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Original Research Article

Antibacterial Activity of Silver Nanoparticles Synthesized Using *Vitex grandifolia* Against Multidrug-Resistant (MDR) Pathogens

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ABSTRACT

Amid escalating antibiotic resistance, the urgency to combat multidrug-resistant (MDR) pathogens calls for innovative solutions. This study explores the potential of silver nanoparticles (AgNPs) synthesized from *Vitex grandifolia* leaves, chosen due to its affordability, accessibility, and therapeutic efficacy. The synthesis involves blending leaf extract with water and silver nitrate ($AgNO_3$). Sunlight exposure led to the biological reduction of $AgNO_3$, resulting in the formation of AgNPs characterized by a distinctive brown hue. These synthesized AgNPs underwent comprehensive characterization using various techniques including ultraviolet-visible spectroscopy (UV Vis), Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), energy-dispersive X-ray spectroscopy (EDX), and X-ray diffraction (XRD). Antibacterial evaluation against multidrug-resistant (MDR) pathogens was conducted using the agar well diffusion method. Characterization studies confirmed the successful synthesis of AgNPs, with UV-visible spectroscopy revealing an absorbance peak at 350 nm. SEM analysis indicated an average particle size of approximately 13.12 nm, predominantly in rod-like shapes. EDX analysis corroborated the presence of silver, oxygen, and carbon, while XRD analysis unveiled a face-centred cubic crystalline structure. FTIR analysis identified various functional groups attributed to phytochemicals in the plant extract, acting as capping and reducing agents. Notably, the AgNPs exhibited a considerable band gap value (3.09 eV). For the antibacterial activity, AgNPs demonstrated significant efficacy against several multidrug-resistant pathogens, exhibiting a zone of inhibition of 15 mm. In summary, this study presents a rapid green synthesis method for AgNPs utilizing *Vitex grandifolia* leaves. The characterized AgNPs show promise in combating MDR pathogens, offering a sustainable and cost-effective solution in nanotechnology.

***Keywords*:** Antibiotic resistance, Multidrug-resistant pathogens, Silver nanoparticles, Synthesis, *Vitex grandifolia*.

Introduction

The recent upsurge in antibiotic-resistant microorganisms has sparked widespread concern and anxiety. Globally, certain pathogens associated with illnesses are leading to higher mortality rates in humans, and the current treatment methods, including the use of multiple antibiotics, are proving ineffective and costly. Therefore, there is a critical need to prioritize the development of innovative antibacterial medicines with high efficacy against multidrug-resistant (MDR) pathogens.[1] Antibiotic resistance represents a significant threat to global health, prompting scientists to explore novel avenues for creating more potent biocidal materials to address these concerns.[2]

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The World Health Organization (WHO) has advocated for evaluating medicinal plants against multidrug-resistant (MDR) pathogens, considering them an important source of bioactive compounds that could serve as a blueprint for antimicrobial drug discovery.[3] Therefore, utilising these compounds as a reducing and capping agent in nanoparticle synthesis could yield better results. Silver nanoparticles have emerged as intriguing subjects in scientific research, offering promising insights into future advancements in medicine and technology. The exceptional antibacterial properties of silver nanoparticles have garnered significant interest due to their numerous distinctive characteristics.[4] The escalating challenge of antibiotic resistance necessitates the exploration of novel therapeutic strategies. The effective utilization of nanoparticles presents a potential solution to this global health crisis.[5] Consequently, the development of new antibiotics that are effective against MDR strains is crucial. As the battle against bacterial infections intensifies, exploring the antibacterial potential of silver nanoparticles has become a scientific and medical imperative. Therefore, harnessing plant-derived compounds for nanoparticle production presents a compelling approach in the field of nanotechnology due to its numerous advantages, including affordability, environmental sustainability, accelerated synthesis, and enhanced yields.

Various biological methods for synthesising silver nanoparticles involve complex procedures, especially when using microorganisms, which require stringent aseptic conditions and maintenance protocols. However, plants offer a more practical alternative due to their less stringent requirements and significant therapeutic qualities. Many plants and their components have been documented to assist in the production of silver nanoparticles. Plant extracts from diverse botanical sources such as *Mentha arvensis* (mint).[7] *Moringa oleifera* flower,[8] *Allium cepa*,[9] *Carduus crispus*,[10] *Silybum marianum*,[11] *Sesuvium portulacastrum*,[12] *Tamarindus indica*,[13] *Tectona grandis*, [14] and *Scutellaria barbata*,[15] have all been used for synthesising silver nanoparticles.

The main aim of this study is to explore the potential of *Vitex grandifolia* leaves as an eco-friendly method for producing silver nanoparticles (AgNPs). This research represents a pioneering effort utilising *Vitex grandifolia* for nanoparticle biosynthesis, although several other *Vitex* plant genera have been employed for similar purposes. This choice stems from the significant medicinal properties demonstrated by plants. *Vitex* is a prominent genus within the Lamiaceae family, primarily comprising of shrubs and small trees that are commonly found in tropical and subtropical regions like Asia, Latin America, and Africa.[16] In traditional medicine, the use of *Vitex grandifolia* bark has been observed to effectively treat various ailments such as stomach aches, diarrhea, bronchial problems, fever, ulcers, and rickets.[17] Furthermore, numerous studies have documented the successful application of *Vitex grandifolia* leaves in treating various diseases, including umbilical cord infections, toothaches, rheumatism, and orchitis.[18,19]

Additionally, the pesticidal properties demonstrated by the pulverized foliage of *V. grandifolia* against *Tribolium castaneum*, a pest frequently found in stored groundnuts, have been well-documented.[20] Surprisingly, despite the various potentials exhibited by this plant, there has been no documentation on the biological synthesis of silver nanoparticles from its leaves. Hence, this study will be the pioneering report on the biosynthesis, characterization, and antibacterial properties of silver nanoparticles mediated by the aqueous leave extract of *V. grandifolia*..

Materials and Methods

Materials

Freshly harvested leaves of *Vitex grandifolia* were sourced from Ilishan Remo, located in the Ikenne Local Government Area of Ogun State, Nigeria (6.8932° N, 3.7105° E) in January 2023, chosen based on considerations of cost-effectiveness, accessibility, and therapeutic efficacy. The leaf sample underwent identification and authentication in the Department of Botany, University of Ibadan, Oyo State, Nigeria. All media used for culturing the isolates were procured from Himedia Laboratories Pvt. Ltd., Mumbai, India. Silver nitrate ($AgNO_3$) was employed, and bacterial cultures, specifically *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Streptococcus pyogenes*, and *Salmonella typhi*, were acquired from the Department of Microbiology, Babcock University, Ilishan-Remo, Ogun State, Nigeria. Solutions were prepared using double-distilled water and stored in a light-free environment to prevent potential photochemical reactions. Before usage, all glassware employed in the experimental procedures was thoroughly washed and dried in a hot oven.

Preparation of plant leaf extracts

The samples of the leaves were dried, blended to powder, and stored in a sealed container at room temperature, 5 g of pulverized leaves were weighed into 50 mL of distilled water and simmered at 60 °C for an hour. The mixture was set aside to cool. The resultant extract was sifted through a No. 1 Whatman filter paper and spun in a Gallenkomp centrifuge (Model 80-2 England) at 3000 revolutions per minute for 2 minutes. The recovered supernatant was stored in an amber container at 4°C for future use.[21]

Silver nitrate solution preparation

A concentration of 1 mM solution of silver nitrate was prepared using commercially purchased silver nitrate, which has a molecular weight of 169.87 g/mol.

Silver nanoparticles biosynthesis

During the nanoparticle synthesis, 1g of the *Vitex grandifolia* extract was dispersed in 10 ml of water. After that, 1 ml of the mixture was introduced into 40 mL of 1 mM $AgNO_3$ in a volumetric flask. The mixture was agitated and afterwards exposed to sunlight for 30 minutes, and colour changes were observed. The bioreduction process of $AgNO_3$ was confirmed upon observing a colour change in the solution, turning it brown. The silver nanoparticles (AgNPs) were washed and obtained after a 10,000-rpm centrifugation for 15 minutes.

Silver nanoparticles characterization

The process of bioreduction of ionic silver (Ag^+) to elemental silver (Ag^0) in the sample was studied using UV-visible spectra analysis conducted with a Shimadzu dual beam spectrophotometer (6500PC) scanning mode between 800 and 200 nm. The shape of the synthesized AgNPs was verified by the JEOL JSM-7600F (United Kingdom) Scanning electron machine, and ImageJ software (2020 version developed by Wayne Rasband was used to determine the sizes. In addition, the composition of the elements was investigated by utilizing the JEOL JSM-7600F (United Kingdom) energy-dispersive X-ray (EDX) machine. The crystallinity was determined using the Rikgaku miniflex XRD (Japan). The biomolecules (capping agents) accountable for the reduction of Ag+ were examined using FTIR-8400S (Model: SHIMADZU European) spectrometer to determine the functional groups.

Screening of antibacterial activity

The antibacterial efficacy assessment utilised the disc diffusion method. Pathogenic bacteria were cultured on Muller Hinton Agar plates using a sterile swab stick at concentrations of 105-106 colony-forming units (CFU/mL). With a sterile cork borer, wells of approximately 6 mm in diameter were carefully created on the agar plates and properly labeled. Various concentrations of 20, 40, 60, 80, and 100 µg/mL of the synthesized nanoparticles were then added to the respective wells. Additionally, standard antibiotics including Gentamicin (10 µg), Cefuroxime (30 µg), Augmentin (30 µg), Nitrofurantoin (300 µg), Cefatrizine (30 µg), and Ceftriaxone (30 µg) were employed to verify the multidrug resistance potential of the pathogens. The plates were incubated in a DNP-9162 Thermostat incubator at 37 °C for 24 hours. Following incubation, the zones of inhibition were measured, serving as a reliable indicator of the antibacterial activity of the synthesized nanoparticles.

Results and Discussion

Ultraviolet-visible spectroscopy analysis

UV–vis spectroscopy is often regarded as the most effective technique for monitoring the conversion of Ag^+ ions derived from $AgNO_3$.[22] The silver nanoparticles synthesized from *Vitex grandifolia* were identified by the colour change owing to surface plasmon resonance development, as shown in Figure 1(A). Nanoparticles exhibit surface plasmon resonance absorption within the ultraviolet-visible spectrum, so UV-visible spectroscopy also validated the confirmation of the synthesized nanoparticles. The sample showed a distinct absorbance at a specific wavelength of 350 nm (Figure 1A), and this result corroborates the findings of previous researchers who reported the UV-VIS Surface Plasmon Resonance (SPR) of AgNPs to be within the ranges from 350 nm to *500 nm*.[23] The optical direct band gap of silver nanoparticles was determined using the Tauc plot method. Figure 1(B) revealed an estimated value of 3.09 eV for the band gap of the synthesized silver nanoparticles. The band gap value exceeds that documented in earlier literature, and this elevated band gap value could be attributed to the phenomenon of quantum confinement.

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SEM analysis of Vitex grandifolia silver nanoparticles

SEM was utilized to assess the morphological characteristics of the synthesized AgNPs. The SEM micrographs of the synthesized silver nanoparticles can be seen in Figure 2A. When a voltage of 15 kV and a magnification of 8000 were used, the size turned out to be in the normal range for nanoparticles, between 0 and 100 nm. The mean particle dimension is around 13.12 nm, as analyzed by ImageJ software, displayed by the normal distribution shown on the histogram (figure 2B). It also exhibits several shapes, but rodlike shapes mostly dominate it. The nanoparticles exhibit a significant degree of polydispersity, characterized by an organic layer enveloping the synthesized silver. The blurring of images with lower particle sizes occurred due to the limited resolution capabilities of the scanning electron microscope (SEM) available at the institute, resulting in the inability to capture photos with clear and distinct outline.

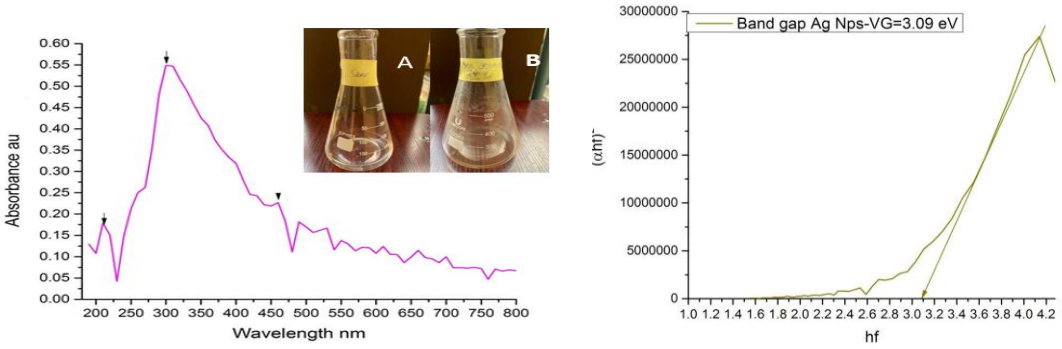

Figure 1A: UV-Vis spectrum Figure 1(B) The Bandgap of the green synthesis of silver nanoparticles from *Vitex grandifolia*. Insert A before (B) after synthesis

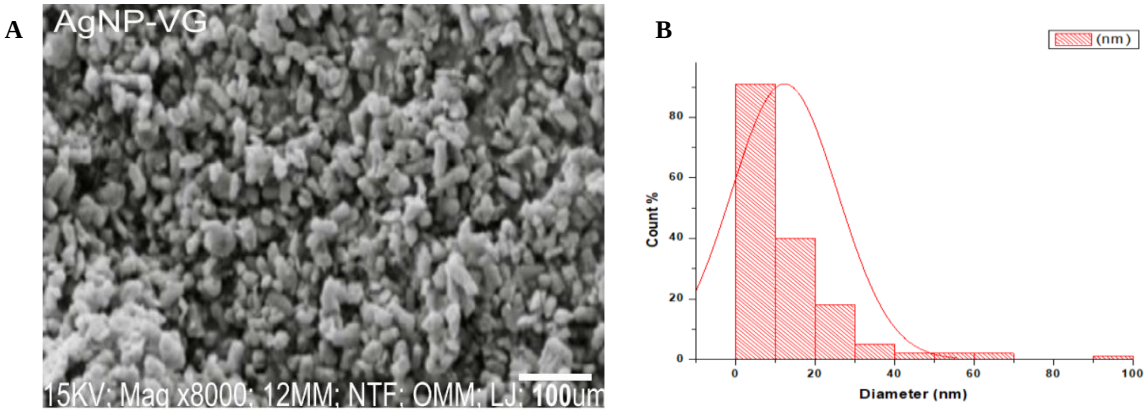

Figure 2: (A): SEM micrograph (**B):** Particle size distribution

EDX Analysis of Vitex grandifolia silver nanoparticles

EDX spectroscopy was used to determine the elemental composition of the produced nanoparticles in conjunction with scanning electron microscopy (SEM), as illustrated in Figure 3. The findings readily showed the presence of a prominent peak in the Ag (68.98%), oxygen (20.60%), and carbon (10.32%), as displayed in Figure 3. The absence of a nitrogen peak in the analysis suggests that there is no presence of trace ions from AgNO3 in the samples.

XRD Analysis of Vitex grandifolia Silver Nanoparticles (AgNps-Vg)

The study employed X-ray diffractometry analysis to examine the crystalline structure of the synthesized silver nanoparticle at the nanoscale level. The patterns displayed in Figure 4 illustrate the characteristic diffraction peaks detected at angles of 30.1° and 35. 2°, 41.1°, 56. 4°, and 66. 2°, these peaks are linked to the crystallographic planes of (110), (111), (121), (200), and (311) in the face-centred cubic structure of the silver nanoparticles (AgNPs) respectively. These diffraction peaks are closely connected to silver nitrate's face-centred cubic (FCC) crystalline structure because of their location and typical peaks, as shown in the International Centre for Diffraction Data (ICDD). (JCPDS card no. 89-3722). The sharpness of the peaks in Figure 4 shows the particles' crystallinity. Thus, the XRD pattern demonstrated that the Ag-NPs generated in this investigation were crystalline, and the results are similar to those of other published literature.[24] Additionally, it was noted that the silver nanoparticles had a size distribution spanning from 0 to 100 nm. The mean dimension of the nanoparticles was determined to be 13.12 nm. The extra peaks observed during X-ray diffraction (XRD) analysis were attributed to other bioorganic constituents inside the silver nanoparticles solution originating from the plant sample. Bioorganic compounds are of significant importance in the process of reducing Ag^{+} as well as ensuring the stability of the produced nanoparticles.[13]

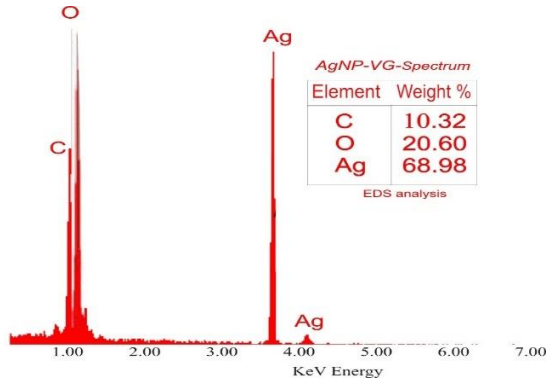

Figure 3: EDX micrograph of the synthesised silver nanoparticles

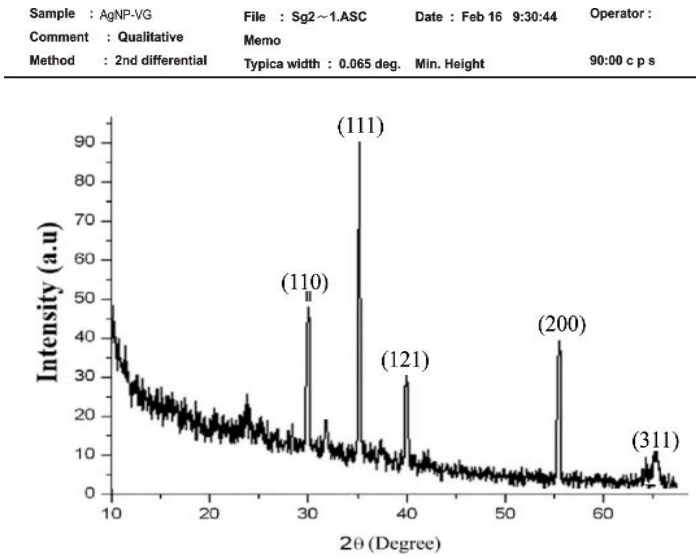

Figure 4: XRD spectrum of the synthesised silver nanoparticles

FTIR spectrum

The functional groups capping the AgNps synthesized using *Vitex grandifolia* were analyzed using FTIR, as depicted in Figure 5. The Fourier transformation infrared spectrum of AgNps-Vg exhibited distinct peaks that correspond to AgNps-Vg at wavenumbers of 3440.10cm $^{-1}$, 2929.52 cm $^{-1}$, 2855.10 cm^{-1}, 2358.37 cm^{-1}, 1613.27 cm^{-1}, 1277.88 cm^{-1}, 1250.65 cm^{-1}, 1042.12 cm^{-1}, 914.90 cm^{-1}, and 470.96 cm^{-1}. The robust and influential band at 3440.10 cm-^{1} signifies the stretching vibration of hydroxyl groups;[25] the bands exhibiting low intensity within the spectral range of 2929.52 cm^{-1} to 2358.37 cm^{-1} are attributed to the elongation of alkane/carboxylic acids within the sample. The intense band at 1613.27 cm^{-1} indicates the existence of chemical compounds, including carboxylic, aldehydes, esters, or ketones, which originate from flavonoids; the weak bands between 1277.88 cm^{-1} and 1042.12 cm^{-1}, is assigned to the N–H stretching vibration that is characteristic of the amide bonds. The band observed between 914.90 cm^{-1} and 470.96 cm-^{1} results from the alkyl group's C-Cl stretching. The FTIR investigation shows the presence of numerous functional groups in AgNps-Vg. Furthermore, these functional groups in AgNps-Vg can be ascribed to the diverse phytochemicals that enclosed the silver nanoparticles and act as capping and reducing agents.[21, 26]

Antibacterial activities

The study aimed to assess the effectiveness of silver nanoparticles (AgNPs) against multidrug-resistant microorganisms, as shown in Table 1 which illustrates the corresponding zones of inhibitory activity. Notably, the results revealed that the nanoparticle displayed no inhibitory activity at concentrations below 20µg/mL, highlighting its concentration-dependent nature. This finding concurs with prior research, indicating a positive correlation between extract concentration and antibacterial efficacy, adding to the existing evidence base in this research area.[27]

Further investigation into the antibacterial activities of AgNPs-VG at a concentration of 40ug/mL, compared to conventional drugs, was conducted, with the zones of inhibition presented in Figure 6. The study specifically evaluated the antibacterial properties of biosynthesized silver nanoparticles (AgNPs) derived from *Vitex gradolofia* against a spectrum of isolated bacteria, including Gram-negative species such as *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Salmonella typhimurium*, as well as Gram-positive strains like *Staphylococcus aureus* and *Streptococcus pyogenes*. The conventional agar well diffusion method was employed to gauge the efficacy of the AgNPs, followed by the assessment of the Zone of Inhibition (ZOI) as depicted in Figure 6.

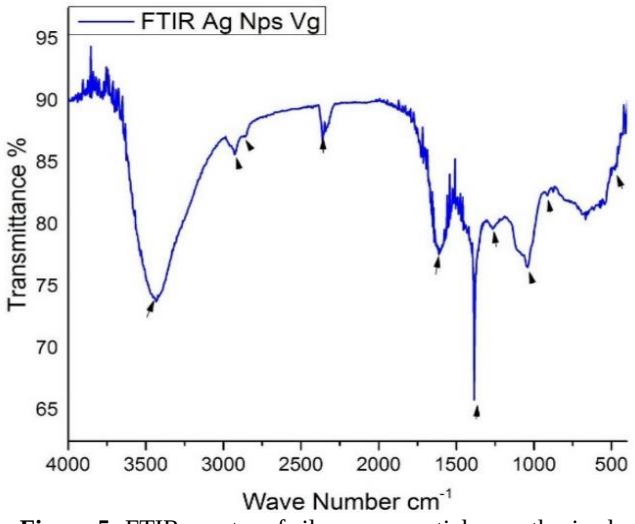

Figure 5: FTIR spectra of silver nanoparticles synthesized

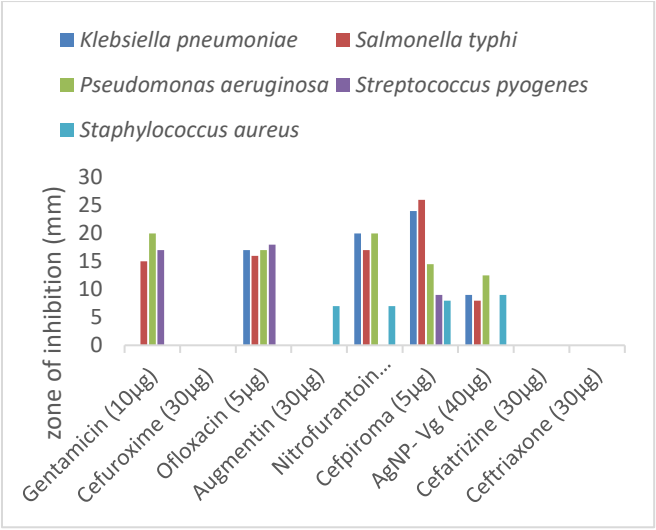

Figure 6: Antimicrobial activities of silver nanoparticles and other commercial antibiotics against the selected isolates

Remarkably, all tested organisms displayed susceptibility to the commercially produced antibiotics, except for Cefuroxime, Cefutrizine, and Cefraxone (Table 1), indicative of the isolates' multidrug-resistant potential, consistent with previous reports.[28, 29]

On the contrary, the increased susceptibility of Gram-negative bacteria in comparison to Gram-positive bacteria observed in Figure 6 can be

attributed to their more rapid susceptibility to penetration by antibacterial agents owing to the composition of their cell walls, thus supporting the conclusions reached by previous findings.[27,30] Moreover, all isolates were susceptibile to AgNP-VG (40ug), except for *Streptococcus pyogenes*, aligning with previous research on the antimicrobial resistance of this bacterium.[31-33] *Streptococcus pyogenes'* resistance may be attributed to intrinsic resistance to low antibiotic concentrations, attributed to limited drug absorption.[34]

In another study involving multidrug-resistant *Pseudomonas aeruginosa*, the application of 5 µg/mL of AgNPs demonstrated significant antibacterial efficacy, resulting in an approximate 99.9% reduction in bacterial viability.[35] Conversely, a lower zone of inhibition (10 mm) was noted when 12.5 µg/mL of AgNPs synthesized from *Phyllanthus amarus* was used in another study on a different strain of *P. aeruginosa*.[36]

Table 1: Inhibition Zones Diameter (mm) of different concentrations of AgNP-VG

Organism	20 µg/mL	40 µg/mL	60 µg/mL	80 µg/mL	100 µg/mL	Gentamicin (10 µg) Control
Klebsiella pneumoniae	0	9	10.5	10	11.5	0
Salmonella typhi	0	8	9	10.5	12	15
Pseudomonas aeruginosa	7	12.5	14.4	14	15	20
Streptococcus pyogenes	0	0	9	9.5	10.5	17
Staphylococcus aureus	0	9	10	13	15	0

These differences could be attributed to variations in the sources of leaves used for AgNP biosynthesis and the diverse strains of *Pseudomonas aeruginosa* employed, highlighting the importance of nanoparticle characteristics in antibacterial efficacy and confirming AgNP activity as concentration-dependent.[37, 25]

Studies have shown that silver nanoparticles (AgNPs) with sizes ranging from 20–80 nm, releasing silver ions, exhibit antimicrobial activity. However, 10 nm AgNPs were found to demonstrate greater toxicity, consistent with this study's findings. This was logical as 10 nm AgNPs had better cell-particle contact, making silver more bioavailable within cells. However, *Streptococcus pyogenes* remained resistant at that concentration. Previous studies have also noted that smaller-sized AgNPs display increased toxicity due to their larger surface area and adsorption capabilities, emphasizing the role of nanoparticle size in toxicity. [1, 38, 39, 40]

Quite a lot of antibacterial activities have been proposed for silver nanoparticles, though the precise mechanism remains elusive. Some researchers proposed that silver nanoparticles continuously release silver ions, which attach to cellular membranes, disrupting cell envelopes and causing cellular content leakage, leading to cell death.[41] Another study posits that liberated silver ions entering cells render respiratory enzymes inactive, generating reactive oxygen species and inhibiting ATP synthesis, altering DNA, and damaging cell membranes.[42] Additionally, earlier research indicates that silver ions impede protein production by disrupting ribosomes in the cytoplasm.[43]

Conclusion

Over time, significant efforts have been dedicated to developing a safer approach for synthesizing silver nanoparticles (AgNPs). This study introduces an efficient and rapid synthesis approach for AgNPs using an extract derived from *V. gradufloxia*. Through various characterization techniques, the morphological analysis of AgNP-VG produced from this plant revealed variations in size, crystallinity structure, and shape distribution, which play a crucial role in their mechanism of action. Additionally, this study also investigates the antibacterial efficacy of the biosynthesized nanoparticles against multidrug-resistant pathogens. However, further research is necessary to ascertain their safety. This study offers the first report on the environmentally friendly synthesis of AgNPs made from *V. gradufloxiaa* leaves.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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