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Research Article

Effect of Phytofabricated Silver Oxide Nanoparticles on Wound Pathogens

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Infection control is a challenging task in the treatment of wounds and a rise in antimicrobial resistance wound pathogens which is a barrier for the wound regeneration rapidly. Therefore, there is an urgent requirement of novel antimicrobial agents to target the wound pathogens and their biofilms. Silver nanoparticles (AgNPs) are the predominant antimicrobial agent for wound treatment due to their broad spectrum antimicrobial potential against various wound pathogens. Silver nanoparticles (AgNPs) were prepared from aqueous *Adenanthera pavonina* seed extract. This extract plays as a reducing agent for reduction of silver nitrate to silver oxide nanoparticles. An absorption peak at 428 nm with characteristic feature of surface plasmon resonance was observed. The average size of AgNPs was found to be ~200 nm and cube in shape of AgNPs. FTIR data revealed the presence of phenolic groups that were responsible for reduction and stabilization of silver nanoparticles. Face centered cubic (FCC) crystalline structure was revealed from XRD relating to silver oxide (AgO) with good antibacterial potential. The antioxidant activity of extract increases when the concentration increases, and it can be used in the inhibition of free radicals at wound site. Antibacterial activities showed effective inhibition against various pathogens such as *E.coli*, *Salmonella typhi*, *Sphingomonas*, and *Bacillus*. Given this correspondence, the green synthesized AgNPs would be a potential antimicrobial agent for various wound treatments.

1. Introduction

In past decades, antimicrobial resistance pathogens are being emerged in the management of wound and soft tissue repair. Silver nanoparticle (AgNP) is a potent antimicrobial agent for wound regeneration and repair and an alternative for antibiotics and has notable properties such as size, shape, and surface charge, giving an outstanding antimicrobial action against the pathogens. The production of antimicrobial action of AgNPs was mechanized via adhesion on the wound pathogens, penetration into the cells, inhibition of free radicals, and modulation of the pathways for signal transduction [1, 2].

The development of green synthesis for silver nanoparticles is an important aspect of current nanotechnology research. The metallic nanoparticles such as gold (Au), copper (Cu), silver (Ag), platinum (Pt), and palladium (Pd) have been synthesized by different green methods such as using fungi, bacteria, and different parts of plants/tree [3–5] [6–8]. Among these metal nanoparticles, AgNPs play a significant role in the field of medicine due to its attractive antimicrobial property.

Metallic oxide nanoparticles are being synthesized via physical, chemical, and biological methods. Zinc oxide has potential applications in food sector, and it can be synthesized via the strain of *Lactobacillus plantarum*. The nanoparticles are spherical with diameter of 7 to 19 nm, giving a potential antimicrobial action against the pathogens [2]. Silver nanoparticles synthesized via microbes were used for degradation of phenol via photochemical effect. These silver nanoparticles also have the catalytic activity for photodegradation of phenol in wastewater.

Previous studies reported that the highly sensitive metal oxide nanoparticles exhibit very good bactericidal action against Gram-positive and Gram-negative bacteria. Physical and chemical methods are the more popular methods for synthesizing nanoparticles, but the usage of toxic chemicals limits their application towards the medical field [9–12] and not economically feasible one. Compared to chemical and physical methods of synthesis, bio/green synthesis methods are established as an alternative and found to be the most efficient one [13–16]. AgNPs possess high surface plasmon resonance (SPR), high extinction coefficient, and antimicrobial properties which are less toxic than the bulk [12].

In this present investigation, green synthesis method of AgNPs from aqueous *Adenanthera pavonina* seed extract is investigated and the characterization of AgNPs was performed and its antimicrobial potential was evaluated against various wound pathogens.

2. Materials and Methods

Silver nitrate ($AgNO_3$) AR grade was purchased from Sigma-Aldrich Chemicals, Mumbai, India. All fresh seeds of *Adenanthera pavonina* were collected from the College of Engineering Guindy (CEG) Campus, Anna University, Tamil Nadu, India.

Fresh seeds of *Adenanthera pavonina* were collected and washed thoroughly first with double-distilled water to remove all the dust and unwanted particles, and then, the seeds were dried at room temperature. The dried seeds were grounded into fine powder using mechanical milling; 5 g of fine powder was transferred into a 250 mL Erlenmeyer flask and then added 100 mL double-distilled water and boiled for 30 min. The sample was then filtered twice by using normal filter paper and then again filtered through Whatman No. 1 filter paper to get clear solution. Then, the aqueous extract was stored in a refrigerator at 4°C in an airtight bottle for synthesis of silver oxide nanoparticle.

1 mM aqueous solution of $AgNO_3$ solution was used for the synthesis of silver nanoparticles. 50 mL of extract was added drop by drop into 250 mL of aqueous solution silver nitrate solution that was heated at 60°C with reflux condition under stirring condition for 30 min. The solution turned from yellowish to dark brown colour confirming the reduction of silver ions into AgNPs. The reaction mixture was centrifuged at 9000 RPM, and the sample was stored for further analysis.

Synthesized silver nanoparticles are initially characterized by UV-visible absorption spectroscopy using the JASCO V 550 spectrophotometer from 200 to 900 nm wavelength. FTIR measurement was used to determine the biomolecules that were present in AgNPs using the PerkinElmer FTIR spectrometer Jusco 5300 model. The analysis was carried out from 400 to 4000 cm^{-1} wavenumber range. SEM analysis was done with Supra Zeiss with resolution of 1 nm at 30 kV. The sample was placed on a copper grid and left to dry for 60 min under vacuum condition before subjecting into instrument. X-ray diffraction (XRD) measurements of the biologically synthesized AgNPs powder cast onto glass slides and analysis were done using X'Pert Pro X-ray diffractometer (PANalytical) at 40 kV and current of 30 mA. AgNPs were air dried overnight, and the powdered samples were used for analysis.

Antibacterial activities of AgNPs were tested against *Escherichia coli*, *Salmonella typhi*, *Sphingomonas*, and *Bacillus subtilis* by agar diffusion method. Wells were made by standard procedure at equal distance and were filled with AgNPs of various concentrations. For cultivating bacterial species, nutrient agar medium was used. The medium was sterilized by autoclave and then transferred to a petri plate. After solidification inoculated with a fresh growth of test stain, different concentrations of 25, 50, 75, and 100 μL AgNPs were loaded. Then, the plates were incubated for 24 h at 37°C after the incubation time, and zone of inhibition was calculated.

The free radical scavenging ability of the extracts against 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical was evaluated. 1 mL of 0.1 mM DPPH dissolved in ethanol was prepared. To this solution, seed extracts from 50 to 250 μg/μL, 1 mL ethanol, and 0.95 mL Tris HCl were added. The mixture was left for 30 min, and the absorbance was measured at 517 nm. The DPPH free radical scavenging activity was subsequently calculated.

$$\%\text{DPPH radical scavenging} = \frac{(\text{Control OD} - \text{Sample OD})}{\text{Control OD}} \times 100, \quad (1)$$

where control OD is the absorbance of the control reaction solution (containing all reagents except the test compound) and sample OD is the absorbance of the test compound, respectively.

3. Results and Discussion

3.1. UV-Visible Spectroscopy. After the addition of aqueous seed extract of *Adenanthera pavonina* into silver nitrate solution, the immediate colour change was observed from colourless to brown confirming the formation of AgNPs due to surface plasmon vibrations. This is confirmed via peak absorbance at 428 nm as shown in Figure 1; narrow peak indicates

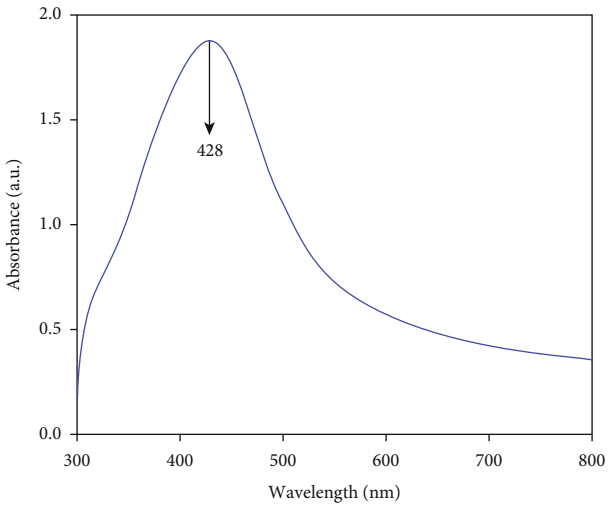

Figure 1: UV-visible spectrum of AgO nanoparticles.

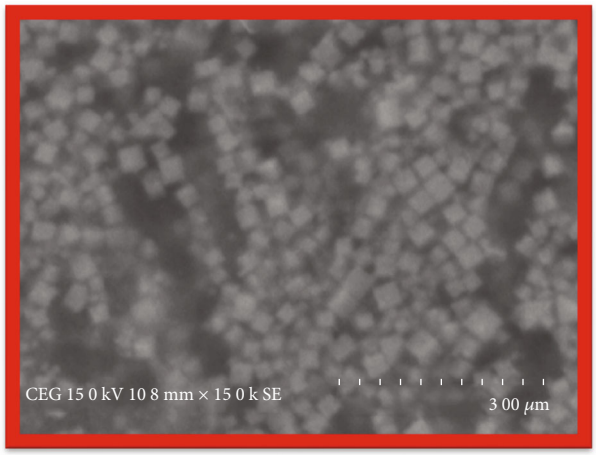

Figure 2: SEM image of synthesized silver nanoparticles.

that the nanoparticles are monodispersed. Absorption spectra of AgNPs revealed the excitation of surface plasmon vibration having maximum values reported in the visible ranges.

3.2. Scanning Electron Microscopy. SEM micrograph reveals AgNPs square shaped with the average size 70 to 100 nm and monodispersed (Figures 2 and 3). Every particle was independent from one another that proved to be well stabilized. The square shape of AgNPs confirms the face centered cubic (FCC) and confirmed via XRD. The shape of nanoparticles does also control the antimicrobial potential of silver nanoparticles. The triangular shape of AgNP synthesized produces good antimicrobial action against *E. coli* than that of spherical- and rod-shaped AgNPs [17]. The shape of the nanoparticle is one of the important properties promoting their antimicrobial action against the wound pathogens [1].

Figure 3 reveals the size distribution of nanoparticles. The size of AgO NP varies from 200 to 250 nm. Due to the agglomeration of nanoparticles, wide size distributions were observed.

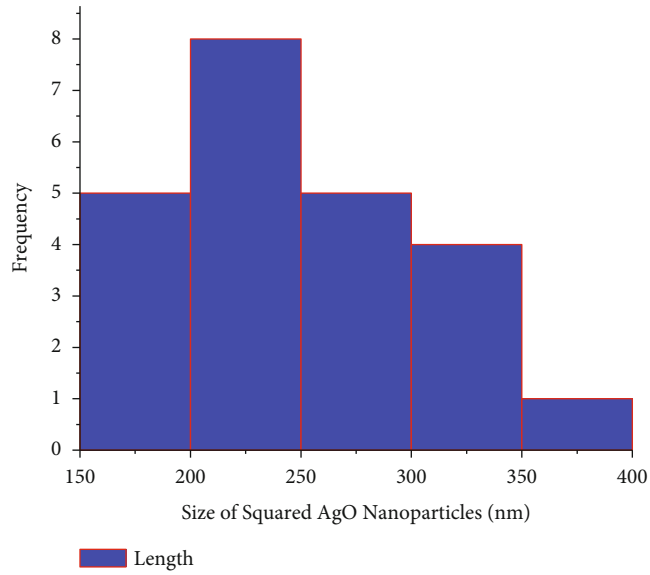

Figure 3: Size distribution of silver nanoparticles.

3.3. X-Ray Diffraction. XRD spectrum showed three distinct diffraction peaks at 37.98°, 46.01°, and 64.41° which indexed the planes 1 1 1, 2 0 0, and 2 2 0 of the face centered cubic (FCC) (Figure 4). The average grain size was determined using Scherrer's formula as shown in equation (2) and estimated as 28 nm. This analysis revealed that biosynthesized AgNPs are in crystalline nature. From these XRD results, it was confirmed that the phytofabricated nanoparticles were silver oxide (AgO). These phytofabricated nanoparticles were found to have silver oxide component.

3.4. Fourier Transform Infrared Spectroscopy (FTIR) Studies. Figure 5 reveals the FTIR spectra of both silver nanoparticles and seed extract confirming that there was presence of various bioactive molecules in the seed extract. These bioactive molecules coat the nanoparticles and make them biological active via the presence of these molecules on the nanoparticles. Table 1 summarizes the peaks in FTIR spectra.

3.5. Antimicrobial Studies. The antibacterial activities of AgNPs were tested against *Escherichia coli*, *Salmonella typhi*, *Sphingomonas*, and *Bacillus subtilis* (Figure 6). Table 2 shows the zone of inhibition of AgNPs on all four bacteria. The concentration of AgNPs was employed in various levels 25, 50, 75, and 100 μg of AgNPs to antibacterial assay against the microbes. The maximum concentration of 100 μL gives good zone of inhibition which was observed to be of 8 mm for *E. coli*, 9 mm for *Salmonella typhi*, 12 mm for *Sphingomonas*, and 13 mm for *Bacillus subtilis*. The zone of inhibition against wound pathogens can be improved by increasing the dose of AgNPs. This test also proves that the bioactive molecules that were coated over the outer surface of the AgNPs also enhanced the antibacterial activity. Table 2 reveals the effect of $AgNO_3$ nanoparticles on the antimicrobial efficacy of the wound pathogens.

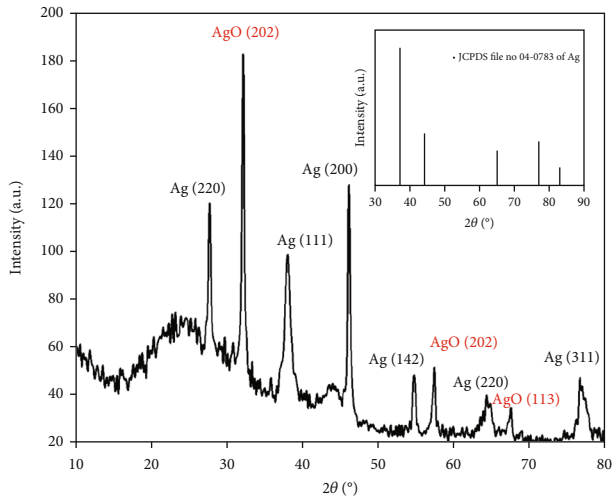

Figure 4: XRD pattern of the silver nanoparticles synthesized from aqueous seed extracts of *A. pavonina*.

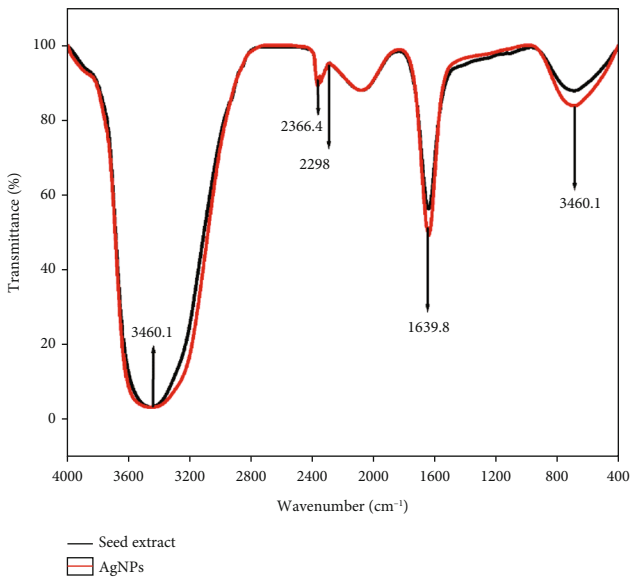

Figure 5: FTIR spectra of silver nanoparticles and seed extract.

Table 1: FTIR peaks.

Shift in peak (cm^{-1})	Stretching bonds	Compounds
3460.1-3463.7	O-H stretch	Alcohols, phenols
2365.7-2366.4	C-H stretching, H–C=O	Aldehyde group
2345.8-2345.7	C=O stretching vibration	Ketone
1639.8-1637.2	N-H bend	Primary amine
690.5-689.7	C-H bend	Alkynes

3.6. Antioxidant Studies. The antioxidant activity was found to be better for the higher concentration of the *Adenanthera pavonina aqueous* extract. The effect of antioxidant activity is due to their hydrogen donating activity of phytochemicals such as flavonoids, polyphenols, alkaloids, saponins, tannins,

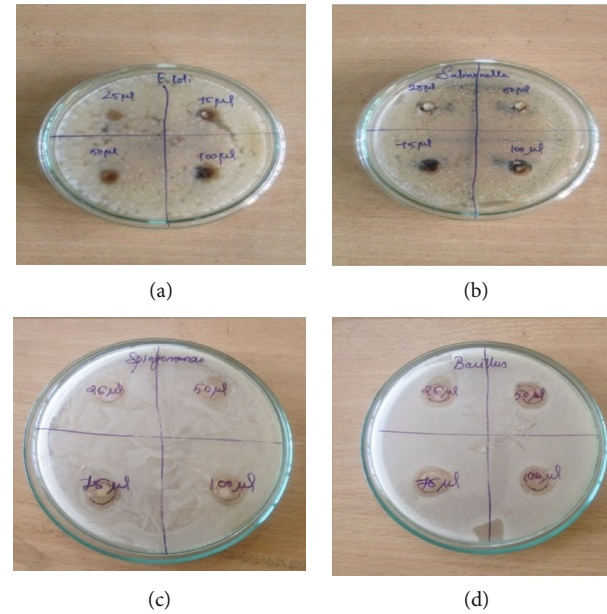

Figure 6: Disc diffusion assay for $AgNO_3$ NP against (a) *Escherichia coli*, (b) *Salmonella typhi*, (c) *Sphingomonas*, and (d) *Bacillus subtilis*.

Table 2: Zone of inhibition for biosynthesized AgNPs from *Adenanthera pavonina* seed extract against different bacterial species (mm).

Wound pathogens	Zone of inhibition (mm) Different concentrations of AgNPs			
	25 μL	50 μL	75 μL	100 μL
Escherichia coli	3	4	5	8
Salmonella typhi	2	3	7	9
Sphingomonas paucimobilis	5	8	11	12
Bacillus subtilis	7	9	10	13

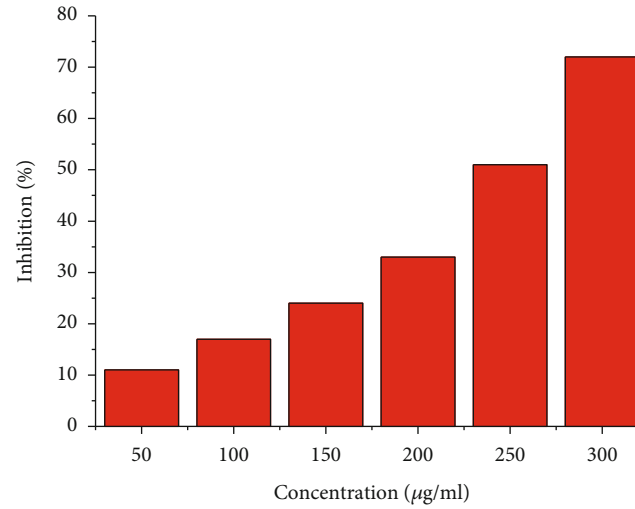

Figure 7: Antioxidant activity of *Adenanthera pavonina* seed extract.

anthraquinones, cardiac glycosides, and cyanogenetic glycosides. Due to the lipophilic nature of DPPH, the electron from the antioxidant bioactive molecule can be accepted by DPPH and gives a colour change from violet to yellow which was observed at 517 nm in a UV-visible spectrophotometer. This assay concludes that AgNPs show very good antioxidant activity. The seed extract of *Adenanthera pavonina* has the capacity to scavenge free radicals. *Adenanthera pavonina* seed extract exhibits good free radical scavenger. The extract gave the highest percentage inhibition of 72% at 300 μg of extract. Figure 7 shows the antioxidant activity of *Adenanthera pavonina* seed extract.

4. Conclusions

The biological synthesis of silver nanoparticles using *Adenanthera pavonina* seed extract was demonstrated and 84.5 nm with square-shaped structure of nanoparticle formed. The antimicrobial screening test indicated that *Bacillus subtilis* showed higher zone of inhibition. The synergistic effect showed higher zone of inhibition effect in all the tested bacterial species. The synthesized silver nanoparticles were found to exhibit a good antioxidant property.

Data Availability

All the data supporting the results of this study were included in the article.

Conflicts of Interest

The authors declare no conflict of interest.

Acknowledgments

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