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Research Article

Characterization and Antibacterial Activity of Biosynthesized Silver Nanoparticles Using the Ethanolic Extract of *Pelargonium sidoides* DC

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Development of cost-effective and eco-friendly methods of nanoparticle synthesis could play a crucial role in integrating nanotechnology and phytomedicine for biological applications. In this study, biogenic silver nanoparticles (AgNPs) were synthesized using the ethanolic extract of *Pelargonium sidoides* DC at 60°C. Formation of nanoparticles was monitored using UV-Visible spectroscopy at different time intervals. A maximum absorption at 456 nm was observed as the reaction time increased, resulting in a red shift of the surface plasmon band (SPB). Attenuated total reflectance Fourier transform infrared spectroscopy (FTIR) revealed the reducing and stabilizing activity of flavonoids, coumarins, tannins, and phenols. Size and morphology of the AgNPs were analysed using scanning electron microscopy (SEM) and transmission electron microscopy (TEM) which indicated the spherical nature of the nanoparticles with sizes ranging from 11 to 90 nm. Further characterization of the AgNPs was carried out using EDS, XRD, and Raman spectroscopy, respectively. Additionally, the AgNPs had a marginally higher antimicrobial activity when compared to the plant extract against Gram-positive *Streptococcus pneumoniae* (ATCC 27336) and *Bacillus cereus* (ATCC 10876) and Gram-negative *Moraxella catarrhalis* (ATCC 25240), *Escherichia coli* (ATCC 25922), and *Pseudomonas aeruginosa* (ATCC 27853).

1. Introduction

Nanoscience offers a range of platforms for the development of novel technological advancements for a broad range of environmental, biochemical, biological, and other applications [1, 2]. Fabrication of materials at the nanoscale using natural or biological sources has rapidly advanced over the past few years [3]. Several routes of synthesizing silver nanoparticles (AgNPs) have been reported previously, classified as chemical, physical, photochemical, and biological methods [4]. Synthesis of silver nanomaterials by means of chemical processes can be further subcategorized into chemical reduction methods, electrochemical techniques, photochemical methods, and pyrolysis whereas physical methods can be subcategorized into physical vapor condensation, inert gas condensation, cocondensation, ultraviolet irradiation, thermal decomposition, laser ablation arc-discharge, sonodecomposition, radiolysis, and direct current magnetron sputtering [5, 6]. Synthesis and fabrication of nanoparticles using either chemical or physical methods pose a significant threat to the environment as their principal contaminants are difficult to purify and often require high energy input [7, 8]. Thus, nanoparticle biosynthesis using plant extracts is by far the most viable method owing to their eco-friendliness, biocompatibility, and low toxicity [9, 10].

Plant extracts of *Rosmarinus officinalis* Linn., *Solanum trilobatum*, *Origanum vulgare*, *Acacia leucophloea*, *Coffea arabica*, *Ficus benghalensis*, and *Azadirachta indica* have

been used as capping and reducing agents in the synthesis of silver (AgNPs) and gold (AuNPs) nanoparticles with potent antimicrobial and anticancer activity [10–14]. Currently, AgNPs are used globally in the production of a wide range of products, such as water treatments, water filters, sprays, detergents, refrigerators, washing machines, paints, cosmetics, and electronics, mainly due to their antimicrobial properties [15–17]. Even so, applications of AgNPs are most advanced in medical devices and supplies, the food industry, and the clothing industry [18].

The use of traditional medicine as an alternative or otherwise, the primary source of health care has been a longstanding practice for decades [19]. The efficiency of medicinal plants mostly depends on the phytochemical constituents that they accumulate through secondary metabolism, and their effectiveness is often rendered by a mixture of various secondary metabolites [5, 20].

Species of Pelargonium (*crispum*, *reniforme*, *sidoides*, *graveolens*, etc.) play an immense role in the basic health care system of a majority of the population of the Southern African regions [21]. *Pelargonium sidoides* DC, of the family Geraniaceae, is a medicinal plant used for the treatment of bacterial and fungal infections such as tuberculosis coughs, diarrhoea, and bronchitis by many South African ethnic groups [22–24]. Phytochemical constituent studies have proven that the roots, stems, and leaves of *Pelargonium sidoides* are rich in tannins, gallic acids and their methyl esters, phenolic compounds, coumarins (scopoletin and umckalin), and flavonoids which contribute to a wide range of pharmacological applications [25–27]. The rising cost of prescription drugs and the emergence of drug-resistant pathogenic infections have brought about the necessity to develop antibacterial substances from plants and other natural sources; thus, the need to develop potent drugs to combat multidrug-resistant microorganisms is imperative [27, 28]. In light of the importance *of P. sidoides* and biogenic silver nanoparticles, this investigation was focused on the effect of biosynthesized nanoparticles against clinically significant, pathogenic bacteria to promote the need of utilizing medicinal plants as natural sources of the alternative to antibacterial drugs. Therefore, herein, we described the green synthesis of AgNPs using *P. sidoides* extracts and the efficacy against Gram-positive and Gram-negative microorganisms.

2. Materials and Methods

2.1. Chemicals, Reagents, and Media. Silver nitrate, solvents, reagents, and culture media used for this study were purchased from Merck, South Africa. Bacterial isolates, Gram-positive *Streptococcus pneumoniae* (ATCC 27336) and *Bacillus cereus* (ATCC 10876), and Gram-negative *Moraxella catarrhalis* (ATCC 25240), *Escherichia coli* (ATCC 25922), and *Pseudomonas aeruginosa* (ATCC 27853) were procured from Davies Diagnostics (Pty) Ltd, South Africa.

2.2. Collection of Plant Samples and Preparation of P. Sidoides Extracts. The roots of *Pelargonium sidoides* were collected from the North-West University (Mafikeng Campus), South Africa, and taxonomically identified. The plant samples were air dried and ground to a fine powder. About 50 g of the powdered plant material was macerated with 80% ethanol (250 mL) at room temperature with constant agitation for 48 hours; this process was duplicated, using fresh solvent each time. The macerate was filtered using Whatman No. 1 filter paper and concentrated to dryness using a rotary evaporator at 65°C for 3 hours. The resultant residue was then stored at 4°C in an airtight bottle until further use.

2.3. Phytochemical Screening. The phytochemical analysis of the ethanolic extracts of *P. sidoides* was carried out by standard procedures described by [26, 27, 29]. The crude extract was screened for the presence of saponins, tannins, phenolic compounds, coumarins, flavonoids, terpenoids, glycoside alkaloids, and proteins.

2.4. Biosynthesis of Silver Nanoparticles (PSAgNPs). The procedure for the synthesis of nanoparticles was adopted from [28] with slight modifications. About 80 mL of 1 mM silver nitrate solution was added to 20 mL (1 mg/mL stock) of the ethanolic plant extract at 60°C with magnetic stirring. A colour change of the reaction mixture from pale yellow to reddish brown after 2 hours served as visual confirmation for the formation of AgNPs.

2.5. Characterization of Silver Nanoparticles

2.5.1. UV-Vis Spectroscopy. The colloidal nanoparticle solution was analysed to monitor the bioreduction of silver ($Ag^+ \rightarrow Ag^0$) using a UV-Visible spectrophotometer (Agilent Technologies, Cary 300) in the wavelength range of 300-800 nm at a resolution of 1 nm. Due to the elevated optical density (OD) of the colloidal suspension, a 1 mL aliquot of the solution was diluted with 3 mL of distilled water. The absorbance spectrum of the silver nanoparticles was monitored periodically for 24 hours. Distilled water was used as a blank.

2.5.2. X-Ray Diffraction. The structural characterization of the AgNPs was carried out using an X-ray diffractometer. XRD analysis was conducted by Bruker equipment using monochromatic Cu kα radiation ($\lambda = 1.5406$ Å) ran at 40 kV. The scanning was controlled in the region of 20°–100°. The attained XRD images were compared with the Joint Committee on Powder Diffraction Standards (JCPDS) library to account for the crystalline structure.

2.5.3. Raman Spectroscopy. Raman spectra were measured using a Bruker Raman spectrometer (model Senterra with laser excitation at 514 nm and laser power at 10 mW). Spectral data were collected using a 50 microscope objective (NA = 0.51) with 30 seconds integration time. The silver nanoparticle samples were prepared by mixing 360 mL of colloidal solution with 40 mL of aqueous solutions of the probe molecule, resulting in a final AgNP concentration of 1.0×10^{-5} mol/L.

2.5.4. ATR-FTIR Spectroscopy. The nanoparticle solution was centrifuged at 6000 rpm for 30 minutes, and the supernatant was discarded. The pellet was resuspended in distilled water and centrifuged further to remove any nonreacting

molecules in the colloidal matrix. A powder sample was obtained by drying the purified pellets in a hot air oven for 2 hours. FTIR studies of the powder AgNPs and crude extracts of *P. sidoides* were performed using the Bruker Platinum-ATR spectrophotometer. FTIR measurements were carried out in the wavenumber range of 4000-400 cm^{-1} with a resolution of 4 cm^{-1} at an average of 32 scans per sample. Both FTIR measurements were carried out in the Attenuated Total Reflectance mode.

2.5.5. Dynamic Light Scattering (DLS). Dynamic light scattering (Malvern Zetasizer Nano-ZS) was used to analyse the zeta potential of the synthesized PSAgNPs. For DLS measurements, powder AgNPs were resuspended in distilled water and sonicated for 15–20 minutes to properly disperse the particles in water. Zeta potential values were obtained from the triplicate analysis of the nanoparticles in the aqueous milieu.

2.5.6. SEM Analysis. Samples were mounted on 12 mm aluminium specimen stubs with double-sided carbon tape, coated with gold palladium, and examined with a FEI Quanta 250 FEG SEM operating at 10 kV.

2.5.7. TEM Analysis. Particles were sonicated for 30 minutes to 1 hour in 100% ethanol. A drop of the suspension was placed on a carbon-coated formvar grid and allowed to dry. Specimens were examined with a FEI Tecnai G2 20 S-Twin transmission electron microscope operating at 200 kV. Micrographs were taken with a Gatan bottom mount camera using Digital Micrograph software.

2.6. Antibacterial Activity

2.6.1. Agar Well Diffusion Assay. The antibacterial activity of *P. sidoides* ethanolic extract and synthesized PSAgNPs was evaluated using the agar well diffusion method against Gram-positive *Streptococcus pneumoniae* (ATCC 27336) and *Bacillus cereus* (ATCC 10876) and Gram-negative *Moraxella catarrhalis* (ATCC 25240), *Escherichia coli* (ATCC 25922), and *Pseudomonas aeruginosa* (ATCC 27853). Pure cultures of these microorganisms were refreshed on nutrient agar medium and incubated at 37°C for 24 hours. Fresh overnight cultures were inoculated on Mueller Hinton agar (MHA) plates using sterile swabs and allowed to stand for 20 minutes. Wells of 6 mm diameter were made on MHA plates with the bacterial lawn. Each well was filled with 50 μL of different concentrations (50, 100, and 150 μg/mL) of PSCE in distilled water and PSAgNPs in dimethyl sulfoxide (DMSO) prepared from 10 mg/mL stock. DMSO (5%) was used as the negative control, and tetracycline (10 μg/mL) served as the reference standard.

The plates were incubated at 37°C for 24 hours and the diameters of the inhibition zones around the wells were measured. Experiments were carried out in triplicates to minimize error.

3. Results and Discussion

3.1. Phytochemical Screening. The phytochemical analysis of the crude ethanolic extracts of *P. sidoides*, shown in Table 1, revealed the presence of a variety of phytochemical compounds including hydrolysable tannins, phenolic compounds, saponins, coumarins, and flavonoids. Compounds such as cardiac glycosides, anthraquinone glycosides, alkaloids, terpenoids, and xanthoproteins were not detected.

Table 1: Phytochemical screening of *P. sidoides* ethanolic extracts.

Phytochemicals	*P. sidoides* ethanolic extract
Saponins	+
Tannins	+
Phenols	+
Terpenoids	−
Coumarins	+
Alkaloids	−
Flavonoids	+
Glycosides	−
Xanthoproteins	−

+: present; −: absent.

3.2. UV-Vis Spectroscopy and Visual Analysis. The addition of silver nitrate to the ethanolic extract of *P. sidoides* resulted in a colour change of the reaction mixture from pale yellow to scarlet brown after 2 hours, as shown in Figures 1(a) and 1(b), which served as visual confirmation for the formation of nanoparticles. The resultant colour change of the colloidal suspension was due to excitation of the surface plasmon resonance (SPR) of the silver nanoparticles [30–32].

The analysis of the colloidal solution by UV-Vis spectroscopy showed a characteristic absorbance peak at 456 nm after 2 hours. As the reaction time increased, a steady shift in the absorbance peak from 456 to 480 nm, shown in Figure 1(c), was observed (Bathochromic effect) which may be due to the formation of larger particles [1, 33]. A directly proportional relationship between the increase in reaction time and intensity of the absorption peak was detected. The γ_{max} values in the 400-500 nm range are specific for the surface plasmon band of AgNPs [34–36].

The results obtained from the spectral analysis of PSAgNPs have a reasonable correlation with the results of *Beta vulgaris* extract-mediated AgNPs by [37] and AgNPs synthesized using ethanolic leaf extracts of *Clausena anisata* by [38].

3.3. X-Ray Diffraction. The AgNP crystalline structure was characterized by X-ray powder diffraction. Figure 2 shows the XRD diffraction pattern of AgNPs which exhibited sharp diffraction peaks corresponding to the crystal planes of (111), (220), and (200) associated with the face-centred cubic lattice of silver. The XRD profile of the nanoparticles indicates a monoclinic phase of the crystalline structure. These findings confirm the formation of silver nanocrystals.

3.4. ATR-FTIR Analysis. The functional groups of the biomolecules responsible for capping and stabilizing the nanoparticles were analysed using FTIR spectroscopy. Peaks at 3182 cm^{-1}, 2922 cm^{-1}, and 2859 cm^{-1} were assigned to the

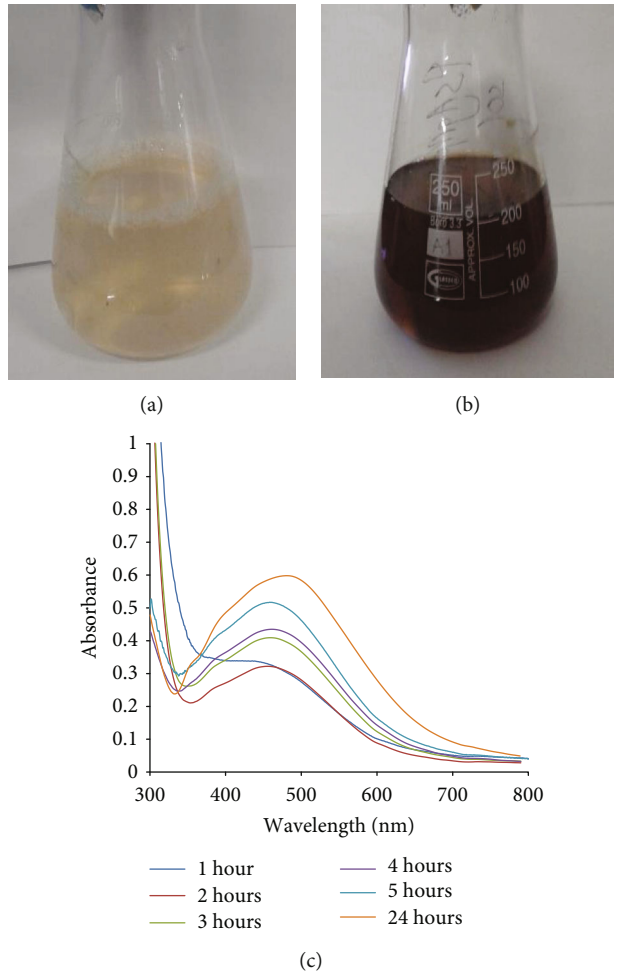

Figure 1: *P. sidoides* ethanolic extract plus 1 mM $AgNO_3$ solution at (a) 0 minutes of reaction time and (b) after 2 hours of reaction time at 60°C. (c) UV-Visible spectra of synthesized AgNPs at different time intervals.

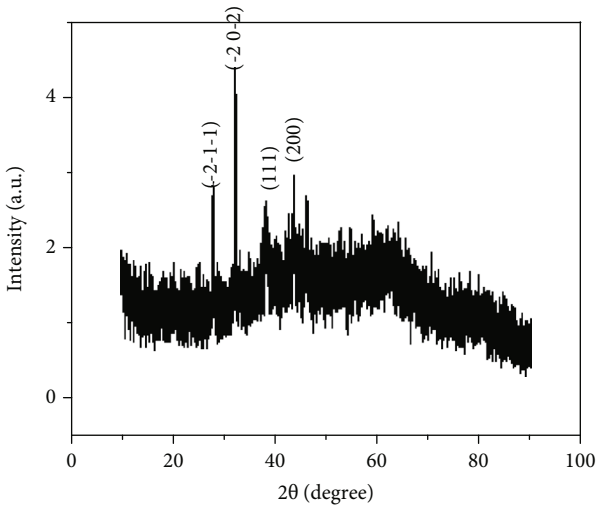

Figure 2: XRD pattern of green synthesized AgNPs using *P. sidoides*.

O–H stretch of carboxylic acids and the C-H stretch of alkanes and alkyls, respectively. The bands at 1028 cm^{-1} to 1328 cm^{-1} correspond to the CO=C–OC and C–O stretching vibrations of esters and alcohols. Peaks at 725 cm^{-1}, 830 cm^{-1}, and 1603 cm^{-1} were connoted to the C–Cl stretch and the C-H stretch of aromatic compounds, alkyl halides, and amines [39]. The chemical alteration of the functional groups of PSCE as a result of the reduction, capping, and stabilization of PSAgNPs is depicted in Figure 3(a).

The FTIR measurements of the purified PSAgNPs showed vibrational peaks at bands at 2914 cm^{-1}, 2848 cm^{-1}, 2675 cm^{-1}, 2348 cm^{-1}, and 2116 cm^{-1}, which are specific for the O-H stretching vibrations of carboxylic acids and alcohols, N-H stretch of amines, C-H bend of aldehydes, and C≡C stretch of alkynes. The C=O, N-H, C-O, C-F, C-H, and ≡C-H stretching vibrations of aldehydes, carboxylic acids, amines, alkyl halides, ethers, aromatic compounds, and alkynes were assigned to the peaks at 1705 cm^{-1}, 1599 cm^{-1}, 1306 cm^{-1}, 1030 cm^{-1}, 828 cm^{-1}, and 610 cm^{-1} [40] (Figure 3(b)). A shift in the intensity of the bands indicates the activity of secondary metabolites in nanoparticle formation. Reduction of ionic silver can

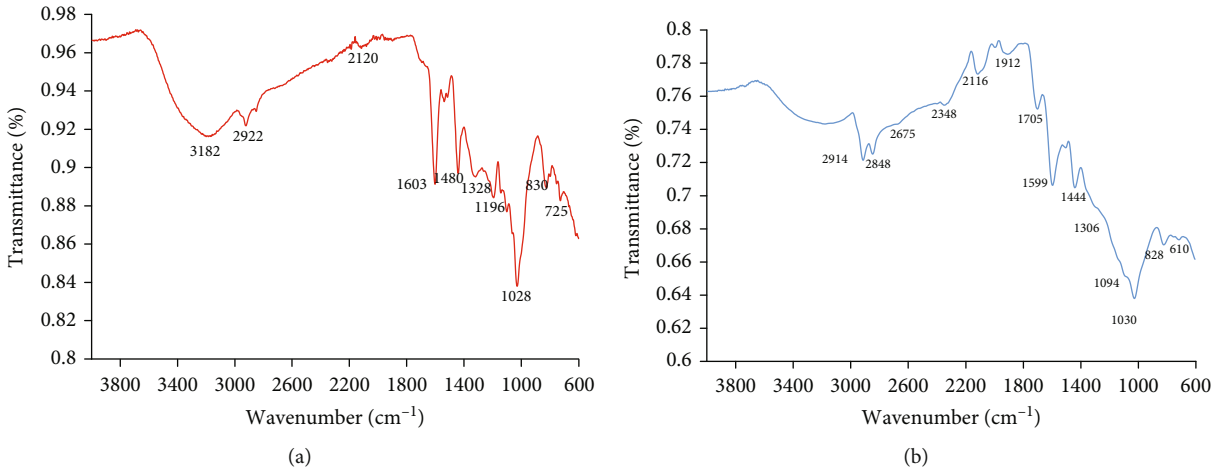

Figure 3: FTIR spectra of *P. sidoides* ethanolic extract (a) and AgNPs (b).

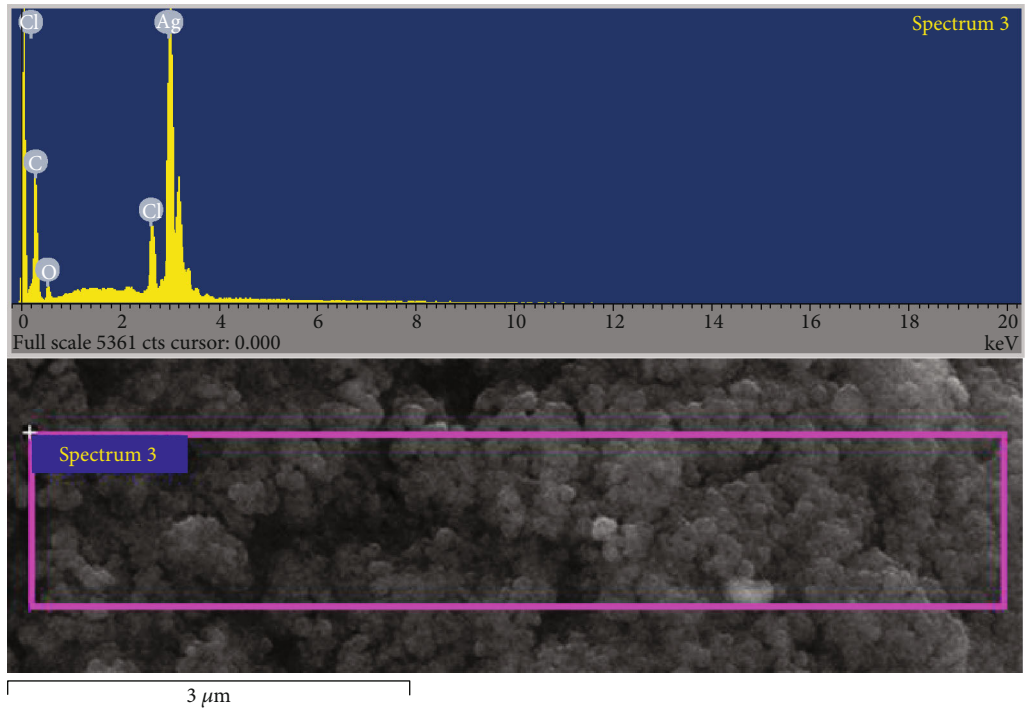

Figure 4: EDX-SEM analysis of *P. sidoides*-mediated AgNPs.

be attributed to coumarins and their methyl esters, flavonoids, tannins, and phenols present in the crude extract of *P. sidoides* (Table 1).

3.5. EDX-SEM and SEM Measurements. The morphology and size of the AgNPs were analysed using EDX-SEM (Figure 4) and SEM images at different magnifications (Figure 5). EDX-SEM analysis (Figure 4) depicts a cluster of relatively spherical and nonuniformly distributed AgNPs with a degree of aggregation. The chemical profile of the synthesized AgNPs was evaluated using EDX-SEM. The EDX pattern of the AgNPs shows high emission energy at 3 keV. The presence of peaks before 5 keV shows the presence of a pure silver metal ion. The pattern also indicates peaks correlating with the binding energies of carbon, chlorine, and oxygen which can be attributed to contaminants during the drying process of the nanoparticles.

3.5.1. TEM Measurements. TEM images of PSAgNPs (Figure 6) revealed the spherical and elliptical nature of nanoparticles ranging from 11 to 90 nm in size. The particles were mostly polydisperse and in direct contact with each other except for a few free floating particles. Formation of bigger particles was due to the agglomeration of smaller particles which may have resulted from evaporating the solvent during the preparation of the powder sample [34, 36]. A translucent layer of biomolecular coating around the nanoparticles serves as evidence of the capping activity of the phytochemical constituents present in the ethanolic extracts of *P. sidoides* which contributes to the stability of the AgNPs [5].

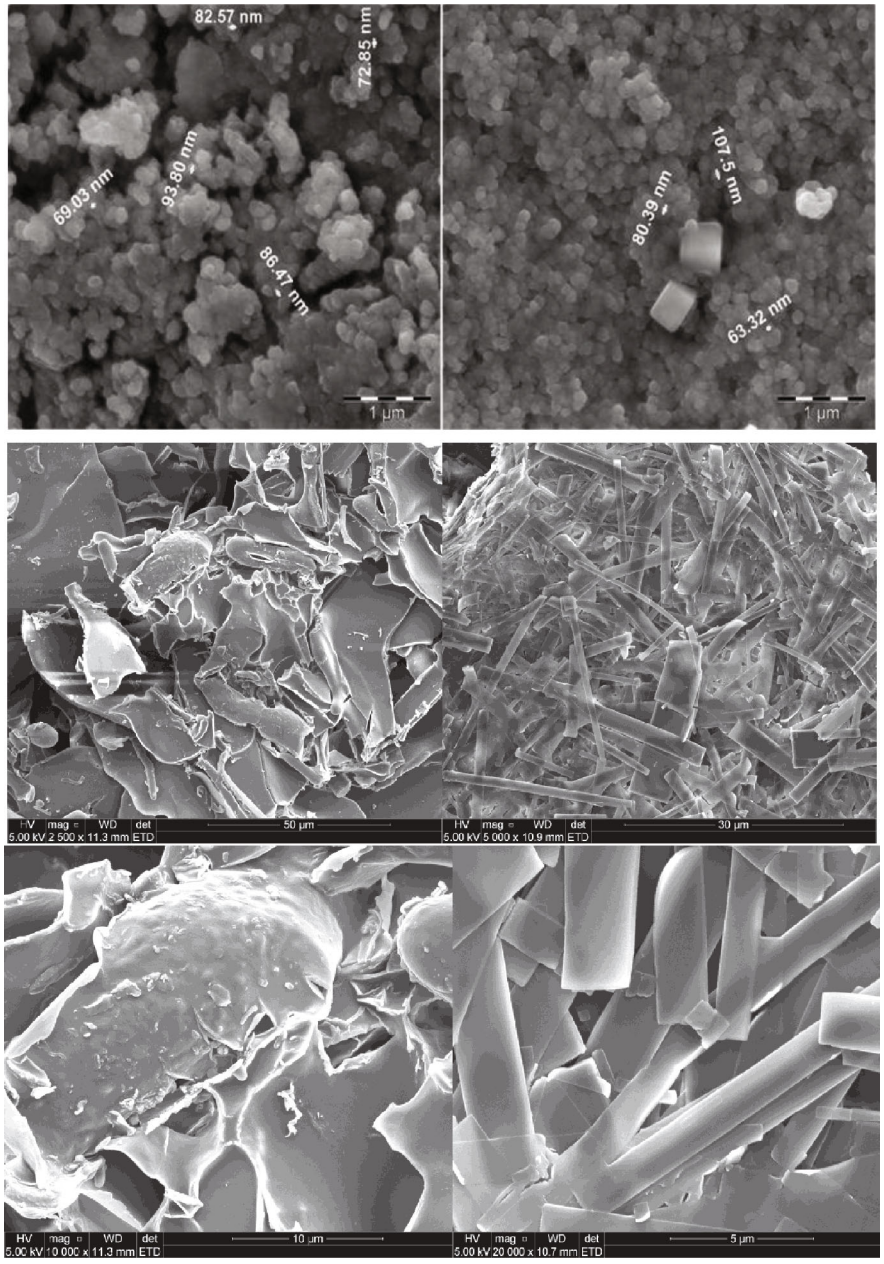

Figure 5: Scanning electron micrographs of synthesized AgNPs at different magnifications (2500x, 5000x, 10000x, and 20000x).

The morphology of PSAgNPs is relatively identical to that of the silver nanoparticles synthesized using *Euphorbia antiquorum* L. latex extract reported by [41].

3.6. Raman Spectroscopy. The Raman spectra of AgNPs, shown in Figure 7, show the intensive peaks at 1595 cm^{-1}, 1361 cm^{-1}, 699 cm^{-1}, and 187 cm^{-1}. These peaks indicate the interaction between the extract and $AgNO_3$ through the carboxylic and hydrophobic group [36, 39]. The band located at 187 cm^{-1} clearly indicates the presence of the silver lattice vibration models [41]. The bands situated at 1595 cm^{-1} and 1361 cm^{-1} indicate the presence of AgNPs.

3.7. Zeta Potential Analysis. The zeta potential value of *P. sidoides*-mediated AgNPs in aqueous suspension was established as −32.3 mV (Figure 8). This suggests that the surface of the nanoparticles is negatively charged and that the particles are uniformly dispersed in the aqueous medium [42]. The high negative value is evident of the extreme stability of the nanoparticles as a result of electrostatic repulsive forces between the particles [43]. A high zeta potential value of about −33 mV ensures a high energy barrier for the stabilization of the nanosuspension [9].

3.8. Antibacterial Activity. The antibacterial potential of PSCE and PSAgNPs was determined against microorganisms that cause lower and upper respiratory tract infections, namely, *Streptococcus pneumoniae*, *Bacillus cereus*, *Moraxella catarrhalis*, and *Pseudomonas aeruginosa*. The resistance or susceptibility of the aforementioned microbes

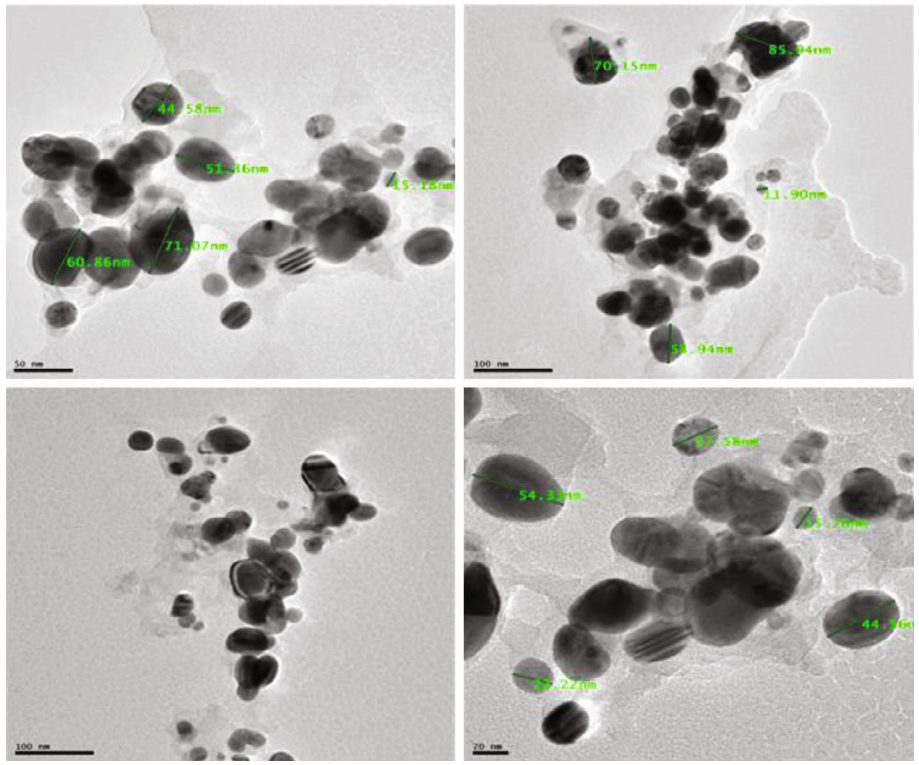

Figure 6: Transmission electron micrographs of AgNPs at different magnifications (20, 50, and 100 nm).

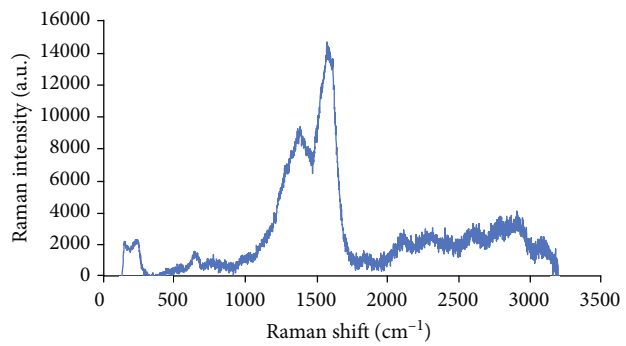

Figure 7: The Raman spectra of the PSAgNPs.

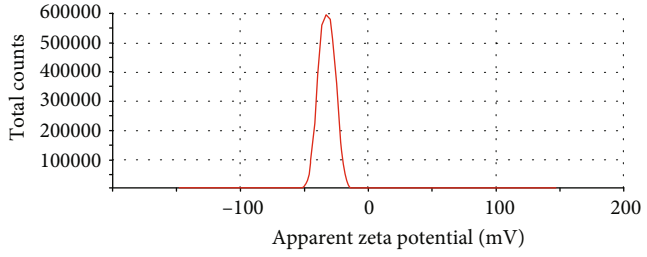

Figure 8: Zeta potential of AgNPs synthesized using *P. sidoides* ethanolic extract.

towards PSCE, AgNPs, and the control antibiotic (tetracycline) was determined by measuring the zones of inhibition around the test compounds, shown in Figure 9. *P. sidoides* extracts showed moderate antibacterial activity against the abovementioned isolates shown in Figure 10(a), with *S. pneumoniae* showing the highest susceptibility, *P. aeruginosa* and *Bacillus cereus* showing the most resistance against different concentrations (50-150 μg/mL) of PSCE. The biogenic PSAgNPs showed a higher potency when compared to the crude extract with a ≥16 mm inhibition zone against *M. catarrhalis*, ≥14 mm against *P. aeruginosa*, and ≥ 13 mm against *S. pneumoniae* at a concentration of a 150 μg/mL, shown in Figure 10(b). AgNPs displayed viable antibacterial efficacy in comparison to the positive control tetracycline [44–47].

4. Conclusion

In this study, the unreported use of the ethanolic extract of *Pelargonium sidoides* as a reducing and capping agent in the quick and eco-friendly synthesis of silver nanoparticles was demonstrated. *P. sidoides*-mediated nanoparticles (PSAgNPs) were characterized using a combination of various techniques, viz., UV-Vis spectroscopy, FTIR, EDS, XRD, SEM, TEM, Raman spectroscopy, and DLS. Formation of PSAgNPs was verified by UV-Visible spectroscopy (λ_{max} at 480 nm) with sizes ranging from 11 to 90 nm. A zeta potential of −32.3 mV confirmed the highly stabilized nature of the nanoparticles. Furthermore, PSAgNPs displayed an elevated antibacterial potential over PSCE. We have demonstrated use of this plant extract as an efficient reducing, capping, and stabilizing agent in AgNPs and their potential value in biomedical and therapeutic applications.

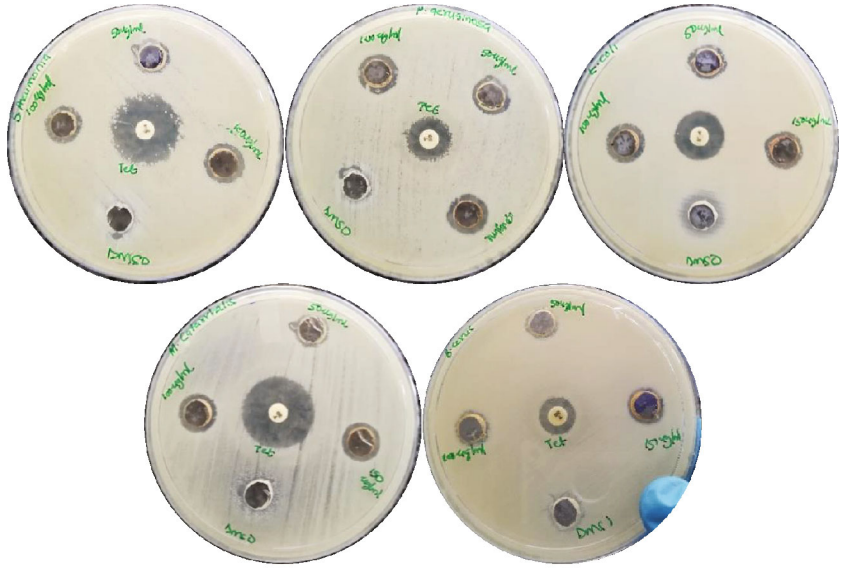

Figure 9: Zones of inhibition of PSAgNPs at different concentrations against various microorganisms.

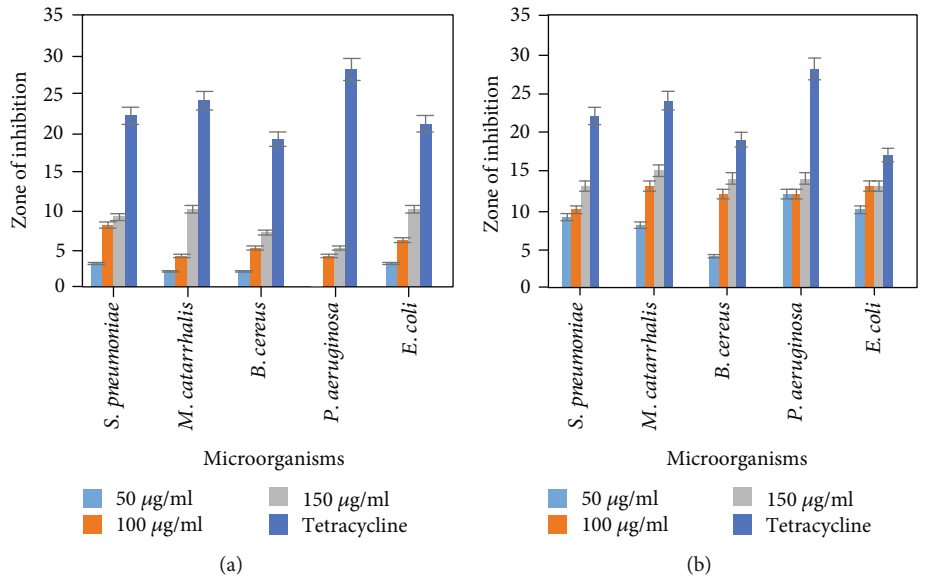

Figure 10: Antibacterial activity of (a) *P. sidoides* ethanolic extract and (b) PSAgNPs at different concentrations.

Data Availability

The data used are in the manuscript and were obtained at the North-West University.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

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Supplementary Materials

Graphical abstract of characterization and antibacterial activity of biosynthesized silver nanoparticles using the ethanolic extract of Pelargonium sidoides DC. *(Supplementary Materials)*

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