

Biosynthesized Silver Nanoparticles from *Strobilanthes attenuata*: Characterization and Enhanced Antibacterial Activity

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Abstract

In this study, silver nanoparticles (AgNPs) were synthesized through a green approach using the aqueous extract of *Strobilanthes attenuata* and their antibacterial potential was investigated. The formation of AgNPs was confirmed by the appearance of a characteristic surface plasmon resonance peak in the UV-visible absorption spectrum, while FTIR analysis indicated that plant-derived functional groups participated in the reduction of silver ions and stabilization of the synthesized nanoparticles. Particle size distribution, surface charge and colloidal stability were evaluated by dynamic light scattering and zeta potential analysis using a Malvern Zetasizer Nano ZS, whereas morphology was examined using scanning electron microscopy. The biosynthesized AgNPs displayed antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis* and *Pseudomonas aeruginosa*, with inhibition zones generally increasing with concentration (25–100 µg/mL). However, the aqueous extract of the plant did not show any activity against all tested bacterial strains. These findings indicated that conversion of the plant extract into nanoparticle form significantly enhanced its antibacterial activity. Thus, *S. attenuata* can be utilized for the green synthesis of nanoparticles with potential antimicrobial applications.

Introduction

Silver nanoparticles (AgNPs) have attracted considerable attention because of their broad-spectrum antibacterial activity and unique physicochemical properties. Owing to their nanoscale size, large surface area and ability to release biologically active Ag⁺ ions, these nanoparticles can interact effectively with bacterial cell surfaces and intracellular components through multiple mechanisms (Mikhailova, 2020; Asif et al., 2022; Eker et al., 2025). As a result, AgNPs are increasingly being explored as potential alternatives or supportive agents to conventional antibiotics, particularly in response to the growing challenge of antimicrobial resistance (Ahmed et al., 2016; Dhir et al., 2024).

Traditional methods used for the synthesis of AgNPs, including physical and chemical approaches, often require high energy input and involve hazardous reducing or stabilizing agents. These limitations raise concerns related to environmental safety, cost and biomedical applicability (Kordy et al., 2022; Fahim et al., 2024). In recent years, green synthesis strategies based on plant extracts have emerged as more sustainable and eco-friendly alternatives. Plant-derived metabolites such as phenolics, flavonoids, proteins and other biomolecules can function as both reducing and capping agents, thereby facilitating nanoparticle formation while improving stability (Ahmed et al., 2016; Vanlalveni et al., 2021). In many cases, plant-mediated synthesis has also been associated with improved biological activity of the resulting nanoparticles.

Although numerous studies have described the biosynthesis of AgNPs using different plant species, their antibacterial activity often varies depending on the phytochemical composition of the extract and the physicochemical characteristics of the nanoparticles produced. *Strobilanthes attenuata*, also called Blue Nettle belongs to the family Acanthaceae is known to contain several important bioactive

constituents (Kaur et al., 2026a; Kaur et al., 2026b), indicating its possible suitability for nanoparticle synthesis. However, its potential for the green synthesis of AgNPs and subsequent antibacterial evaluation remains largely unexplored.

Therefore, the present study was undertaken to synthesize AgNPs using the aqueous extract of *S. attenuata*, characterize the synthesized nanoparticles using spectroscopic and microscopic techniques and compare their antibacterial activity with that of the corresponding plant extract. The work aims to assess whether nanoparticle formation can enhance the antibacterial potential of this plant and to explore its value in sustainable antimicrobial nanotechnology.

Materials and Methods

Collection and preparation of plant extract

Aerial parts of *S. attenuata* were collected from Chamoli district, Uttarakhand, India (30.4025° N, 79.4364° E; 2115 m above sea level). Plant material was authenticated by the Botanical Survey of India (BSI), Dehradun and a voucher specimen was deposited under accession number 1435. The collected plant material was thoroughly washed with distilled water, shade-dried at room temperature for a week and ground into a fine powder. For extraction, 5 g of the powdered aerial parts were packed into a cellulose thimble and subjected to Soxhlet extraction using distilled water as the extraction solvent. The extraction was carried out continuously for 6 h using a heating mantle. After completion, the aqueous extract was concentrated under reduced pressure using a rotary evaporator and the dried extract was collected and stored at 4°C until further use.

Green synthesis of AgNPs

For biosynthesis of AgNPs, 400 mg of the dried extract was dissolved in 10 mL of distilled water and mixed with 90 mL of 5 mM silver nitrate (AgNO₃) solution. The pH of the reaction mixture was adjusted to approximately 9.0 using 0.1 M NaOH. The mixture was maintained at 60°C under continuous stirring at 150 rpm for 1 h. Formation of AgNPs was indicated by a visible color change from reddish brown to dark brown/black (Fig. 1). The synthesized samples were stored in amber-colored vials at 4°C till further analysis (Constantin et al., 2023).

UV-Visible spectroscopic analysis

Formation of AgNPs was confirmed by UV-visible spectroscopy. The absorption spectrum was recorded over the wavelength range of 200–700 nm using a double-beam UV-visible spectrophotometer (Systronic-2202). Distilled water was used as the reference blank. The characteristic surface plasmon resonance (SPR) peak was monitored as sign of nanoparticle formation.

FTIR analysis

Fourier transform infrared (FTIR) spectroscopy was performed to identify functional groups involved in reduction of Ag⁺ ions and stabilization of nanoparticles. Spectra of both the aqueous extract and synthesized AgNPs were recorded using a Nicolet Summit LITE spectrometer in the range of 4000–400 cm⁻¹ with a resolution of 1 cm⁻¹. The spectra were compared to determine possible biomolecular interactions associated with nanoparticle synthesis and capping.

Dynamic light scattering and zeta potential analysis

Hydrodynamic particle size, polydispersity index (PDI) and zeta potential were measured using a Malvern Zetasizer Nano ZS at 25°C. Prior to analysis, the AgNP suspension was appropriately diluted with distilled water to minimize multiple scattering effects. These analyses were used to assess particle size distribution, surface charge and colloidal stability.

Scanning electron microscopy

The morphology of the synthesized nanoparticles was examined using a field emission scanning electron microscope (JEOL JSM-7610F, Japan). Diluted AgNP suspensions were drop-cast onto clean glass substrates and allowed to air dry before gold sputter coating. Imaging was carried out under high-vacuum mode using a secondary electron detector at an accelerating voltage of 20 kV and a working distance of approximately 7.9 mm. Micrographs were recorded at magnifications ranging from 12,000× to 80,000× with scale bars between 1 µm and 100 nm. Particle diameter was determined manually using ImageJ software.

Antibacterial activity

Antibacterial activity of aqueous extract and the synthesized AgNPs was evaluated by the agar well diffusion method against *Escherichia coli* (MTCC 452), *Staphylococcus aureus* (MTCC 3160), *Bacillus subtilis* (MTCC 441) and *Pseudomonas aeruginosa* (MTCC 424). Mueller-Hinton agar plates were inoculated with freshly prepared bacterial cultures using sterile cotton swabs. Wells were made using a sterile cork borer and samples of aqueous extract and AgNPs at concentrations of 100, 50 and 25 µg/mL were added to the wells. Ciprofloxacin (100 ppm) was used as the positive control, while distilled water served as the negative control. Plates were incubated at 37°C for 24 h, after which inhibition zones were measured in millimetres. All experiments were carried out in triplicate and results were expressed as mean ± standard deviation (Wiegand et al., 2008).

Statistical analysis

All antibacterial assays were performed in triplicate and the results were presented as mean $\pm$ standard deviation (SD). Statistical analysis was carried out using GraphPad Prism 10.6.0. Differences among concentrations within each bacterial strain were analyzed by one-way ANOVA followed by Tukey's multiple comparison test. Differences were considered statistically significant at $p \leq 0.05$.

Results and Discussion

UV-visible spectral analysis

The reduction of Ag^+ ions and subsequent formation of AgNPs were confirmed by UV-visible spectroscopy through the appearance of a characteristic SPR band (Fig. 2). The absorption spectrum showed a distinct peak centred at 407 nm which is attributed to the collective oscillation of conduction electrons on the nanoparticle surface after interaction with incident light (Mikhailova, 2020). This optical response is a well-recognized feature of metallic silver nanostructures (Vanlalveni et al., 2021).

The presence of a single dominant SPR peak indicates successful formation of AgNPs and is generally associated with predominantly spherical particles. In colloidal silver systems, the position and shape of the SPR band are strongly influenced by particle size, morphology, aggregation state and the surrounding dielectric environment. The observed peak within 400–450 nm range agrees well with previously reported values for plant-mediated AgNPs and reflects the combined influence of nanoscale particle dimensions and biomolecular capping from the extract (Ahmed et al., 2016; Vanlalveni et al., 2021; Wangkheirakpam et al., 2014; Hanafiah et al., 2023; Javed et al., 2023).

Importantly, the absence of secondary absorption band or long wavelength shoulder in the visible region of 300–600 nm suggests minimal particle aggregation and excludes the formation of anisotropic structures such as rods or triangular plates which typically produce multiple plasmon bands (Mikhailova, 2020). The moderate broadening of the peak may be attributed to polydispersity and surface bound stabilizing molecules, a common feature in green synthesized AgNPs where organic capping layers influence local refractive index and plasmon damping (Vanlalveni et al., 2021; Fahim et al., 2024).

Thus, the UV-visible spectral profile confirmed successful nanoparticle synthesis under the optimized conditions and supported the presence of stable, predominantly spherical AgNPs suitable for further characterization and antibacterial evaluation.

FTIR analysis

FTIR spectroscopy was used to identify functional groups involved in nanoparticle formation and stabilization (Fig. 3; Table 1). The spectra of the aqueous extract and synthesized AgNPs displayed comparable band positions with slight shifts and changes in intensity, indicating that phytochemicals from the extract remained associated with the nanoparticle surface after synthesis. A broad absorption band around 3273 cm^{-1} corresponds to O-H/N-H stretching vibrations, commonly associated with

phenolics, flavonoids and proteins present in plant extracts (Pasieczna-Patkowska et al., 2025; Kordy et al., 2022). Broadening and slight shifting of this band in the AgNP spectrum suggest the participation of hydroxyl and amine containing compounds in reduction of silver ions and surface capping.

The band near 1635 cm^{-1} assigned mainly to carbonyl or conjugated $\text{C}=\text{C}$ stretching vibrations also showed minor variation after nanoparticle formation indicating possible coordination of carbonyl containing compounds with the silver surface. Changes observed in the fingerprint region further support adsorption of organic molecules onto the nanoparticles. These results indicate that plant-derived biomolecules acted as both reducing and stabilizing agents during synthesis consistent with the commonly proposed mechanism of green nanoparticle formation.

Table 1
FTIR peak positions and spectral changes associated with AgNP formation.

Functional group	Aqueous extract (cm^{-1})	AgNPs (cm^{-1})	Observed change	Interpretation
O-H/N-H stretching	3273.20	Slightly shifted (lower wavenumber)	Minor peak shift and intensity reduction	Participation of hydroxyl and/or amine groups in Ag^+ reduction and surface coordination.
C = O/C = C stretching	1635.61	Slightly shifted (lower wavenumber)	Peak shift and band sharpening	Coordination of carbonyl containing groups with AgNP surface (capping/stabilization)
Fingerprint region (C-O, C-N)	1000–1100 (broad)	Broadened/less resolved	Band modification	Contribution of organic moieties to nanoparticle surface stabilization

DLS and zeta potential

DLS analysis showed that the synthesized AgNPs had an average hydrodynamic diameter of 140.1 nm with a polydispersity index (PDI) of 0.38 (Fig. 4), indicating moderate dispersity and acceptable uniformity for biologically synthesized nanoparticles (Fahim et al., 2024). The intensity-weighted size distribution exhibited a dominant nanoscale population with a minor secondary peak, suggesting slight heterogeneity arising from phytochemical capping.

The hydrodynamic diameter obtained by DLS was considerably larger than the particle size observed later by SEM. This difference is expected because DLS measures the hydrodynamic size of nanoparticles, including the surrounding solvation layer and surface-bound biomolecules, whereas SEM determines the physical core diameter of dried particles. Similar discrepancies have been widely reported for plant-mediated nanoparticles and are generally considered indicative of successful surface functionalization rather than particle aggregation (Alzubaidi et al., 2023).

Zeta potential analysis showed a surface charge of -30.05 ± 6.34 mV (Fig. 5), indicating good colloidal stability of the AgNP suspension. The negative surface charge is likely associated with ionizable phytochemicals adsorbed onto the nanoparticle surface, which generate electrostatic repulsion and help prevent aggregation (Vanlalveni et al., 2021; Fahim et al., 2024; Tarannum et al., 2019). This observation is consistent with the FTIR results which confirmed the presence of hydroxyl, amine and carbonyl-containing functional groups on the nanoparticle surface.

SEM and particle size

SEM micrographs recorded at different magnifications (Fig. 6a-f) illustrates that the biosynthesized AgNPs were predominantly spherical in morphology with minimal visible agglomeration. The particles appeared discrete and well distributed, suggesting that phytochemicals present in the aqueous extract of *S. attenuata* contributed to nanoparticle stabilization during synthesis. The observed morphology is consistent with the UV-visible and zeta potential analysis which indicated the formation of stable and well-dispersed nanoparticles.

Quantitative particle size analysis was performed using ImageJ software and the resulting histogram (Fig. 7) showed a unimodal size distribution. Most nanoparticles were distributed within the range of 22–55 nm, with an average diameter of 38.67 ± 7.8 nm. The relatively narrow size distribution indicates moderate uniformity of the synthesized nanoparticles. Similar particle sizes have been reported for other plant-mediated AgNPs, where phytochemicals regulate nanoparticle nucleation and growth, resulting in controlled particle formation (Alzubaidi et al., 2023; Alharbi et al., 2022).

The average particle size obtained by SEM was smaller than the hydrodynamic diameter measured by DLS, a commonly reported observation for green-synthesized nanoparticles arising from the different measurement principles of the two techniques (Alzubaidi et al., 2023). Spherical AgNPs in similar size ranges (< 100 nm) have been repeatedly associated with strong antibacterial activity because smaller spherical nanoparticles interact more effectively with bacterial membranes, penetrate cell walls and induce oxidative stress through reactive oxygen species generation and Ag⁺ ion release (Franci et al., 2015; More et al., 2023). Many studies have reported that spherical morphology leads to higher surface reactivity and facilitates membrane contact (Alharbi et al., 2022), supporting the biological relevance of the morphology observed in the present study.

Antibacterial activity

The antibacterial activity of *S. attenuata*-mediated AgNPs and the corresponding aqueous extract was evaluated against two Gram-positive bacteria (*B. subtilis* and *S. aureus*) and two Gram-negative bacteria (*E. coli* and *P. aeruginosa*) using the agar well diffusion method (Table 2; Fig. 8–9). The biosynthesized AgNPs exhibited antibacterial activity against all tested bacterial strains, whereas the aqueous extract did not suppress bacterial growth at any of the tested concentrations (25–100 µg/mL).

The antibacterial effect of AgNPs was concentration dependent with inhibition zones generally increasing with increasing concentration. Among the tested microorganisms, *B. subtilis* was the most susceptible displaying the largest inhibition zone of 22.16 ± 1.25 mm at 100 $\mu\text{g/mL}$ against the synthesized AgNPs. *E. coli* and *P. aeruginosa* also showed susceptibility with inhibition zones of 20.66 ± 1.15 mm and 20.33 ± 0.57 mm, respectively at 100 $\mu\text{g/mL}$. In contrast, AgNPs exhibited similar inhibition zones of 19.66 mm against *E. coli* and *S. aureus* at 50 $\mu\text{g/mL}$. Effective bacterial growth inhibition was also observed at the lowest tested concentration of 25 $\mu\text{g/mL}$ which indicates strong inhibitory potential of the synthesized nanoparticles. The antibacterial activity of the AgNPs was comparable to that of the standard antibiotic ciprofloxacin which exhibited inhibition zones of 21.33–25.33 mm at 100 ppm.

Nevertheless, the complete absence of antibacterial activity in the aqueous extract of *S. attenuata* indicates that the phytoconstituents alone were insufficient to suppress bacterial growth under the tested conditions. However, following their conversion into AgNPs, these biomolecules likely acted as reducing and stabilizing agents, improving the physicochemical properties and biological activity of the synthesized nanoparticles.

The improved antibacterial activity of AgNPs relative to the crude plant extract can be attributed to the unique properties acquired at the nanoscale. Reduction in particle size increases the surface-to-volume ratio, resulting in enhanced surface reactivity and improved interaction with microbial cells. In addition, AgNPs may act as reservoirs for the gradual release of Ag^+ ions, which further contributes to their antimicrobial effect (Shaik et al., 2018; Hassan Afandy et al., 2023). Similar findings have been reported for plant-mediated AgNPs synthesized using *S. glutinosus*, *S. crispus* and *S. flaccidifolius* (Javed et al., 2023; Hanafiah et al., 2023; Wangkheirakpam et al., 2014), highlighting the potential of *Strobilanthes* genus in green nanotechnology-based antimicrobial applications.

The synthesized AgNPs demonstrated antibacterial activity against both Gram-positive and Gram-negative bacteria which indicates broad-spectrum antibacterial potential. Previous studies have also documented plant-mediated AgNPs synthesized using different plant species which have shown inhibitory activity against a wide range of bacterial pathogens (Liaqat et al., 2022; Musimun et al., 2022; Nallusamy et al., 2025). The observed antibacterial activity may be explained by the well-established bactericidal mechanisms of AgNPs, which include disruption of cell membrane integrity, generation of reactive oxygen species, inactivation of proteins and enzymes and interference with DNA replication (Rai et al., 2012; Morones et al., 2005; Qayyum et al., 2017). The spherical morphology and sub-50 nm particle size observed in the present study are especially conducive for such activity as smaller spherical nanoparticles possess greater contact efficiency with bacterial surfaces and can penetrate cell walls more readily (Hassan Afandy et al., 2023).

Furthermore, FTIR analysis confirmed the presence of surface bound functional groups such as -OH, -NH and C = O indicating the formation of a phytochemical capping layer or bio-organic corona around the nanoparticles. This organic coating may improve nanoparticle dispersion, enhance colloidal stability and regulate interactions with microbial cell surfaces. Such an organic-inorganic interface likely contributed

to the sustained antibacterial activity observed in this study, which is also supported by the absence of aggregation as indicated by UV-visible spectroscopic analysis.

Table 2
Antibacterial activity of *Strobilanthes attenuata*-mediated silver nanoparticles and aqueous extract against selected bacterial strains evaluated by agar well diffusion method.

Sample	Concentration (µg/mL)	Zone of inhibition (mm)			
		<i>B. subtilis</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>
AgNPs	100	22.16 ± 1.25 a	20.66 ± 1.15 a	20.33 ± 0.57 a	20.00 ± 0.00 a
	50	21.66 ± 0.57 ab	19.66 ± 1.52 a	19.00 ± 1.00 ab	19.66 ± 3.21 a
	25	20.00 ± 0.00 b	18.66 ± 1.52 a	16.66 ± 1.15 b	18.33 ± 2.88 a
Aqueous extract	100	Nil	Nil	Nil	Nil
	50	Nil	Nil	Nil	Nil
	25	Nil	Nil	Nil	Nil
Positive control (Ciprofloxacin)	100 ppm	25.33 ± 0.28	21.33 ± 1.15	21.33 ± 0.57	23.50 ± 0.86
Negative control (Distilled water)	-	Nil	Nil	Nil	Nil

Values represent mean of triplicates ± SD. Means followed by the same letter within columns are not significantly different ($p \leq 0.05$) using Tukey’s multiple comparison test.

Nil indicates no zone of inhibition.

Conclusion

In the present study, stable AgNPs were successfully synthesized using the aqueous extract of *S. attenuata* through a simple, green and environmentally friendly approach. Characterization by UV-visible spectroscopy, FTIR, DLS, zeta potential analysis and SEM confirmed the formation of predominantly spherical and stable nanoparticles. While the crude aqueous extract did not suppress bacterial growth, the synthesized AgNPs showed concentration-dependent antibacterial activity against both Gram-positive and Gram-negative bacteria. This demonstrates that nanoparticle synthesis substantially enhances the antibacterial potential of *S. attenuata* and highlights its promise as a sustainable source of antimicrobial nanomaterials. The results of our study provide a foundation for further investigation of *S. attenuata*-derived AgNPs for antibacterial applications and for future research aimed at exploring their biological properties and underlying mechanisms of action.

Declarations

Ethics declaration

Not applicable.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author Contribution

A.K. collected the study plant, performed experimental analysis, data analysis, wrote the manuscript text and also prepared figures. S.K. designed the study, conceptualization and reviewed and edited the manuscript. H.W. provided supervision, review and editing of the manuscript. All authors read and approved the final version of the manuscript.

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Data Availability

All the data supporting the study is provided within the manuscript.

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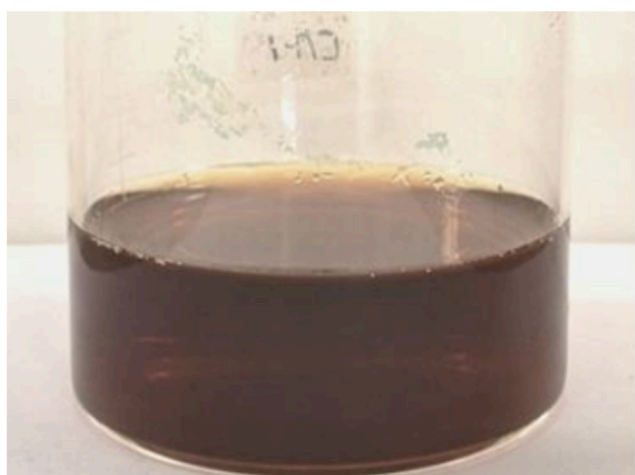
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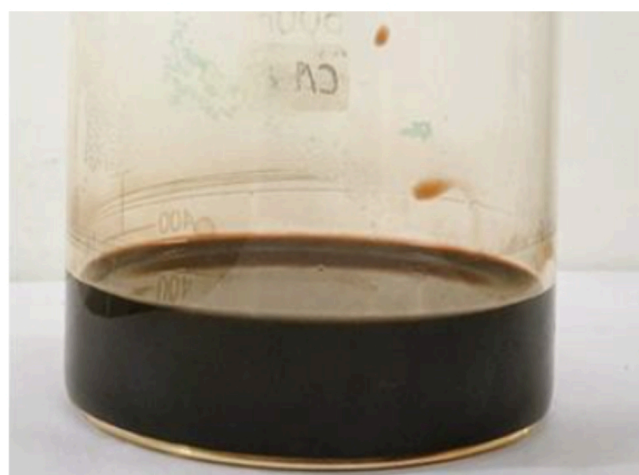
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Figures



[A]



[B]

Figure 1

Visual color change observed during biosynthesis of silver nanoparticles [A] reaction mixture before synthesis and [B] after nanoparticle formation.

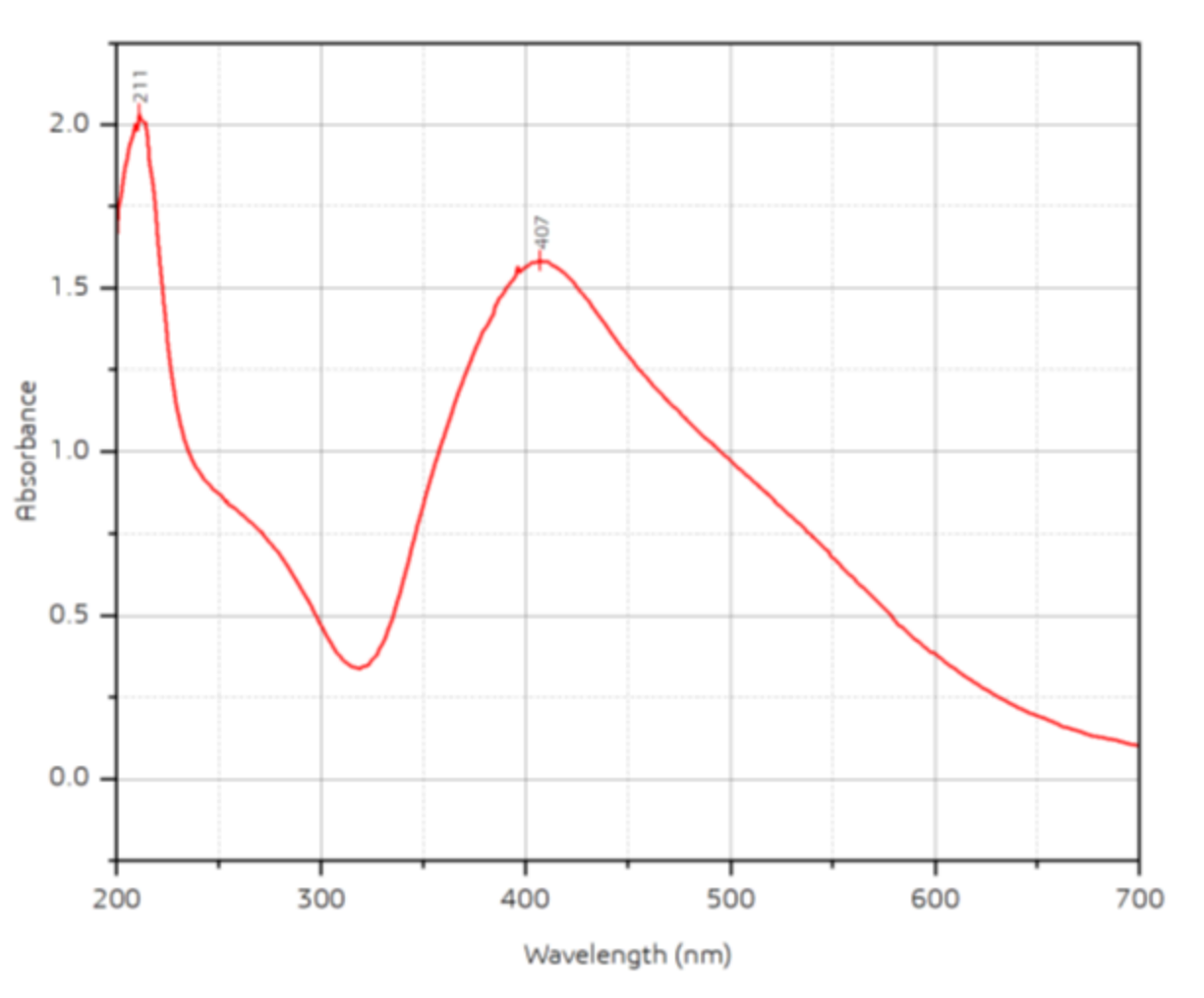


Figure 2

UV-Vis absorption spectra of reduction of silver ions to silver nanoparticles by aqueous extract of *Strobilanthes attenuata*.

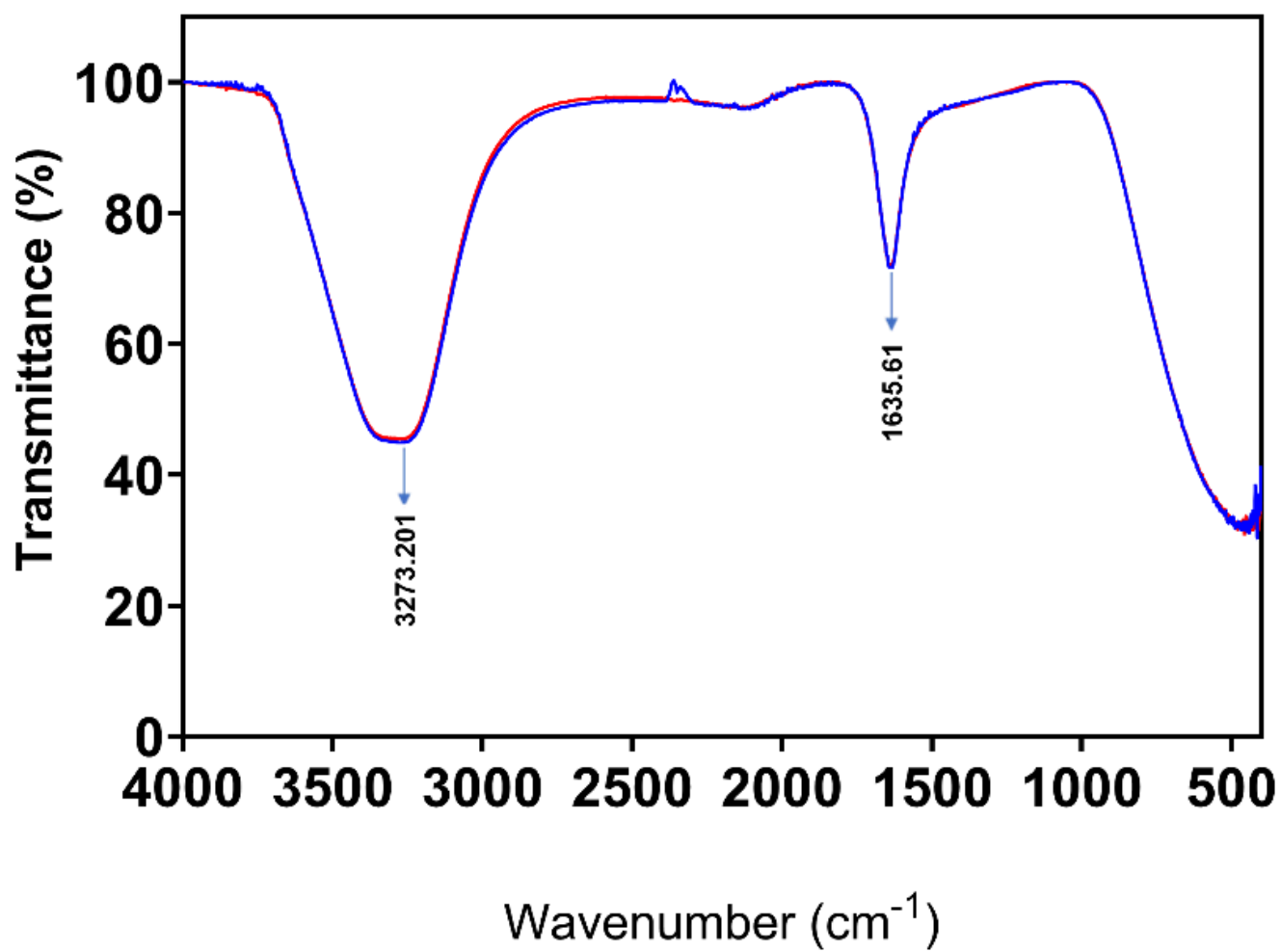


Figure 3

FTIR spectra of *Strobilanthes attenuata* aqueous extract (red) and synthesized AgNPs (blue).

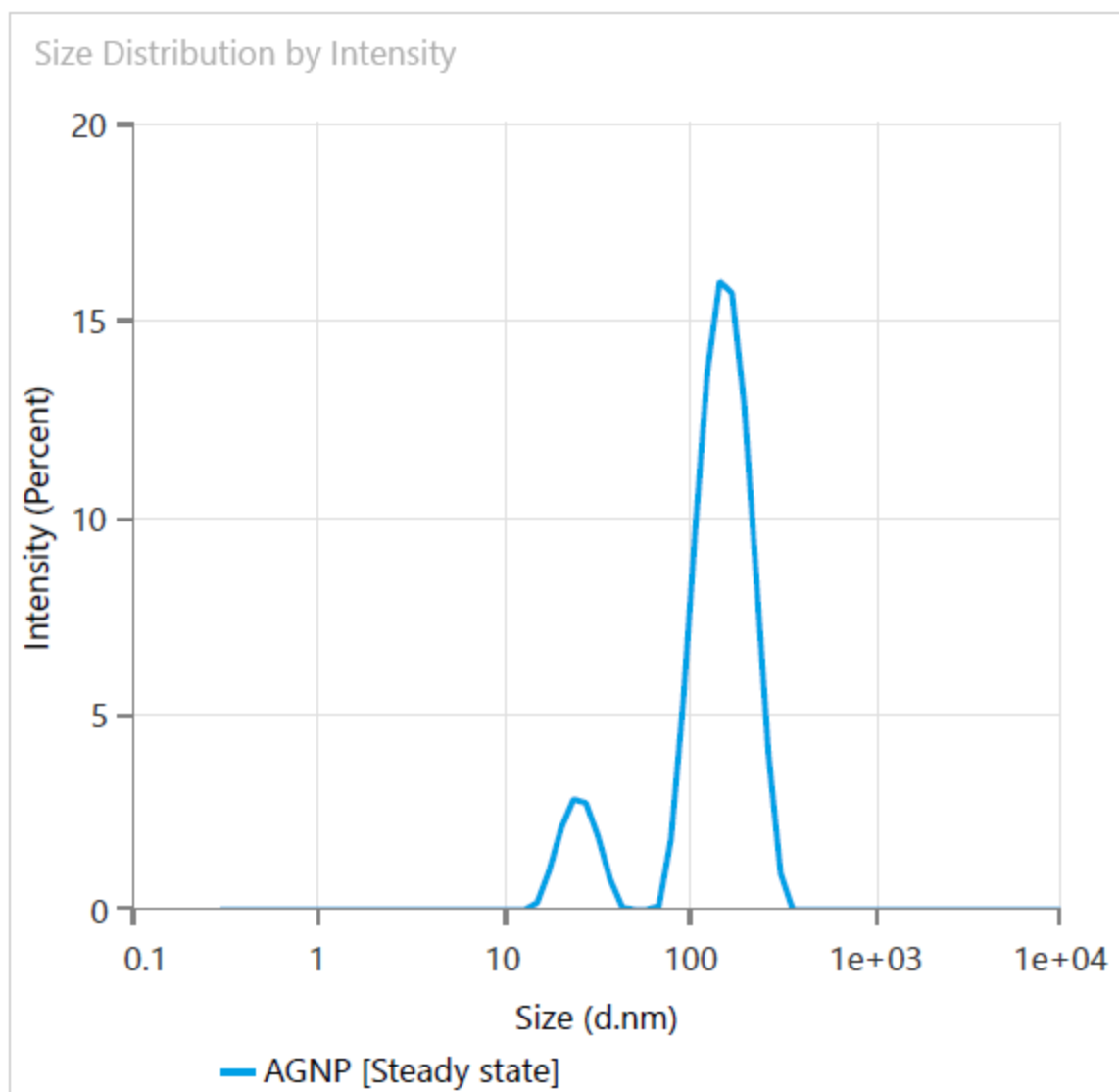


Figure 4

Nanoparticle size and PDI analysis.

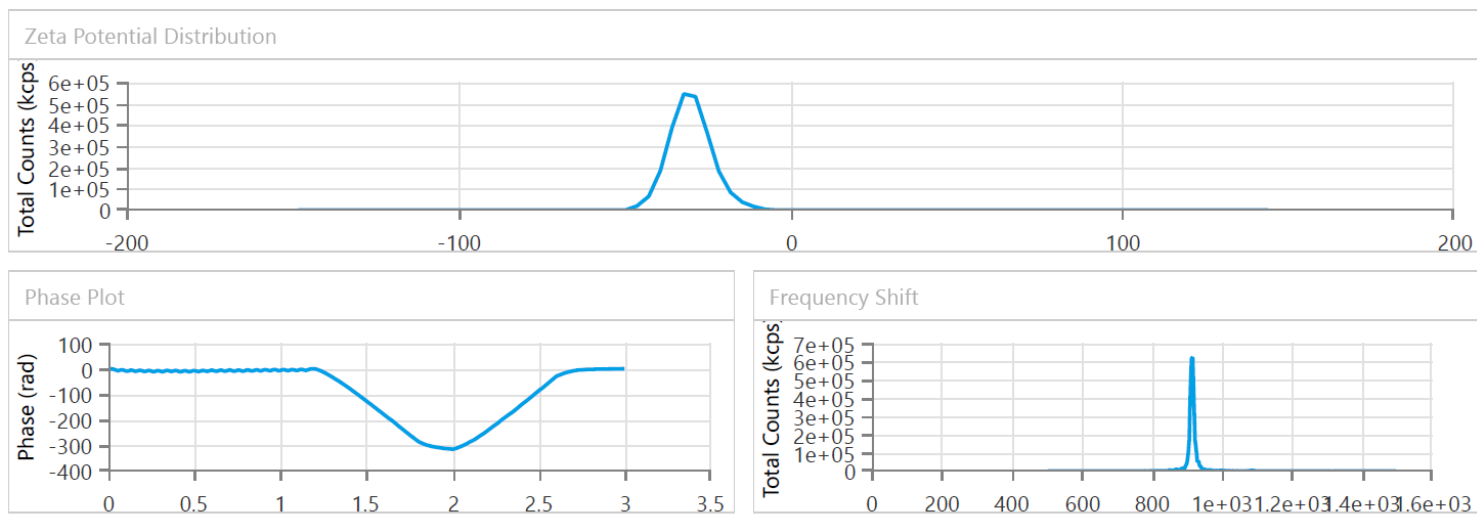


Figure 5

ζ -potential of the biosynthesized AgNPs.

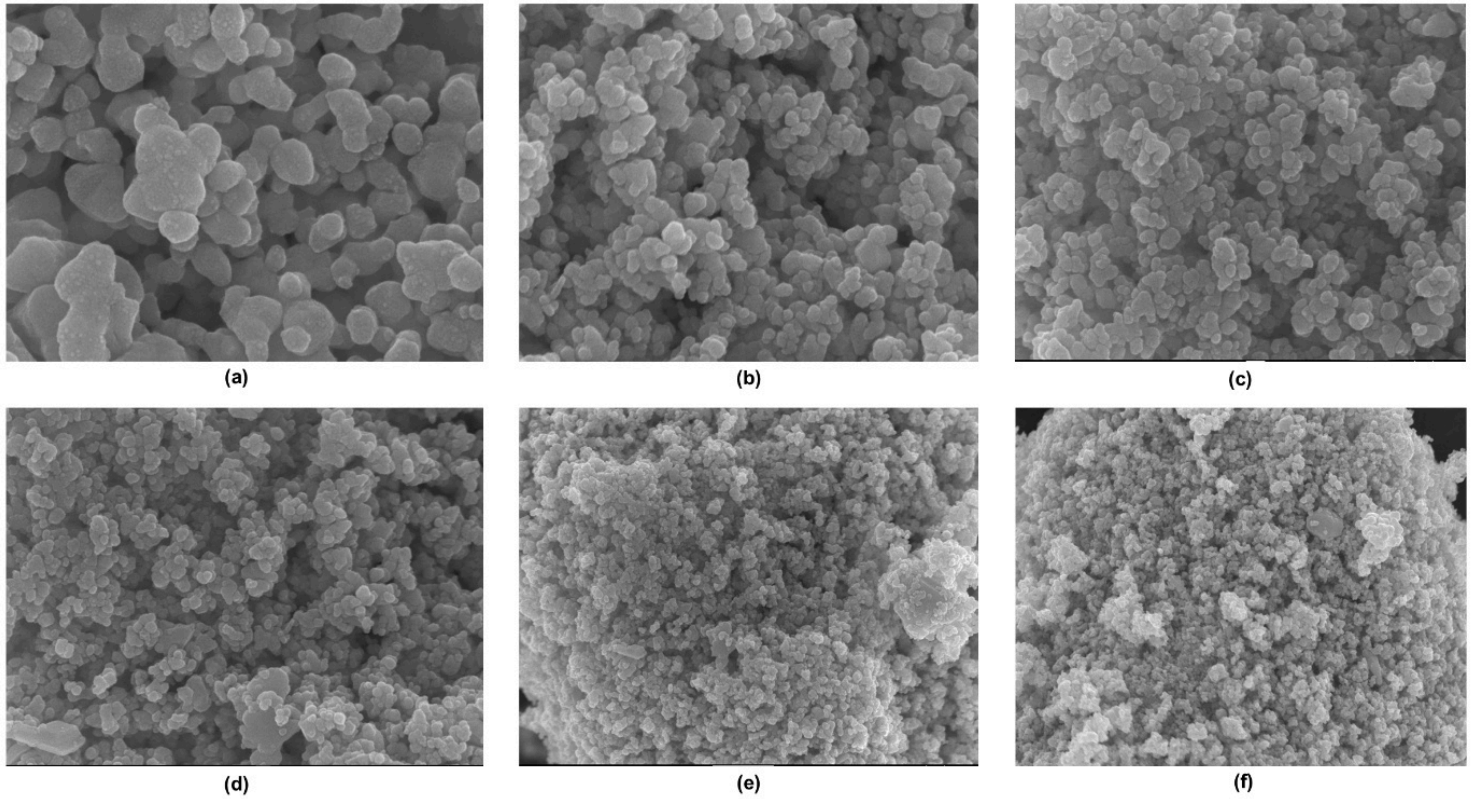


Figure 6

Scanning electron micrographs of *Strobilanthes attenuata*-mediated silver nanoparticles at different magnifications. (a) at higher magnification 80,000x, (b) at 60,000x, (c) at 50,000x, (d) at 37,000x, (e) at 16,000x, (f) at 12,000x.

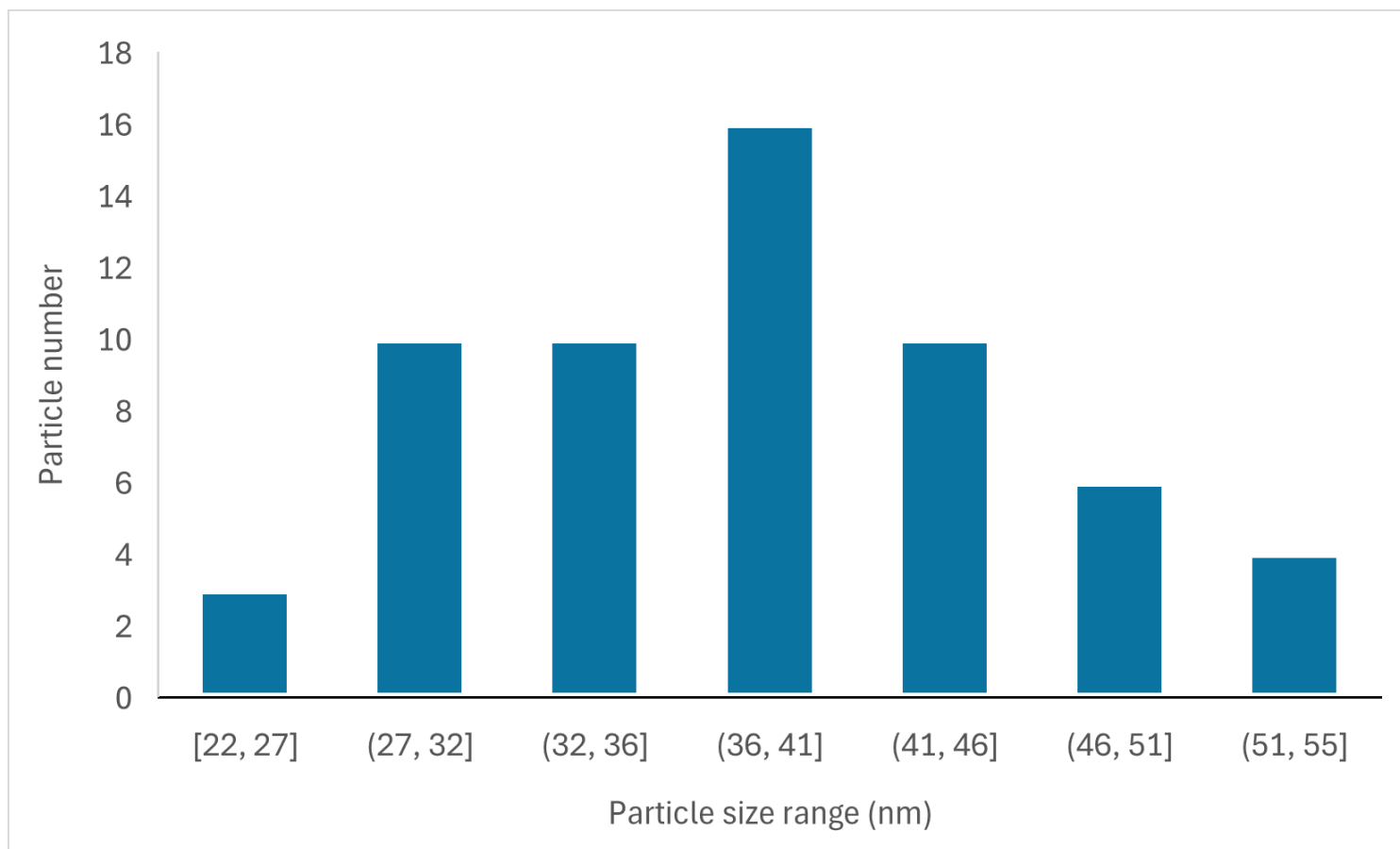
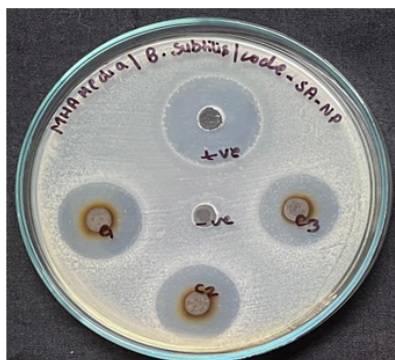


Figure 7

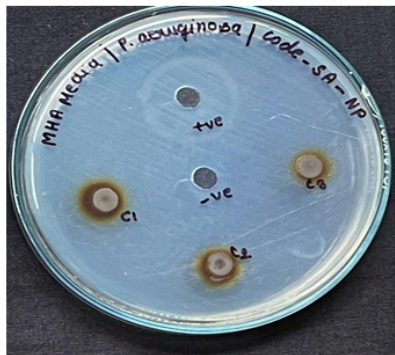
Particle size distribution of biosynthesized silver nanoparticles.



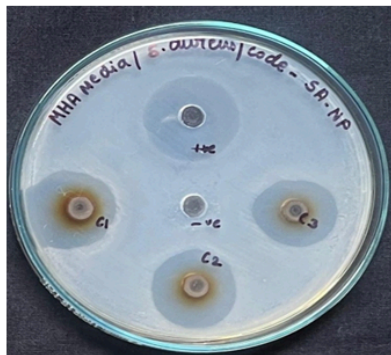
Bacillus subtilis



Escherichia coli



Pseudomonas aeruginosa



Staphylococcus aureus

Figure 8

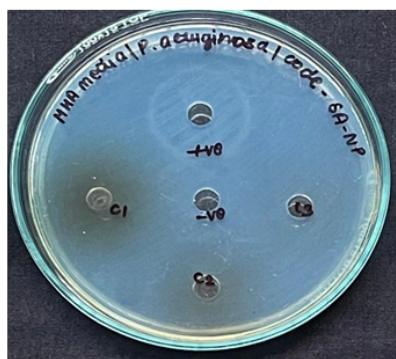
Antibacterial activity of synthesized nanoparticles against four different bacterial strains by agar well diffusion method. C1: 100 µg/mL; C2: 50 µg/mL; C3: 25 µg/mL; +ve control: Ciprofloxacin; -ve control: distilled water.



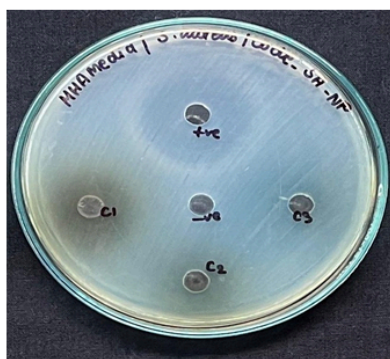
Bacillus subtilis



Escherichia coli



Pseudomonas aeruginosa



Staphylococcus aureus

Figure 9

Antibacterial activity of aqueous extract of *Strobilanthes attenuata* against four different bacterial strains by agar well diffusion method (+ve control: Ciprofloxacin; -ve control: distilled water).