrobial activity against gram-positive and gram-negative bacteria. Plant-mediated synthesis using *Musa paradisiaca* extracts has shown effective nanoparticle formation with enhanced biological activity [12]. All these nanoparticles are combined and formulated into a gel fit for wound healing purposes. A hydrogel is a type of 3D water-formulated gel, made from synthetic or natural substances like chitosan, silver nanoparticles, alcohol, etc. [13]. A silver mixed with CNP was formulated and mixed in a chitosan-based hydrogel, where most of its individual properties, like fast regeneration, wound dressing and antimicrobial properties, were retained. The investigation especially evaluated its time kill kinetic assay, antimicrobial, and antibiofilm properties. Antimicrobial property and wound regeneration, and also helps with regeneration. The motive of adding AgNCs was to increase the range of AgNCs gel-based wound-care treatments by improving therapeutic efficacy through synergistic biological effects [14]. The novelty of the study lies in the development of a bioactive, eco-friendly nanocomposite gel incorporating silver nanoparticles, CNPs, and chitosan in a single formulation using *Musa paradisiaca* leaf extract for potential wound management applications.

The present study aimed to synthesise and evaluate green-mediated silver-carbon-chitosan nanocomposite wound healing gel using *Musa paradisiaca* leaf extract against wound pathogens. The primary objective of the study was to evaluate the antimicrobial activity of the synthesised nanocomposite wound healing gel. The secondary objective was to assess the antibiofilm and time-kill kinetic activities of the developed formulation.

MATERIALS AND METHODS

This in-vitro experimental study was conducted at the Department of Microbiology, Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical Sciences, Chennai, Tamil Nadu, India, over a period of 3 months from January 2026 to March 2026.

Sample size: As this was an in-vitro experimental study, no formal sample size calculation was performed. All experiments were carried out independently in triplicate (n=3) following previously published protocols to ensure reproducibility and statistical reliability.

Inclusion criteria: Standard laboratory reference strains of wound-associated pathogens (Pseudomonas aeruginosa, Acinetobacter baumannii, Enterococcus faecium, Escherichia coli, and Staphylococcus aureus) that showed adequate growth and were suitable for antimicrobial evaluation were included in the study.

Exclusion criteria: Contaminated microbial cultures, expired reagents, damaged culture media, and samples showing contamination or experimental inconsistency were excluded from the study.

Study Procedure

The study involved the green synthesis of silver-carbon-chitosan nanocomposites using *Musa paradisiaca* leaf extract, followed by the evaluation of antimicrobial, antibiofilm, and time-kill activities against selected wound pathogens. All experiments were performed under controlled laboratory conditions to ensure reproducibility.

Chemical reagents: High-purity chemicals, solvents, and reagents were sourced from Sigma-Aldrich and Merck (Germany), ensuring consistency and reliability throughout the experimental procedures. Silver nitrate (AgNO3, ≥ 99.0%) and Medium molecular weight of chitosan ($C6H11O_4$, ≥ 99.9%).

Plant extract preparation: After gathering fresh *Musa paradisiaca* leaves from their natural environment, Dust and other surface contaminants were eliminated by thoroughly cleaning them under running tap water and then rinsing them with distilled water. After being shade-dried at room temperature until they reached a consistent weight, the leaves were mechanically ground into a fine powder. The extract was prepared by weighing and dissolving 3g of powdered leaf material in 150 mL of distilled water in a conical flask. With continuous stirring, the mixture was heated on a heating mantle at 60°C for 20 minutes. Once the solution had cooled to room temperature, it was filtered through Whatman No. 1 filter paper. The resulting filtrate is collected and kept in an airtight container at 4°C for future experimentation [15].

Silver nanoparticle preparation: For the green synthesis of silver nanoparticles (AgNPs), 1mM of 0.033g silver nitrate solution was prepared by dissolving the required volume of silver nitrate in 80 mL of distilled water. Subsequently, 20 mL of the filtered *M. paradisiaca* leaf extract was added to this solution. After that, the nanoparticle solution was placed in continuous stirring at 450 rpm using a magnetic stirrer for uniform mixing for 24 hours. The resulting solution was then centrifuged at 8000rpm for 10 minutes. To collect the pellet from the supernatant. The collected pellet was carefully stored in an airtight container for further biomedical applications [15].

Chitosan preparation: 0.5 g of chitosan was dissolved in 1mL of glacial acetic acid and kept in a beaker. Then, the remaining 49 mL of distilled water is mixed into this solution and is kept for 24 hours in a magnetic stirrer [16].

Carbon Nanoparticles (CNP) preparation: Freshly prepared plant extract of *M. paradisiaca*, where 130 mL of extract is taken in a China crucible and kept in a muffle furnace for 2 hours at 250°C [17].

Preparation of nanocomposite: For the preparation of the novel nanocomposite of C-AgNCs were prepared 100 mg of CNPs were dissolved in 5 mL of distilled water. To synthesise a nanocomposite solution, 2.5 mL of AgNPs and 2.5 mL of chitosan solution were added to this dispersion and carefully mixed with 2.5 mL of CNPs. After that, the nanocomposite solution was kept in a magnetic stirrer at 450 rpm at 60°C for five hours [16].

Preparation of wound healing gel using AgNCs: The wound healing gel was developed by dissolving 2.5 g of Carboxymethyl Cellulose (CMC) in 25 mL of distilled water and 2.5 g of Hydroxypropyl Methylcellulose (HPMC) in 25 mL of distilled water. One mL of biologically synthesised C-AgNCs was then added. After that, the two gels were homogenised together to develop a consistent formulation of wound healing gel [18].

Antimicrobial activity: The antimicrobial activity of green-synthesised C-AgNCs wound healing gel using *M. paradisiaca* leaf extract was evaluated using the agar plate diffusion method using Mueller-Hinton agar plates. The Mueller-Hinton agar plates were sterilised using an autoclave at 121°C for 15 minutes. After this, the medium was poured into a sterile petri dish. It was made to cool at room temperature, on the agar plates, bacteria *Pseudomonas* sp., *Acinetobacter* sp., *E. faecium*, *E. coli*, and *S. aureus* were

spread evenly, and a 9mm diameter well was created using a sterile polystyrene tip where different concentrations (25,50,100 µg/mL) of the nanocomposite were added. An empty gel formulation without C-AgNCs was used as the control during antimicrobial evaluation, and the plates were incubated at 37°C for 24 hours (bacterial culture) and 48 hours (fungal cultures). The antimicrobial activity was determined by measuring the diameter of the inhibition zone around the well in millimetres (mm). The antibacterial activity was interpreted based on the size of the inhibition zone, where larger inhibition zones indicated greater antimicrobial efficacy, as reported by Munusamy T and Shanmugam R (2023) [19].

Antibiofilm assay: By using a procedure, the antibiofilm assay aids in evaluating the effectiveness of biofilm eradicating, which was described by Munusamy T and Shanmugam R (2023), where the biofilm was made to mature within a 96 micro plate at first then followed by protocols over a 72 hours incubation time, after the media was removed, non adherent cells were removed from the wells by washing them with Phosphate Buffered Saline (PBS), 300 µL of these CNPs, *M. paradisiaca* C-AgNCs wound healing gel were placed in the wells, and after specific durations, the nanocomposite solution was discarded, and the biofilm adhered to the wall was washed with PBS, the biofilm was scraped and diluted with PBS and evaluated at 590 nm using an ELISA reader [19].

Time kill kinetic assay: A kill time curve examination was used to determine the *M. paradisiaca* C-AgNCs wound healing gel antibacterial effectiveness against the bacteria, as well as their characteristics and concentration-based association with *M. paradisiaca* C-AgNCs wound healing gel. The net growth rate of *Pseudomonas* sp., *Acinetobacter* sp., *E. faecium*, *E. coli*, and *S. aureus* was measured at regular intervals. The assay was done in a Mueller-Hinton broth and different concentrations (25, 50 and 100 µg/mL) of *M. paradisiaca* C-AgNCs wound healing gel, followed by a kill time assay. After pre-incubating the cultures for four hours in antimicrobial-free medium, we employed ciprofloxacin, an antibiotic, as a standard. An empty gel formulation without C-AgNCs was used as the control during time kill curve evaluation. PBS was used to aseptically create a 0.5 McFarland inoculum from colonies cultured on Mueller-Hinton agar at 37°C for 18-20 hours. Next, we dispersed 90µL of this inoculum onto each ELISA plate well after diluting 30 µL of it in 15 mL of Mueller-Hinton broth that had been heated without the use of antibiotics. Lastly, 10 µL of *M. paradisiaca* C-AgNCs wound-healing gel was applied to each well at varying concentrations, while the control well remained untreated. Bacterial growth inhibition during the time-kill kinetic assay was evaluated using optical density measurements at 600 nm (OD600) with a microplate reader. Readings were recorded at 0, 1, 2, 3, and 4 hours after treatment exposure. All experiments were carried out in triplicate (n=3). The percentage reduction in bacterial growth was calculated by comparing the OD600 values of treated samples with the untreated control using the following equation:

Percentage reduction (%) = $\{(OD_{control} - OD_{treatment}) / OD_{control}\} \times 100$,

where $OD_{control}$ represents the optical density of the untreated bacterial culture, and $OD_{treatment}$ represents the optical density of the treated bacterial culture at the corresponding time interval [19].

Physicochemical characterisation

The synthesised *Musa paradisiaca* leaf extract, chitosan, silver nanoparticles (AgNPs), CNPs, and chitosan-coated carbon-supported silver nanocomposites (Cs/C-AgNCs) were characterised using a range of physicochemical techniques to confirm their successful synthesis and assess their structural properties. Ultraviolet-visible (UV-Vis) spectroscopy was used to record the absorption spectra over a wavelength range of 200–800 nm. Fourier-Transform Infrared (FTIR) spectroscopy was performed using a Bruker Tensor 37 instrument to identify the functional groups present in the *Musa paradisiaca* leaf extract and chitosan and to evaluate their involvement in nanoparticle formation and stabilisation. X-Ray Diffraction (XRD) analysis was carried out using a BRUKER-binary V4 diffractometer to determine the crystalline nature and average particle size of the synthesised materials. The surface morphology and particle size distribution of the C-AgNCs were further examined using Scanning Electron Microscopy (SEM).

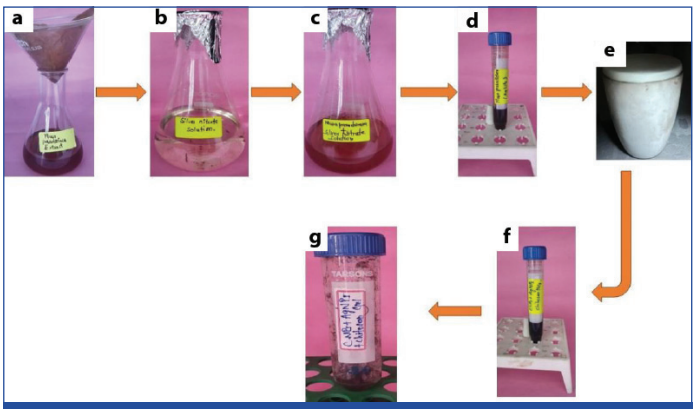
STATISTICAL ANALYSIS

Statistical analyses were performed using GraphPad Prism version 8.4.2.679 (GraphPad Software, San Diego, CA, USA). Experimental data were analysed by two-way analysis of variance (ANOVA) to evaluate the effects of treatment groups and concentrations. For repeated measurements, a two-way repeated-measures ANOVA with row matching was applied, followed by Tukey's multiple comparisons test for post-hoc analysis. Data are presented as mean±Standard Deviation (SD), and differences were considered statistically significant at $p<0.05$.

RESULTS

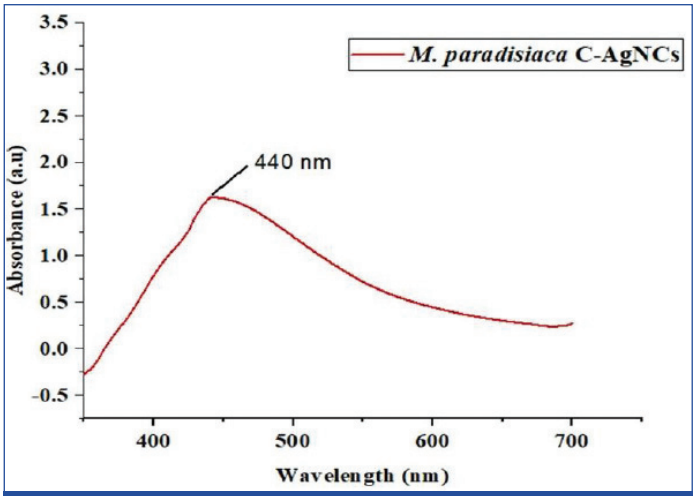
Visual observation: Biosynthesised silver nanoparticles were initially dark yellow. *M. paradisiaca* AgNPs gently changed to a dark brown colour after 2 hours of synthesis shown in [Table/Fig-1a-g]. The colour changes indicate the reducing ability of *M. paradisiaca* leaf extract, resulting in the reduction of silver nitrate nanoparticles. To improve stability and biocompatibility, the biosynthesised AgNPs were next mixed with CNPs made from *Musa paradisiaca* leaf extract and then distributed in a chitosan solution while being constantly stirred. To create a consistent wound-healing gel formulation, the resultant AgNP/CNP–chitosan combination was mixed into a hydrogel matrix. The gel appears smooth, homogeneous, and free of air bubbles, indicating that the nanocomposite was uniformly dispersed inside the polymeric matrix. The addition of biologically developed C-AgNCs resulted in a light brownish-grey colour, indicating the effective incorporation of silver and CNPs into the gel matrix.

UV-Vis spectral analysis of AgNCs: Using the UV-Vis spectral analysis, it was possible to validate the reaction colour shift in the mixture made from *M. paradisiaca* leaf extract and silver nitrate solution. This validated the effective preparation of silver nanocomposites (C-AgNCs) by using chitosan and CNPs, SPR, which occurs throughout the reaction period, observed colour shift from dark brown to dark black [Table/Fig-1f]. This method yields important details about the nanomaterial's optical characteristics. C-AgNCs development and stability are primarily confirmed by the UV-Visible spectrum, which displays a clear SPR absorption peak at about 440 nm, as seen in [Table/Fig-2].

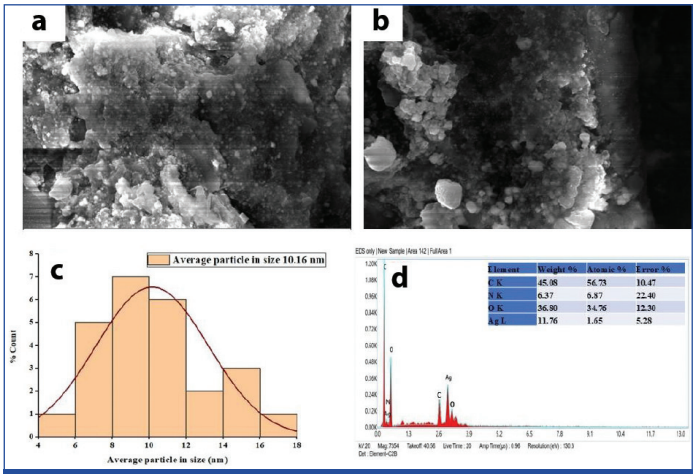


[Table/Fig-1]: Preparation of the wound healing gel by using M. paradisiaca-mediated silver and Carbon Nanoparticles (CNP): a) Filtered M. paradisiaca; b) Silver nitrate solution; c) Silver nitrate + M. paradisiaca solution; d) green synthesised AgNPs; e) CNPs development; f) Carbon-based AgNCs + Chitosan; g) C-AgNCs with wound healing gel.

Morphological analysis of C-AgNCs: Morphological analysis of C-AgNCs synthesised by using *M. paradisiaca* leaf extract, which shows [Table/Fig-3a-d]. The average particle size was determined



[Table/Fig-2]: UV-visible absorption spectral analysis of green synthesised C-AgNCs using M. paradisiaca leaf extract



[Table/Fig-3]: SEM images at different scale bars of C-AgNCs using M. paradisiaca leaf extract: a) 1 µm magnification; b) 500 nm magnification; c) Histogram images of C-AgNCs; d) Elemental analysis.

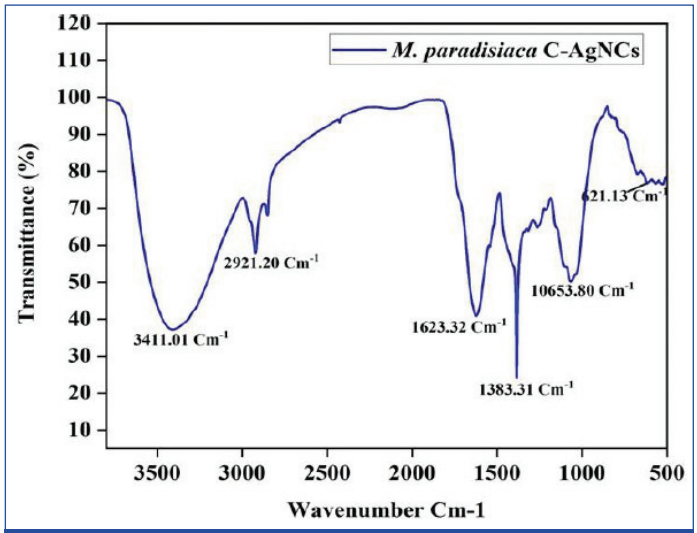
from SEM micrographs by measuring 25 randomly selected particles using ImageJ software (NIH, USA). The particle size distribution histogram was generated using OriginPro software (2024), and the mean particle size was calculated from the obtained measurements. Biologically synthesised C-AgNCs using *M. paradisiaca*, from the microscopic images, were observed to be spherical in shape, and the CNPs synthesised from *M. paradisiaca* are capped and aggregated with silver nanoparticles, indicating the nanocomposite is stabilised by chitosan coating. The emergence of some massive particles can be attributed to the aggregation and overlap of tiny particles. Given the feather nature of the particles and their tendency to aggregate and agglomerate, it was evident that the particles had taken on an irregular shape with varying sizes. Additionally, the typical size is between 5.85 and 16.84 nm. In their investigation, it was revealed that utilisation of *M. paradisiaca* leaf extract facilitated the synthesis of C-AgNCs, with an average nanoparticle size of ~ 10.16 nm. These findings strongly suggest that *M. paradisiaca* leaf extract could potentially be employed in the production of C-AgNCs as a reduction and capping agent.

Elemental analysis by using EDX

Energy-dispersive X-ray (EDX) analysis was carried out to determine the elemental composition of the synthesised C-AgNCs. The spectrum confirmed the presence of carbon, nitrogen, oxygen, and silver in the prepared nanocomposite. Carbon was detected as the major element with a weight percentage of 45.08%, followed by oxygen (36.80%), silver (11.76%), and nitrogen (6.37%). The characteristic silver signal verified the successful formation of silver-containing nanocomposites. The presence of carbon and oxygen peaks may be related to the carbon nanoparticles and chitosan matrix involved in the synthesis process. These findings confirmed the successful synthesis of the C-AgNCs nanocomposite [Table/Fig-3d].

FTIR Spectral analysis of C-AgNCs using *M. paradisiaca*

The Fourier Transform Infrared (FTIR) spectroscopy was used to identify the functional groups present in the synthesised *M. paradisiaca* C-AgNCs [Table/Fig-4]. The spectrum showed a broad peak at 3411.01 cm^{-1}, which may be assigned to hydroxyl (O-H) stretching vibrations. The absorption peak at 2921.20 cm^{-1} indicated C–H stretching vibrations. A distinct band observed at 1623.32 cm^{-1} corresponded to amide or carbonyl groups. The peak at 1383.31 cm^{-1} was related to C–N stretching vibrations, while the absorption band near 1053.80 cm^{-1} represented C–O stretching vibrations. The detected functional groups suggest the involvement of phytochemicals and chitosan components in the synthesis and stabilisation of the nanocomposite, while the peak at 621 cm^{-1} may be attributed to metal-oxygen interactions, indicating the presence of silver nanoparticles in the nanocomposite. These findings confirmed the successful synthesis and stabilisation of the *M. paradisiaca*-mediated C-AgNCs.

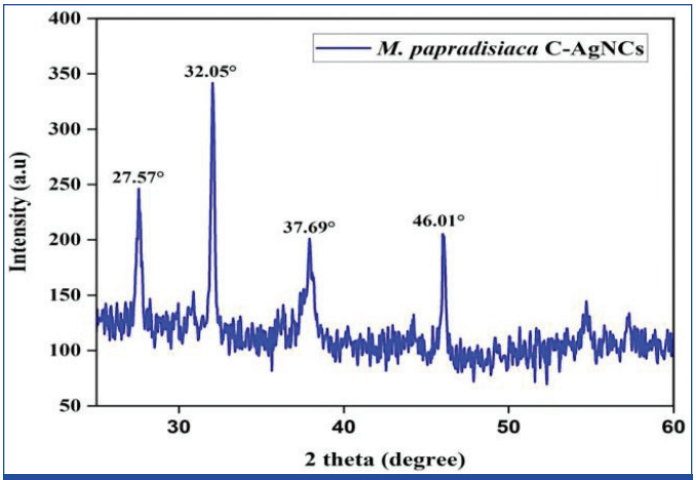


[Table/Fig-4]: FTIR spectral analysis of green synthesised C-AgNCs using M. paradisiaca leaf extract.

X-Ray Diffraction (XRD) Analysis of C-AgNCs using *M. paradisiaca*

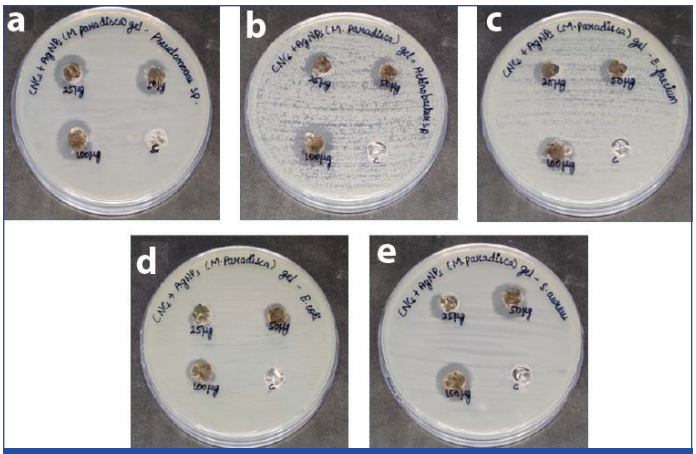
The crystalline nature of the synthesised *M. paradisiaca* C-AgNCs was analysed using XRD analysis. The diffraction pattern showed characteristic peaks at 2θ values of 27.57°, 32.05°, 37.69°, and 46.01°, and a lattice plane of the monoclinic silver nanocomposite structure is represented by the diffraction peaks found to be plane of (600), (-421), (121), (-211), respectively (JCPDS Ref. No. 00-026-0339, 00-024-1899) [Table/Fig-5]. The presence of distinct diffraction peaks confirmed the crystalline structure of the synthesised nanocomposite. The sharp diffraction peak observed at 32.05° indicated good crystallinity of the nanoparticles. These diffraction peaks may be attributed to the formation of silver-containing carbon nanocomposites stabilised by chitosan. The obtained XRD pattern confirmed the successful synthesis of crystalline *M. paradisiaca*-mediated C-AgNCs.

Agar well-diffusion method: The antimicrobial activity of *M. paradisiaca*-derived carbon-based silver nanocomposites (C-AgNCs) mixed into a wound healing gel was evaluated using the agar well-diffusion method. The experiment was conducted against bacteria that cause wounds, including *Pseudomonas* sp., *Acinetobacter* sp., *Enterococcus faecium*, *Escherichia coli*, and *Staphylococcus aureus*. The wound healing gel demonstrated antibacterial activity at doses of 25, 50, and 100 µL. The highest inhibitory effect was observed at 100 µL, producing inhibition zones of 18.0±0.82 mm



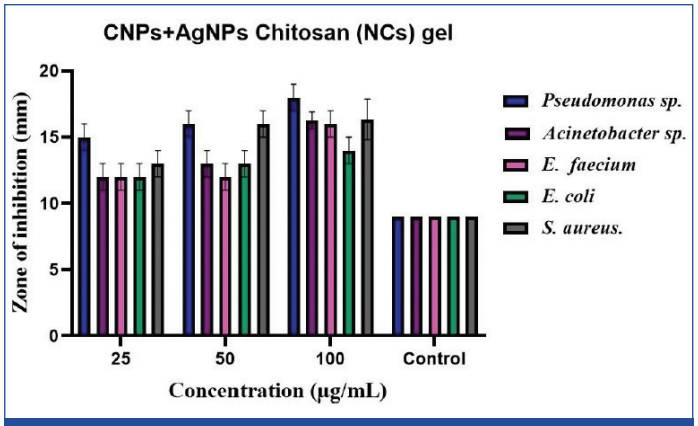
[Table/Fig-5]: X-Ray Diffraction (XRD) spectral analysis of green synthesised C-AgNCs using M. paradisiaca leaf extract.

against *Pseudomonas* sp., 16.33±1.53 mm against *S. aureus*, 16.27±0.52 mm against *Acinetobacter* sp., 16.0±0.82 mm against *E. faecium*, and 14.0±0.82 mm against *E. coli*. Comparatively smaller inhibition zones were recorded at 25 and 50 µL. The control group exhibited a consistent inhibition zone of 9.0±0.0 mm for all tested microorganisms. Values are expressed as mean±SD (n=3). Statistical analysis was performed using two-way repeated-measures ANOVA followed by Tukey's multiple-comparisons test. Significant effects were observed for concentration p=0.0080, and bacterial strain p=0.0160 [Table/Fig-6]. The nanocomposite formulation demonstrated high antibacterial effectiveness, with maximum inhibition observed at a concentration of 100 µL. The results obtained show that *M. paradisiaca*-C-AgNCs wound healing gel has strong and concentration-dependent antibacterial activity, with specific efficacy against *Pseudomonas* sp., an increasingly prevalent and persisting wound-related infection [Table/Fig-7].

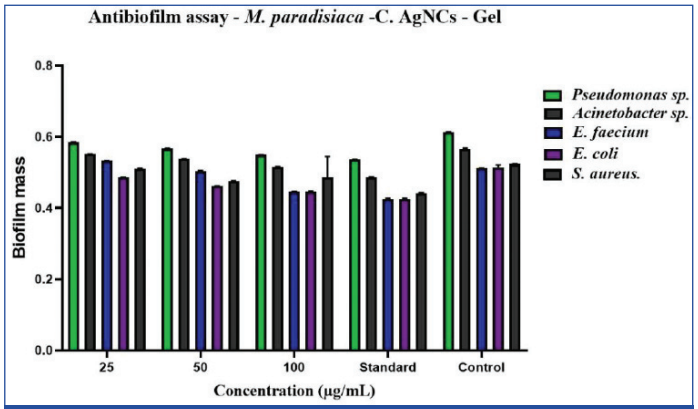


[Table/Fig-6]: The antimicrobial activity of M. paradisiaca C-AgNCs using the agar well diffusion method against wound pathogens is: a) Pseudomonas sp., b) Acinetobacter sp., c) E. faecium; d) E. coli; e) S. aureus.

Evaluation of antibiofilm activity using C-AgNCs wound healing gel: The antibiofilm effectiveness of the green-synthesised C-AgNCs wound-closure gel against wound pathogens, such as *Pseudomonas* sp., *Acinetobacter* sp., *E. faecium*, *E. coli*, and *S. aureus*, was assessed by measuring absorbance values, as shown in [Table/Fig-8]. All pathogens showed concentration-varying antibiofilm activity with C-AgNCs wound healing gel, with larger concentrations often resulting in more pronounced decreases in biofilm mass. A concentration-dependent reduction in biofilm formation was observed for all tested wound pathogens following treatment with C-AgNCs wound-healing gel. For *Pseudomonas* sp., the absorbance value decreased from 0.584667±0.000471 at 25 µg/mL to 0.549667±0.000471 at 100 µg/mL, while the standard control recorded 0.536333±0.000471. *Acinetobacter* sp. showed a reduction in absorbance from 0.551667±0.000471 at 25 µg/mL to



[Table/Fig-7]: Graphical presentation of antibacterial activity of M. paradisiaca C-AgNCs against wound pathogens, Pseudomonas sp., Acinetobacter sp., E. faecium, E. coli, and S. aureus. Values are presented as mean±SD (n=3). Statistical significance was determined using two-way ANOVA followed by Tukey's multiple-comparison test (p<0.05).



[Table/Fig-8]: Graphical presentation of antibiofilm activity of biologically synthesised M. paradisiaca C-AgNCs against wound pathogens Pseudomonas sp. Acinetobacter sp., E. faecium, E. coli, S. aureus. Values are expressed as mean±SD (n=3). Statistical analysis was performed using two-way repeated-measures ANOVA followed by Tukey's multiple comparison test. Differences were considered statistically significant at p<0.05. Significant differences were observed between treatment concentrations and bacterial strains (p<0.05). Showed statistically significant differences with p=0.005, respectively

0.515667±0.000471 at 100 µg/mL. In *E. faecium*, the absorbance values decreased from 0.532667±0.000471 to 0.446333±0.000471 with increasing concentration. Similarly, *E. coli* demonstrated a decrease in absorbance values from 0.486333±0.000471 at 25 µg/mL to 0.447000±0.000816 at 100 µg/mL. *S. aureus* also showed reduced biofilm formation, with absorbance values decreasing from 0.511000±0.000816 to 0.448333±0.047849. The 100 µg/mL concentration showed antibiofilm activity comparable to the standard control. These findings indicate that the *M. paradisiaca* C-AgNCs wound-healing gel possesses dose-dependent antibiofilm activity against wound-associated pathogens. All values are presented as mean±SD of three independent experiments (n=3). Statistical analysis was performed using two-way repeated-measures Analysis of Variance (ANOVA), and differences were considered statistically significant at p<0.05.

These findings indicate that the C-AgNCs gel exhibits a dose-dependent antibiofilm activity, which demonstrates its potential as a promising therapeutic agent for wound management applications. Change the concentration to 25, 50, 100, and standard control, then change the *M. paradisiaca*-C-AgNCs wound healing gel. The antibiofilm assay is used to determine whether the given nanocomposite can degrade the biofilm formed by wound pathogens. A 25µg/ml concentration did not exhibit significant antibiofilm activity. Standard had high inhibition of biofilm activity. A concentration of 100 µg/ml showed some inhibition, similar to the standard.

Time-kill curve analysis of wound healing gel: The green-synthesised C-AgNCs wound healing gel using *M. paradisiaca* against the 5 wound pathogens, *Pseudomonas* sp. *Acinetobacter*

sp., *E. faecium*, *E. coli*, and *S. aureus* demonstrated bactericidal properties that are dose-dependent in comparison to the typical control group. Throughout the experiment period, a decrease in *Pseudomonas* sp. counts was noted at all concentrations of *M. paradisiaca* C-AgNCs wound healing gel (25 µg/mL, 50 µg/mL, and 100 µg/mL) in comparison to the control group [Table/Fig-9a]. The maximum dose of 100 µg showed a gradual decline in *Pseudomonas* sp. counts during the first two hours, indicating moderate bactericidal action. [Table/Fig-9b] shows that *Acinetobacter* sp., C-AgNCs wound repair gel had concentration-dependent bactericidal activity. C-AgNCs for wound healing gels reduced *Acinetobacter* sp. counts significantly at all dosages when in comparison with the control group. At the maximum dose of 100 µg, there was a considerable reduction in *Acinetobacter* sp. counts during the first hour, demonstrating fast antibacterial effectiveness. [Table/Fig-9c] shows that the C-AgNCs wound healing gel also has an antibacterial action that is concentration-dependent. The antibacterial effect against *S. aureus*, although the reduction in bacterial growth was comparatively standard and the control group. Notably, at the highest concentration (100 µg), a significant decline in *S. aureus* counts was observed within the first two hours, suggesting rapid bactericidal activity. In [Table/Fig-9d], as in *E. faecium*, C-AgNCs exhibited rapid, concentration-dependent bactericidal effects against *E. faecium*. Compared with the untreated group, *E. faecium* numbers decreased significantly at all concentrations. C-AgNCs also demonstrated concentration-dependent antibacterial properties against *E. coli* in [Table/Fig-9e]; however, the decrease in bacterial numbers was rather slight. *E. coli* counts declined less sharply even at the highest dose (100 µg), suggesting that it is less susceptible to C-AgNCs than the other *Pseudomonas* sp. pathogens. However, over time, a discernible decline in *E. coli* levels was observed compared with the control group. Together, our results highlight how C-AgNCs antibacterial qualities against the studied wound pathogens are concentration-dependent. While *E. coli* and *Pseudomonas* sp. responded more slowly to C-AgNCs, *Acinetobacter* sp., *S. aureus*, and *E. faecium* showed a quick responsiveness. The comparison with the control group demonstrates the potential of C-AgNCs as antibacterial agents for wound care and emphasises their notable effect in lowering bacterial counts.

DISCUSSION

Skin is a barrier to many pathogens that cause infections and injuries, hindering human activities. Antimicrobial medicines based on metallic nanoparticles are a promising alternative to traditional antibiotics, with clinically significant outcomes. The formation of the nanocomposite is confirmed using a double-beam UV spectrophotometer, where the maximum peak is 440 nm.

Previous studies have shown that green-synthesised silver nanoparticles typically exhibit absorption peaks within the 420-450 nm range, which are associated with nanoparticle formation and stability. In the present investigation, the detected absorption peak was in agreement with earlier reports on chitosan-coated silver nanoparticles synthesised using banana peel extract, where an absorption maximum at approximately 434.5 nm was attributed to the successful formation of silver nanoparticles [20].

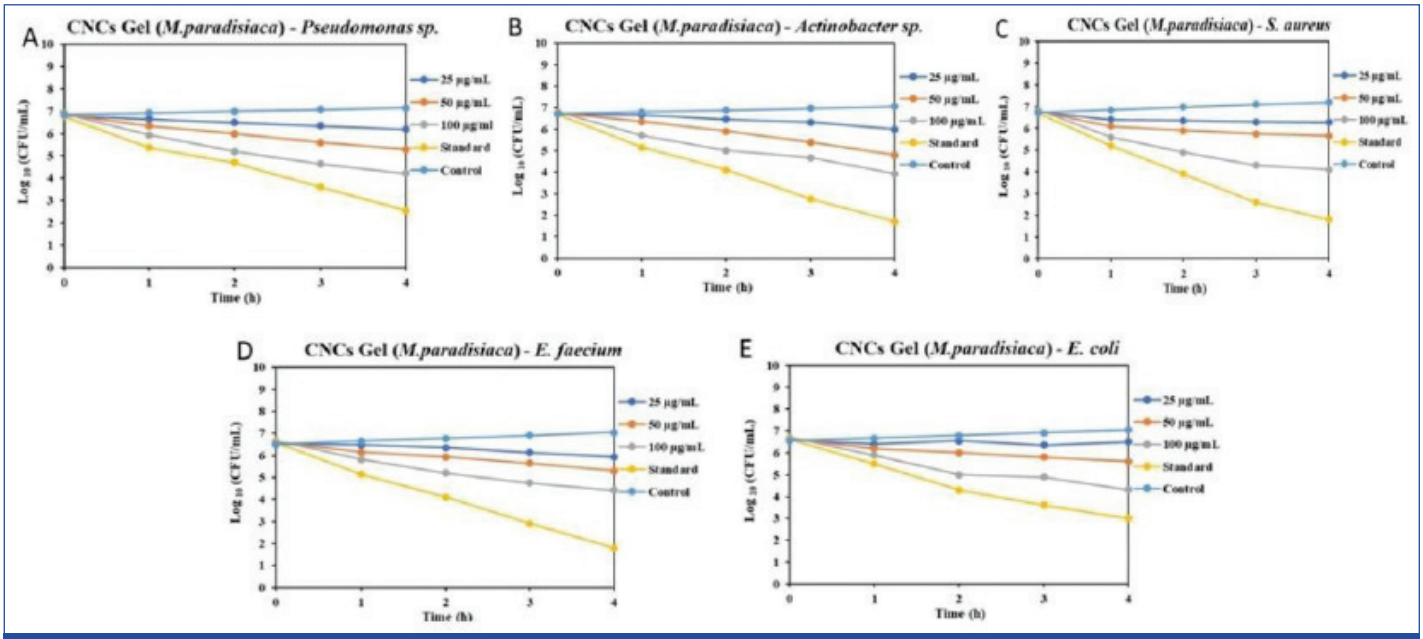
Biologically synthesised *M. paradisiaca* C-AgNCs wound healing gel. Morphological images of the novel nanocomposite, which are spherical in shape, show an average particle size of 10.16 nm [Table/Fig-3]. The spherical shape observed in the present study agrees with earlier reports describing chitosan-stabilised silver nanoparticles developed for wound healing purposes [21].

In the agar gel diffusion method, both 25 µg/mL and 100 µg/mL concentrations of *Pseudomonas* sp. have the maximum inhibition, whereas *E. coli* has the lowest. In the time kinetic kill assay, 25µg/ml concentration showed bacteriostatic property, and a 100 µg/mL concentration showed potent bactericidal property. A 25 µg/mL concentration didn't show much anti-biofilm activity, but 100 µg/mL showed a significant increase in anti-biofilm activity.

Previous studies have demonstrated that gram-negative bacteria, especially *Pseudomonas aeruginosa*, are highly susceptible to silver nanoparticles, primarily due to the ability of silver ions to interact with cell membrane components and intracellular targets [19].

The agar well-diffusion method involves having wells in plates; in each well, different concentrations of dental varnish and inoculum were added, and a standard was added in another well. When the inhibition zone is larger, the antimicrobial activity is greater in *Pseudomonas* sp., showing a greater inhibition zone. The time kinetic kill assay involves the action of the minimum time required to kill the pathogen, where different concentrations were used to see how much inhibition had occurred. In the antibiofilm assay, the biofilm was matured, washed with PBS, and the nanocomposite gel was added. And after some intervals, it was scrapped and used as a test [22].

Previous studies have demonstrated that chitosan-silver nanoparticle composites can effectively inhibit biofilm development by disrupting



[Table/Fig-9]: Graphical representation of Time-kill curve assay of synthesised M. paradisiaca C-AgNCs wound healing gel against wound pathogens: a) Pseudomonas sp. b) Acinetobacter sp. c) S.aureus; d) E. faecium; e) E. coli, Values are presented as mean±SD (n=3). Bacterial growth was monitored by measuring OD600 at different time intervals (0-4 h).

the structural integrity of established biofilms and reducing bacterial attachment. The observed antibiofilm activity may be attributed to the capacity of silver nanoparticles to penetrate the Extracellular Polymeric Substance (EPS) layer, impair cell-to-cell communication mechanisms, and prevent microbial adhesion and subsequent biofilm formation. In addition, chitosan may contribute to this effect by interacting with bacterial cell surfaces, modifying their surface properties, and weakening the stability of the biofilm matrix [21].

Previously investigated, silver nanoparticles were greenly synthesised using an aqueous extract from *Scutellaria barbata*, and their biological activity was then assessed. The biosynthesised nanoparticles demonstrated potent antibacterial activity and dramatically reduced the production of biofilms in both susceptible and resistant microbial strains. This antibacterial activity was maintained in cotton fabrics coated with the nanoparticles, indicating their potential use in biomedical materials. Additionally, the wound scratch assay verified the nanoparticles' significant wound-healing capacity, bolstering their promise as a natural wound-healing agent [23].

In another study, Chitosan with silver nanoparticles demonstrated antibacterial efficacy against *S. aureus* and *Pseudomonas*. sp using a resazurin-based microtiter assay, with the strongest impact at 12.5 µg/mL. The nanocomposite also showed strong antifungal efficacy against *Candida albicans* and *Aspergillus* species, as evidenced by measured inhibition zones. Overall, the synthesised chitosan-silver nanocomposite showed excellent antibacterial, antifungal, antioxidant, and wound-healing activities that are appropriate for biomedical applications [24]. The present study showed that C-AgNCs have antibacterial action against wound pathogens like *S. aureus*, *P. aeruginosa*, and *E. coli* in both the time-kill curve and agar well diffusion tests. Higher concentrations of C-AgNCs led to more pronounced decreases in bacterial counts, demonstrating their dependence on the concentration of antibacterial properties. In the previous study reported, silver nanoparticles were used with *Senna auriculata* and *Symplocos racemosa* extract. Time-kill kinetic studies validated the embedded nanofilm C-AgNCs antibacterial action, which was concentration- and time-dependent. Microbial viability was significantly reduced at 100 µg/mL within 3-4 hours, particularly in *E. coli* and *S. aureus*, with Colonies Forming Units (CFUs) dropping to nearly undetectable levels. The study found that silver nanoparticles at 100 µg/mL completely killed *E. coli* within four hours and reduced *S. aureus* vitality similarly. *Pseudomonas* sp. demonstrated increased resistance to C-AgNCs, requiring longer exposure or higher dosages for substantial CFU reduction. *P. aeruginosa* biofilms also showed delayed susceptibility compared to planktonic forms [24].

In another study, Soni M et al., investigated green-synthesised silver nanoparticles that are potentially used to treat chronic wounds. The antibiofilm activity was mostly strong and effectively disturbed, followed by wound pathogens, such as *S. aureus* and *Pseudomonas* sp. *Acinetobacter* sp., *E. faecium*, and *E. coli*. The treatment resulted in a considerable reduction in biofilm biomass, comparable to the inhibitory effects of standard antibiotics. Biologically synthesised AgNPs showed remarkable antibiofilm activity, especially against gram-positive pathogens such as *S. aureus* and E. faecalis, reducing biofilm mass by over 65% at comparable concentrations. Combining bioactive phytochemicals with AgNPs enhances antibacterial activity, especially in breaking biofilms [25].

Limitation(s)

The present study was limited to the in-vitro evaluation of antimicrobial, antibiofilm, and time-kill activities of the developed C-AgNCs using *M. paradisiaca* nanocomposite gel. Although the formulated gel exhibited promising antimicrobial activity against wound-associated pathogens, direct wound-healing assays such as scratch assay, fibroblast migration assay, cell proliferation studies, and in-vivo wound models were not performed. Therefore, the wound-healing potential of the gel remains to be confirmed and will be investigated in future studies. Cytotoxicity assessment, stability studies, and in-vivo wound healing analysis were not carried out. Future studies are required to validate the biocompatibility, safety, and clinical applicability of the formulation.

CONCLUSION(S)

In conclusion, the biologically synthesised C-AgNCs wound healing gel using *Musa paradisiaca* leaf extract provided a simple and eco-friendly method for the synthesis of silver nanoparticles. The synthesised C-AgNCs showed effective antibacterial, antibiofilm, and time-kill activities against wound pathogens. The antibacterial activity was found to be concentration-dependent, demonstrating both bactericidal and bacteriostatic effects. The findings of the present study suggest that plant-mediated C-AgNCs may serve as potential alternatives for antimicrobial wound dressings and coatings in managing wound infections and antibiotic resistance. Further investigations, including cytotoxicity studies and in-vivo evaluation, are necessary to confirm their therapeutic applicability and safety.

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