



Functional Characterization and Wound-healing Applications of Green-synthesized Silver Nanoparticles Using *Phyllanthus emblica* Bark Extract

S. Karthikeyan¹, A. Gokulkrishnan¹, D. Kanimozhi¹, S. Sujitha¹, G. Sameetha¹, S. Madhu Bala¹ and S. Venkatesa Prabhu^{1,2*}

¹Department of Biotechnology, Faculty of Engineering, Karpagam Academy of Higher Education, Coimbatore, TN, India

²Centre for Natural Products and Functional Foods, Karpagam Academy of Higher Education, Coimbatore, TN, India

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*venkatchemdata@gmail.com



ABSTRACT

This study focuses on the green synthesis of AgNPs using *Phyllanthus emblica* bark extract, aiming to enhance antimicrobial activity and promote tissue regeneration. The synthesized AgNPs were characterized using UV-Visible spectroscopy, confirming the formation of nanoparticles with a characteristic surface plasmon resonance peak at 439 nm. Zeta-potential analysis indicated high colloidal stability, while dynamic light scattering (DLS) revealed an average particle size of 96.60 nm for the synthesized AgNPs. Furthermore, Fourier-transform infrared spectroscopy (FTIR) and scanning electron microscopy (SEM) were performed to examine particle morphology and confirm the involvement of plant-derived phytochemicals in nanoparticle reduction and stabilization. In addition, the biological efficacy of the AgNPs was evaluated through antibacterial assays and *in vitro* wound-scratch assay, where enhanced cell migration and accelerated wound closure were observed.

Keywords: *Phyllanthus emblica*; Silver nanoparticles; Wound healing; Characterization; *in vitro*.

1. INTRODUCTION

Wound healing is a complex and highly regulated biological process that involves a coordinated sequence of cellular and molecular events, typically categorized into four overlapping and interdependent phases: hemostasis, inflammation, proliferation, and remodeling or maturation. Each phase plays a critical role in restoring the structural and functional integrity of damaged tissue. While acute wounds typically progress through these stages in an orderly manner, chronic or infected wounds often stall during the inflammatory phase, leading to delayed healing, tissue necrosis, and increased susceptibility to secondary infections. The growing incidence of chronic wounds, particularly among diabetic, elderly, and immunocompromised patients, poses a significant global healthcare challenge (Gonzalez *et al.* 2016). Conventional wound management strategies, such as the application of topical antibiotics and synthetic dressings, are increasingly constrained by factors including microbial resistance, cytotoxicity, and inadequate tissue integration. These limitations have prompted the pursuit of novel therapeutic agents capable of simultaneously combating infection and promoting tissue regeneration without eliciting adverse side effects. (Panchve *et al.* 2025). In this line, silver nanoparticles (AgNPs) have gained prominent research interest due to their remarkable anti-

inflammatory, antimicrobial, and pro-angiogenic properties. AgNPs exhibit potential to disrupt the microbial membranes and interfere with cellular metabolism, thereby inhibiting a broad spectrum of pathogens. Their nanoscale size enables improved tissue penetration and prolonged interaction with cellular components, thereby enhancing their therapeutic potential. So far, different traditional chemical and physical methods for AgNP synthesis have been reported. However, chemical and physical methods for AgNP synthesis often require toxic reducing agents and are typically energy-intensive processes that pose significant environmental hazards (Singh *et al.* 2022).

To address these limitations, green synthesis methods for nanoparticles have garnered considerable interest among researchers, as they provide a sustainable and biocompatible alternative to conventional approaches. In green synthesis, plant extracts function as both reducing and capping agents, enabling the eco-friendly production of nanoparticles. Moreover, the phytochemicals present in these extracts enhance the bioactivity of the nanoparticles, thereby increasing their effectiveness in biomedical applications (Vijapur *et al.* 2025). In this vein, various researchers have investigated different plant extracts for the green synthesis of AgNPs. In the present study, *Phyllanthus emblica* (commonly known as Indian gooseberry or amla) bark extract was

utilized for the synthesis of AgNPs. *P. emblica*, a plant widely used in ayurvedic medicine, has immense therapeutic potential. The bark contains bioactive compounds, such as polyphenols, tannins, flavonoids, and gallic acid, which possess potent antioxidant, antimicrobial, and anti-inflammatory activities (Prananda *et al.* 2023). Despite its rich phytochemical profile, the use of *P. emblica* bark extract for nanoparticle synthesis has not been studied yet. Hence, this study is aimed at developing a cost-effective and straightforward method for synthesizing AgNPs using *P. emblica* bark extract. Further, the synthesized AgNPs were characterized using different analytical techniques, including their wound-healing potential through an *in vitro* biological assay. By integrating the natural therapeutic phytochemical properties of *P. emblica* bark with the nano-enabled capabilities of AgNPs, this study explores a novel and synergistic approach to wound care (Chinnasamy *et al.* 2021).

2. MATERIALS AND METHODS

2.1 Preparation of *P. emblica* Bark Extract and Synthesis of AgNPs

P. emblica barks were collected from a local garden in Coimbatore, Tamil Nadu, India. The barks were washed, shade-dried, and ground into a fine powder. The powdered bark was further screened to obtain uniform fine particles. These fine particles were then subjected to extraction using distilled water as the solvent, with continuous stirring at 60 °C for 4 h. The resulting extract was filtered through Whatman No. 1 filter paper. The filtrate was subsequently concentrated using a rotary evaporator (Büchi Labortechnik, R-300, Switzerland) to increase yield. Finally, the concentrated aqueous extract was stored at 4 °C for further analysis. The aqueous extract of *Phyllanthus emblica* bark was treated with silver nitrate (AgNO_3) solution to synthesize silver nanoparticles via a green synthesis approach. In this process, 5 mL of silver nitrate solution (prepared at an appropriate concentration) was mixed with the plant extract at three different volume-to-volume ratios of extract to silver nitrate: 1:1, 5:1, and 9:1. The mixtures were thoroughly vortexed to ensure homogeneity. Each reaction mixture was then incubated at room temperature (25 °C) under static conditions for 24 h to allow the reduction of silver ions (Ag^+) to elemental silver (Ag^0) nanoparticles facilitated by phytochemicals present in the extract. Successful synthesis was preliminarily indicated by a visible color change of the solutions from pale yellow or colorless to brown, a characteristic feature arising from the surface plasmon resonance effect of AgNPs. The resulting brown colloidal solutions were further subjected to characterization to confirm nanoparticle formation and to analyze their properties. Figure 1 presents a schematic representation of the overall workflow for this study (Suvandee *et al.* 2022).

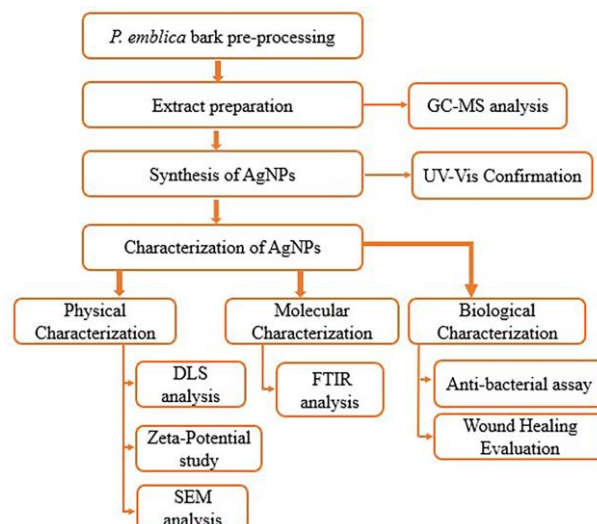


Fig. 1: Schematic representation of the overall workflow of the study

2.2 GC-MS Analysis of *P. emblica* Bark Extract

Phytochemical profiling of the extract was performed using a gas chromatography–mass spectrometry system (Shimadzu, GC-MS-QP2020 NX, Japan). Helium (99.999% purity) was used as the carrier gas at a constant flow rate of 1.0 mL/min. Separation was carried out using an Rxi-5Sil MS capillary column with dimensions of 30 m × 0.25 mm ID and 0.25 µm film thickness. The GC oven temperature program was set at an initial temperature of 70 °C (held for 2 min), ramped at 10 °C/min to 280 °C, and held for 10 min. The injector temperature was maintained at 250 °C, and the injection volume was 1.0 µL in split mode (split ratio 10:1). The MS was operated in electron ionization (EI) mode at 70 eV, scanning from m/z 40–600. Compound identification was achieved by comparing mass spectra with the NIST library database.

2.3 Molecular and Morphological Characterization of AgNPs

UV–Vis spectrophotometry is a rapid, non-destructive analytical technique commonly employed to confirm the formation of AgNPs. During synthesis, AgNPs exhibited a distinct surface plasmon resonance (SPR) phenomenon due to the collective oscillation of conduction band electrons when excited by incident light in the visible range. This SPR effect produced a characteristic absorption peak, typically observed between 400 and 450 nm, depending on particle size, shape, and the surrounding medium. In this study, UV–vis spectrophotometry (PerkinElmer Lambda 25, PerkinElmer Inc., Waltham, MA, USA) was employed to analyze the formation of AgNPs in the different treated solutions. The maximum amount of AgNPs produced using different extract treatments can be determined by

comparing the absorbance intensity at the characteristic SPR wavelength (typically 400–450 nm). A higher absorbance value at this peak corresponds to a greater concentration of AgNPs. The size distribution of the synthesized AgNPs was analysed using a particle size analyser (Malvern Zetasizer, MAL1111244, Malvern, UK). The measurements were performed in electrolyte water as the dispersant at 25 °C. The dynamic light scattering (DLS) technique was employed to determine the Z-Average size and polydispersity index (PDI) of the produced nanoparticles (Shamhari *et al.* 2018). To evaluate the surface charge and colloidal stability, the Zeta potential of the AgNPs was assessed using the same instrument (Masum *et al.* 2019).

The measurements were performed in electrolyte water at 25 °C. Samples were loaded into a disposable folded capillary cell, and three independent measurements were taken to ensure reproducibility. The zeta potential readings were recorded as the mean $\pm$ standard deviation. Data analysis was conducted using Malvern Zetasizer software. SEM (Hitachi, S-4800, Japan) and FTIR (Bruker Optik GmbH, Alpha II, Germany) were employed to characterize the morphological and functional group analysis of AgNPs (Sivalingam and Pandian, 2024). For SEM, a drop of the nanoparticles sample solution was poured into clean silicon water, then dried, and a thin layer of gold was used to coat, which will enhance the conductivity for the analysis. Further, with the SEM instrument, at various magnifications, images of the nanoparticle were captured to analyze its surface characteristics and size of the nanoparticles (Sivalingam and Pandian, 2024). For FTIR, the AgNPs solution was treated with potassium bromide (KBr) to form a pellet. Then, IR spectra were observed at room temperature with the spectral range of 4000 – 400 cm^{-1} (Alwarhi *et al.* 2022).

2.4 Characterization Studies for Biological Activities

2.4.1 Antibacterial Activity

The well-diffusion method was used to assess antibacterial activity, wherein agar plates were prepared using nutrient agar and agar-agar. Further, using the injection, wells were created in the plates. In this work, bacterial culture against *Staphylococcus aureus* was examined to check the antibacterial activity using the spread plate technique (Salayová *et al.* 2021). In this study, *S. aureus* was selected for antibacterial studies due to its status as a major human pathogen responsible for a wide range of infections, including wound and skin infections, pneumonia, and sepsis. It is particularly notorious for its ability to rapidly develop resistance to antibiotics, including methicillin-resistant strains (MRSA), which pose significant challenges in clinical treatment. Furthermore, *S. aureus* is one of the leading

causes of hospital-acquired infections and wound infections, contributing to increased morbidity, mortality, and healthcare costs globally. Its prevalent role in wound infections and its clinical significance make it an essential target for evaluating the antibacterial efficacy of AgNPs produced in this study.

Samples at different concentrations, silver nitrate solution without sample, positive control, and negative control were added to the well and incubated for 24 hours at 37 °C. After the incubation, the zone of incubation was measured and compared with the controls (Meena *et al.* 2020).

2.4.2 In Vitro Wound Healing Evaluation

From the National Centre for Cell Sciences (NCCS), Pune, India, the L929 mouse fibroblast cell line was obtained to investigate the *in vitro* wound-healing evaluation of synthesized AgNPs. The study was carried out using Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS) and antibiotics (100 U/mL streptomycin and 100 U/mL penicillin). The cells were cultured under a humidified atmosphere at 37 °C with 5% CO₂. Once the culture reached 80% confluency of cells, it was sub-cultured, and the viability of the cells was assessed with a hemocytometer (Marienfeld Superior, Lauda-Königshofen, Germany) (Torabi *et al.* 2023).

2.4.3 Scratch Assay

As described by the *in vitro* scratch assay was assessed for the test sample containing green AgNPs. For this assay, at a density of 10,000 cells/well, L929 cells were seeded in a 6-well plate and incubated overnight to adhere. After the incubation, a linear scratch was made with a sterile pipette tip in the cell monolayer, and the detached cells were washed with Dulbecco's Phosphate Buffered Saline (DPBS) (Pitz *et al.* 2016). Further, the cells were treated with the 1000 $\mu\text{g/mL}$ test sample of biosynthetic AgNPs and 5 $\mu\text{g/mL}$ positive control [Cipladine (Cipla Ltd.)]. Images of scratch healing were captured at 0, 24, 36, 48, and 72 h using a digital camera (Nikon DS-Fi3, Tokyo, Japan) attached to an inverted microscope. Then, it was quantified with ImageJ software (Version 1.54f, National Institutes of Health, USA) to assess the wound area. The percentage of wound closure was calculated using Equation (1).

$$\text{Wound Closure (\%)} = \frac{\text{Initial Wound Area (A}^0\text{)} - \text{Remaining Wound Area (A}_t\text{)}}{\text{Initial Wound Area (A}^0\text{)}} \dots (1)$$

where, A⁰ is the wound area at the initial time point (0 h), A_t is the wound area at a specific time point (24, 36, and 48 h).

3. RESULTS

3.1 Characteristics of Synthesized AgNPs

The synthesis of AgNPs was initially indicated by a color change of the reaction mixture to brown, signifying the reduction of silver ions. UV–Visible spectrophotometric analysis (Figure 2) further confirmed nanoparticle formation, with a prominent peak observed at 439 nm (Sivalingam and Pandian, 2024). Among the tested ratios, the solution containing a 1:1 ratio of silver nitrate to *P. emblica* bark extract exhibited the most intense and broad peak, indicating optimal nanoparticle synthesis and stability at this proportion.

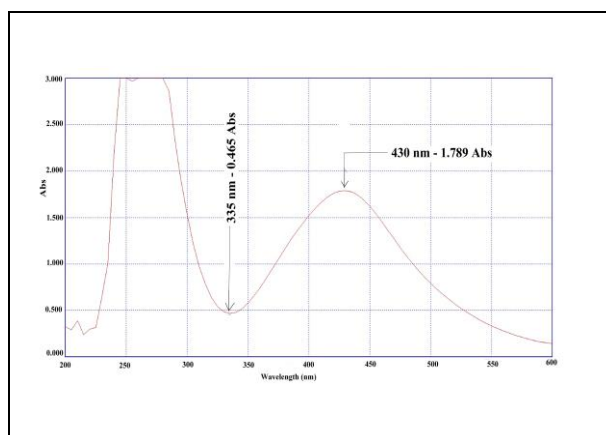


Fig. 2: UV-Vis absorption peak was observed at 439 nm for the 1:1 ratio sample

3.2 GC-MS Analysis of *P. emblica* Bark Extract

Phytochemicals of *P. emblica* bark extract were analyzed using GC-MS, and a graph was generated to illustrate the bioactive compounds present in the extract.

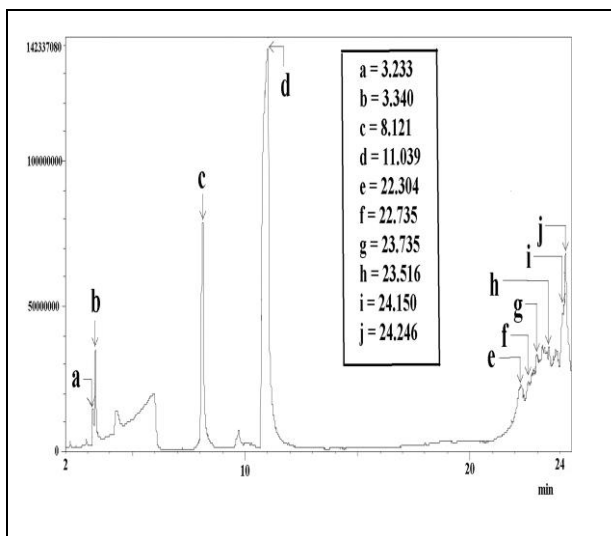


Fig. 3: GC-MS chromatogram of *P. emblica* bark extract

Table 1. The bioactive compounds identified using GC-MS analysis

Peak	Retention time	Area%	Height%	A/H	Name
1	3.233	1.12	3.65	3.61	Ethanol, 2,2'-oxybis-
2	3.340	3.74	9.37	4.69	Bromonitromethane
3	8.121	12.31	21.99	6.57	2-Methylmercaptoaniline
4	11.039	67.81	40.90	19.46	Bronopol
5	22.304	1.45	2.17	7.86	Tetrapentacontane
6	22.735	0.48	0.89	6.34	Tetrapentacontane, 1,54-dibromo-
7	23.010	0.63	1.32	5.58	Tetrapentacontane, 1,54-dibromo-
8	23.516	0.58	1.50	4.49	Erythro-7,8-Bromochlorodisparlure
9	24.150	3.77	6.42	6.89	1-Chloroeicosane
10	24.246	8.10	11.78	8.07	Tris(2,4-di-tert-butylphenyl)phosphate

Figure 3 presents the GC-MS chromatograph, in which peak shows the presence of bioactive compounds. The highest peak was observed at the retention time of 11.039 minutes; further, MS data were compared with the NCL library to identify the bioactive compounds. Table 1 lists the compounds identified using GC-MS. This analysis helped in identifying the bioactive compounds involving the bio-reduction of Ag^+ ions and the capping of AgNPs that can enhance the stability and bioactivity (Shamhari *et al.* 2018).

3.3 Particle Size Distribution and Zeta Potential Analysis

The synthesized AgNPs were analyzed using DLS and zeta potential. The size distribution was analyzed using DLS, which provided the Z-average diameter and Pdl (Shamhari *et al.* 2018). The results are provided in Figure 4. The nanoparticles showed a Z-average size of 96.60 nm and a Pdl of 0.219, indicating a relatively uniform size distribution and monodispersity. The intensity-based DLS profile displayed a dominant peak at 127.3 nm with an intercept value of 0.919, suggesting high measurement reliability. Although a minor presence of aggregates was observed, it did not significantly affect the overall homogeneity or functionality of the nanoparticles (Houghton *et al.* 2024). These results confirmed that the synthesized AgNPs possessed suitable dimensions and stability for potential applications in antimicrobial and biomedical fields.

The surface charge and colloidal stability of the synthesized AgNPs were evaluated using zeta potential analysis. The nanoparticles exhibited a zeta potential value of -25.7 mV, indicating moderate to good colloidal stability due to sufficient electrostatic repulsion between particles. The zeta potential distribution revealed a single, sharp peak centered at -25.7 mV, with 100% area

coverage and a standard deviation of 8.30, confirming a uniform surface charge across the nanoparticle population (Salayová *et al.* 2021). Additionally, the nanoparticles demonstrated a conductivity of 1.54 mS/cm, which further supported the stable ionic environment of the colloidal system. Overall, the negative surface charge suggests effective capping by phytochemicals present in *P. emblica* bark extract, contributing to the stability and dispersion of the nanoparticles, making them suitable for biomedical and antimicrobial applications (Masum *et al.* 2019).

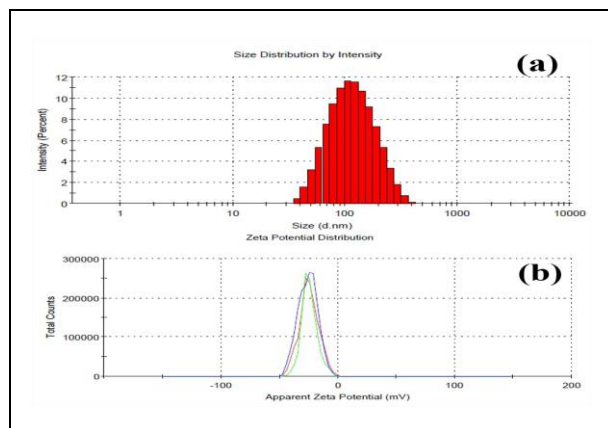


Fig. 4: Functional characterization of green-synthesized AgNPs: (a) DLS analysis and (b) Zeta potential peak.

3.4 FTIR Analysis

Structural analysis of the synthesized AgNP was conducted by FTIR to reveal the presence of functional groups on the surface of AgNPs (Zhang *et al.* 2016). Figure 5 presents the FTIR spectrum of the AgNPs sample. The results showed that the presence of alcohols and phenolic groups that were identified by observing the prominent peak at 3340 cm^{-1} (O–H stretching). The peak at 1637 cm^{-1} (C=O stretching) showed the amide or carboxyl functional group in the sample, and the peak at 1380 cm^{-1} indicated the C–N stretching of aromatic amines.

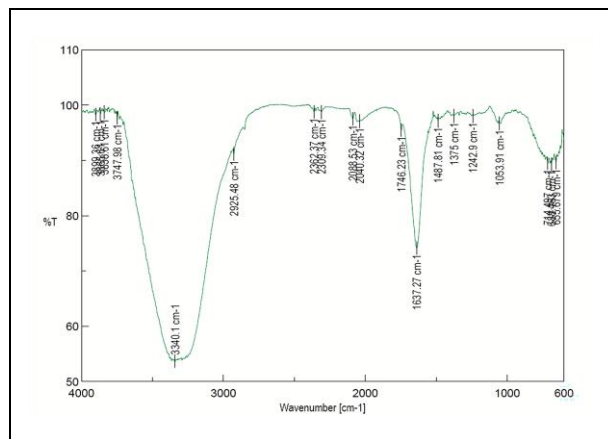


Fig. 5: FTIR spectrum of synthesized AgNPs

3.5 SEM Analysis

SEM was used to study the surface morphology of AgNPs. From this analysis, the shape of the nanoparticle was observed to be irregular with a uniform size distribution. Figure 6 shows that the AgNPs were moderately agglomerated, which could be attributed to the presence of residual plant biomolecules acting as capping agents (Huang *et al.* 2024). Further, the figures illustrated the particle sizes ranged approximately between 40 and 70 nm, confirming the nanoscale nature of the synthesized AgNPs. The morphological features indicated successful green synthesis and stabilization of AgNPs (Ali *et al.* 2024).

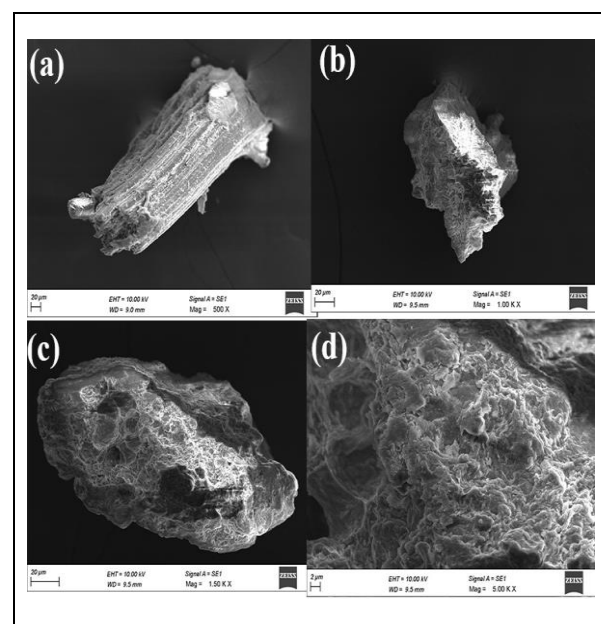


Fig. 6: SEM images of synthesized AgNPs: (a) 500× magnification, (b) 1000× magnification, (c) 1500× magnification, and (d) 5000× magnification.

3.6 Antibacterial Activity

The antibacterial activity was evaluated for the AgNPs using the zone incubation method. The results showed an excellent antibacterial activity against *S. aureus*. Four wells were created on the spread plate to add the control and samples (Rajeshkumar, 2016; Aghili *et al.*, 2016). Figure 7 illustrates the zone of inhibition against the bacterial culture, where the maximum zone of inhibition can be identified for the synthesized silver nanoparticle with bark extract. Negative control in the plate confirms that the assay was standard, and there were no environmental factors inhibiting the growth. This analysis showed the prominent approach to utilize the synthesized AgNPs for wound infection, which can reduce the bacterial infection and prevent environmental pathogens (Paladini and Pollini, 2019).

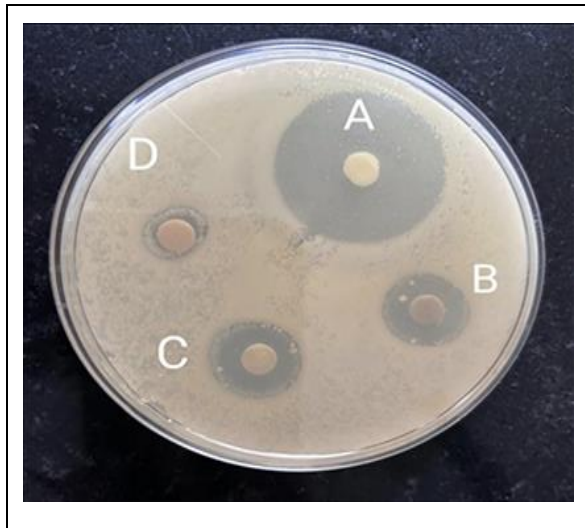


Fig. 7: Antibacterial assay plate: (a) sample, (b) AgNO₃ (c) bark extract, and (d) negative control

3.7 Wound-healing Evaluation (Scratch Assay)

The wound-healing properties of the test sample and the positive control (Cipladine) were evaluated using the L929 cell line. Wound closure was observed over 72 h, and the extent of cell migration and proliferation was measured at 24, 36, 48, and 72 h. The initial wound area was consistent across all experimental groups, measuring approximately 16 cm² (Veeraraghavan *et al.* 2021). Table 2 summarizes the wound-closure measurements and percentages, highlighting the accelerated healing properties of the AgNPs sample. Figure 8 demonstrates the wound-closure rates with comparison to the control. Observations revealed that the sample exhibited a prominent effect within the initial 24 h, healing the wound more effectively than the control, with a high percentage of wound closure. This effect increased in efficiency over time, with each 24-h period showing further improvement. It was found that the sample of AgNPs healed the wound faster than the control. It results in a more prominent approach for wound healing than existing drugs and ointments. Figure 9 presents the cell line study of synthesized green AgNPs. Microscopic images captured during the experiment reveal significant cell migration and proliferation in both the test and control groups. At 36 h, a marked reduction in the scratch area was observed, which was more pronounced in wound closure using the test sample group compared to the control. By 48 h, complete wound closure was observed in both groups, demonstrating comparable wound-healing efficacy. The results suggested that the test sample can be used to serve as a promising natural alternative for promoting wound-healing applications (Teymoorian *et al.* 2024). However, further studies, including *in vivo* evaluations and molecular analyses, are required to elucidate the underlying mechanisms and confirm these findings.

Table 2. The wound-closure measurements and percentages

Wound area measurement		
Time points (h)	Cipladine as control (cm)	Test sample used for 1000 µg/mL (cm)
0	16	16
24	14	10
36	10	7.0
48	4.0	1.0
72	0.0	0.0
Percentage (%) of wound closure		
Time points (h)	Cipladine as control (%)	Test sample used for 1000 µg/mL (%)
24	12.5	37.5
36	37.5	56.3
48	75	93.7
72	100	100

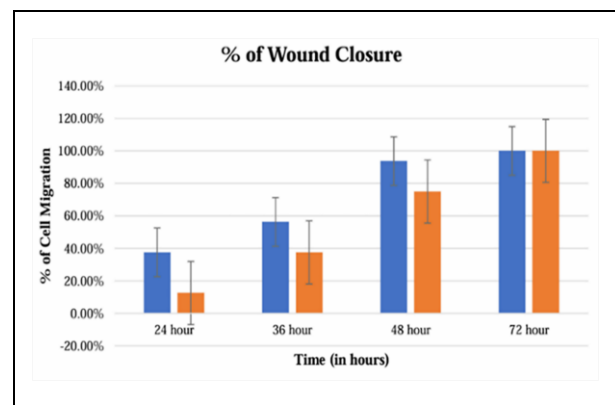


Fig. 8: Graphical representation of wound-closure rates compared with the control (red bar)

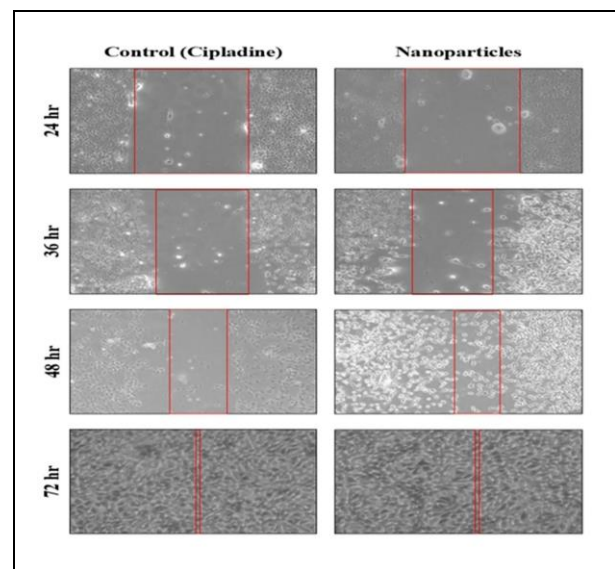


Fig. 9: Cell line study of synthesized green AgNPs

4. DISCUSSION

The present study demonstrated the successful biosynthesis of AgNPs utilizing *P. emblica* bark extract as a reducing and stabilizing agent. The synthesis process, monitored through UV-Visible spectrophotometry, revealed a distinct surface plasmon resonance (SPR) peak, indicative of AgNPs formation (Sivalingam and Pandian, 2024). Among the various ratios, the 1:1 ratio of bark extract to silver nitrate proportion yielded the most pronounced SPR intensity, suggesting optimal conditions for enhanced nanoparticle formation. This enhanced SPR intensity can be attributed to the balanced availability of phytochemicals, providing sufficient reducing and stabilizing capacity for efficient Ag⁺ ion reduction. This proportion likely promoted an optimal nucleation–growth balance, producing monodisperse nanoparticles within the size range favourable for maximal plasmonic resonance. Excess extract might result in over-capping, while lower extract content might limit complete ion reduction, both leading to reduced SPR intensity. Physicochemical characterization further supported the successful synthesis and stability of AgNPs. Particle size analysis and zeta potential analyses confirmed the synthesis of well-dispersed nanoparticles with a mean hydrodynamic diameter in the optimal nanoscale range and a zeta potential indicative of colloidal stability (Houghton *et al.* 2024). SEM analysis corroborated the formation of predominantly irregular-shaped nanoparticles with minimal agglomeration (Huang *et al.* 2024). Furthermore, FTIR spectral analysis identified key phytochemical functional groups (such as hydroxyl, carbonyl, and amine moieties) that likely contributed to the reduction of Ag⁺ ions and stabilization of the nanoparticle surface through electrostatic or hydrogen-bonding interactions (Zhang *et al.* 2016).

The synthesized AgNPs exhibited significant biological efficacy. In the antibacterial assay, the AgNPs demonstrated broad-spectrum activity against tested pathogenic strains, surpassing the efficacy of *P. emblica* bark extract alone (Rajeshkumar, 2016). More notably, the wound scratch assay revealed a marked enhancement in fibroblast migration and wound closure in AgNP-treated cultures compared to both untreated controls and Cipladine-treated groups. This observation suggested a pro-healing effect, potentially mediated by AgNP-induced modulation of reactive oxygen species (ROS), upregulation of growth factors, or promotion of extracellular matrix remodelling (Torabi *et al.* 2023).

5. CONCLUSION

This study demonstrated the green synthesis of AgNPs using a plant bark extract of *P. emblica*. The synthesized AgNPs exhibited favourable physicochemical properties, including stable zeta potential, nanoscale particle size, and irregular

morphology as confirmed through multiple analytical techniques. FTIR analysis verified the involvement of phytochemicals in reduction and stabilization processes. The AgNPs showed significant antibacterial activity against common wound pathogens and accelerated wound closure in an *in vitro* scratch assay, indicating their efficacy in promoting tissue regeneration. The validation further provided valuable insights into the bioactive interactions between phytochemicals and key molecular targets involved in wound-healing and antimicrobial processes, elucidating potential mechanisms underlying their therapeutic effects. Collectively, the findings highlighted the therapeutic potential of biosynthesized AgNPs using bark extract of *P. emblica* as a promising wound-healing agent. However, future studies involving *in vivo* validation and formulation into topical delivery systems could pave the way for clinical translation of this nanobiotechnology-based approach in regenerative medicine.

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CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

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