

GREEN SYNTHESIS, CHARACTERIZATION, AND ANTIBACTERIAL ACTIVITY OF SILVER NANO PARTICLES AGAINST SOME BACTERIAL ISOLATES

Gabi Baba^{1,3,5}, *Aishatu M. Aliyu^{2,4}, Jonathan Tersur Orasugh^{5,6,7} and Zakari Abdullahi^{5,8}

¹Department of Biochemistry, Kaduna State University, Kaduna

²Department of Microbiology, Kaduna State University, Kaduna

³Department of Applied Chemistry, Kaduna Polytechnic, Kaduna

⁴Department of Applied Biology, Kaduna Polytechnic, Kaduna

⁵Department of Polymer and Textile Technology, Kaduna Polytechnic, Kaduna

⁶Department of Chemical Sciences, University of Johannesburg, Doornfontein, Johannesburg, 2028, South Africa

⁷DST-CSIR National Centre for Nanostructured Materials, Council for Scientific and Industrial Research, Pretoria, 0001, South Africa

⁸National Board for Technical Education(NBTE) Kaduna, Nigeria

*Corresponding Author Email Address: aisha2zra@yahoo.com

Phone: +2348035890640

ABSTRACT

Nanotechnology has emerged as a promising field for the development of novel antibacterial agents with reduced environmental impact. In this study, we present a novel green synthesis approach for the production of silver nanoparticles (AgNPs) using a plant-based extract. These AgNPs were subsequently characterized using various analytical techniques, including UV-Vis spectroscopy, X-ray diffraction (XRD), transmission electron microscopy (TEM), and Fourier-transform infrared spectroscopy (FTIR). UV-Vis spectroscopy confirmed the formation of AgNPs by exhibiting a characteristic surface plasmon resonance peak at around 401 and 420 nm. XRD analysis revealed the crystalline nature of the AgNPs, with distinct diffraction peaks corresponding to the face-centered cubic structure of silver. TEM analysis demonstrated that the synthesized AgNPs were predominantly spherical in shape and exhibited an average size within the nanoscale range. FTIR analysis was employed to elucidate the potential bioactive compounds present in the plant extract responsible for the reduction and stabilization of AgNPs. Furthermore, we evaluated the antibacterial activity of these synthesized AgNPs against a panel of bacterial isolates. All the bacterial isolates were sensitive to the silver nanoparticles. *Staphylococcus aureus* as found to be most resistant, while *E. coli* as found to be the most sensitive.

Keywords: Bacterial, Silver nanoparticles, Green Synthesis, Antibacterial.

INTRODUCTION

In recent years, the development of nanotechnology has opened up new avenues for addressing critical challenges in various scientific domains, including healthcare and environmental science. Among the myriad of nanoparticles explored for their diverse applications, silver nanoparticles (AgNPs) have emerged as promising candidates due to their unique physical and chemical properties (Abboud *et al.*, 2013; Yin, *et al.*, 2020). Their antimicrobial potential, in particular, has garnered significant attention in the context of combating bacterial infections and addressing the growing concern of antibiotic resistance.

While silver nanoparticles exhibit remarkable antibacterial properties, their conventional synthesis methods often involve the

use of chemical reducing agents and stabilizers, raising concerns regarding environmental sustainability and potential toxicity. In response to these challenges, researchers have turned to "green synthesis" approaches, which harness the reducing capabilities of natural compounds, such as plant extracts, and emphasize eco-friendliness and biocompatibility (Abboud *et al.*, 2013; Yin, *et al.*, 2020).

This study delves into the realm of green nanotechnology, focusing on the synthesis, characterization, and antibacterial activity of silver nanoparticles. By employing a biocompatible and environmentally benign synthesis route, this research seeks to not only create nanoparticles with enhanced antibacterial efficacy but also mitigate the adverse ecological impacts associated with conventional synthesis methods.

Silver in all its forms or combined with other technologies has been historically used as an antimicrobial agent. This metal has taken advantage of its ability to inhibit bacterial growth by incorporating it as silver nitrate or silver sulfadiazine in creams and dressings to treat burns and ulcers, in food packaging to prevent contamination, in-home appliances as refrigerators and washing machines, and several applications in the industrial area (Bruna *et al.*, 2021).

The antibacterial activity of Ag at the nanoscale has been most valuable in medical and healthcare areas, where the incorporation of AgNPs into hundreds of products has been studied, including surgical and food handling tools, clothing, cosmetics, dental products, catheters, and dressings (Bruna *et al.*, 2021). The potential of AgNPs as antibiotics is related to their various mechanisms of action, which attack microorganisms in multiple structures at a time and give them the ability to kill various types of bacteria (Bruna *et al.*, 2021).

Silver nanoparticles contain 20 to 15,000 silver atoms, and their diameters are usually smaller than 100 nm. Due to a large surface-to-volume ratio, silver nanoparticles exhibit antimicrobial activity, even at a low concentration. They are low cost and have shown low cytotoxicity and immunological response (Yin *et al.*, 2020). Previous study have shown that antimicrobial formulations in the form of nanoparticles (NPs) could be used as effective antibacterial materials due to their enhanced reactivity, resulting from their high

surface/volume ratio. Particularly, silver in the form of NP (AgNP) is known to exhibit strong biocidal effects on different bacterial species including multidrug resistant bacteria. It is generally accepted that free silver ions, present or released from the nanomaterials, are able to bind cell membrane structures, destabilizing the membrane potential and causing proton leakage (Losasso *et al.*, 2014).

Development of multidrug resistance has become a worldwide issue with serious consequences in the management of infectious diseases caused by pathogenic bacteria. Indiscriminate use of antibiotics in human healthcare, agriculture, and veterinary medicine being the main cause. The most common multidrug-resistant pathogens are *Acinetobacter baumannii*, ES β L-producing *E. coli*, penicillin-resistant *Streptococcus pneumoniae*, *Klebsiella pneumoniae*, vancomycin-resistant *Enterococcus*, methicillin-resistant *S. aureus*, and extensively drug-resistant *Mycobacterium tuberculosis* (Qais *et al.*, 2019).

The aim of the study was to synthesize, characterize, and determine the antibacterial activity of silver nanoparticles against some bacterial isolates. The objectives of this study were multifaceted:

1. Explore a green synthesis approach that utilizes a plant-based reducing agent for the fabrication of silver nanoparticles.
2. Comprehensively characterize the synthesized nanoparticles through various analytical techniques, including UV-Vis spectroscopy, X-ray diffraction (XRD), and transmission electron microscopy (TEM).
3. Assess the antibacterial activity of the green-synthesized silver nanoparticles against a selection of bacterial isolates.
4. Elucidate the potential applications of these nanoparticles in the context of addressing bacterial infections, antibiotic resistance, and environmental sustainability.

This research will contribute to the growing body of knowledge surrounding green nanotechnology, offering a sustainable and biocompatible solution to the urgent global health challenge posed by bacterial infections and antibiotic resistance.

MATERIALS AND METHODS

Green Synthesis of Silver Nanoparticles (AgNps) Using Scent Leaf

Preparation of Leaf Extracts and Silver Nanoparticles

The fresh leaf of a scent leaf (*Ocimum basilicum*) broth solution was prepared by taking 10 g of thoroughly washed and finely cut leaves in a 300-mL Erlenmeyer flask along with 100 mL of sterilized double-distilled water, and then the mixture was boiled for 5 minutes before finally decanting it. The extract was filtered through Whatman filter paper No. 1 and stored at -15°C. Leaf extract obtained was used as green reductant and capping agents for the biosynthesis of AgNPs (Ghaffari-Moghaddam *et al.*, 2014; Ojo *et al.*, 2017). The biosynthesis of AgNPs was carried out as described by Okaiyeto *et al.* (2015), with some slight modifications. Fifty milliliters (50 mL) of aqueous leaf extract were added to 450 mL of 1 mM silver nitrate (AgNO₃) solution and the resultant mixture was stirred in the dark, at 300 rpm for 1hr at 70 OC. The mixture was stirred continuously till the completion of synthesis. On cooling, the

reaction media was centrifuged at 1500 rpm for 15 min (Beckman Coulter Allegra 64R, California, USA). The pellets were rinsed three times with 10 ml of ultrapure water, dried in an oven over night, and stored at room temperature (Baran, 2019; Mohammadi *et al.*, 2019). Physicochemical Characterizations of the Synthesized Silver Nanoparticles (AgNps).

Utilizing UV-visible spectroscopy, the optical characteristics of the biosynthesized nanoparticles were studied. A Shimadzu UV-2550 spectrophotometer (Shimadzu, Kyoto, Japan) was used to record the spectra after diluting the samples with ultrapure water. The spectra were recorded as a function of wavelength from 300 to 700 nm, with a resolution of 1 nm. For UV-Vis tests, ultrapure water was utilized as the control (White *et al.*, 2012; Jain and Mehata, 2017). Transmission electron microscopy (TEM; Zeiss Libra-120 kV TEM microscope, Carl Zeiss, Oberkochen, Germany) was used to analyze the morphology and form of the particles. For the TEM experiment, the diluted samples were dropped onto copper TEM grids that had been coated with carbon. The grids were air-dried at room temperature for an entire night before particle visualization, and any surplus liquid was absorbed using filter paper. To estimate the sizes of observed nanoparticles, ImageJ-win32 was employed (Orasugh *et al.*, 2023) Log-normal fitting, a probability function frequently used to evaluate skewed size distributions, was used to estimate the mean diameters.

Sample Collection (Bacterial Isolates)

The test organisms were obtained from the Microbiology laboratory of the Department of Microbiology Kaduna State University (KASU) Kaduna.

Standardization of Inocula

A 0.5 McFarland standard was prepared using BaCl₂ and H₂SO₄ One (1) ml of concentrated H₂SO₄ was added to 99 ml of distilled water in a conical flask and mixed well (1 % v/v solution of H₂SO₄). Then 0.5 g of dihydrate barium Chloride salt (BaCl₂.2H₂O) was dissolved in 50 ml of distilled water (1 % w/v of BaCl₂₀). Aliquot of 0.6 ml of BaCl₂ solution was added to 99.4 ml of H₂SO₄ solution to make up to 100 ml. The solution was mixed well. This is the stock solution of the 0.5 McFarland turbidity standards. Normal saline solution was prepared (0.9% sodium chloride (NaCl)) by dissolving 9g of NaCl in 100ml of distilled water in a clean beaker and autoclaved at 121°C for 15 minutes. A standard inoculum was prepared using overnight broth culture of each of the test organism by diluting with sterile saline solution to match 0.5 McFarland turbidity standards in front of light against a white background with contrasting black lines. Overnight nutrient broth cultures of *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, and *Staphylococcus aureus* were adjusted using sterile distilled water to match with the standard (Akinpelu and Onokoya 2006).

Bioassay

Agar well diffusion was adopted. Thus, each standardized inoculum of *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, and *Staphylococcus aureus* were separately inoculated into sterile Mueller Hinton plates (90mm) radially using sterile swab sticks in duplicate. The inoculated plates were allowed to diffuse (5 minutes). Subsequently, a sterile cork borer (6mm) was used to dig five wells per plate. The prepared sample concentrations were pipetted and introduced into designated wells with the central as control (Gentamycin Sulphate 20mg/ml). The

preparations were incubated aseptically at 37 °C for 24 hours. Thereafter, zones of inhibition (mm) were measured and recorded.

RESULTS AND DISCUSSION

Synthesized AgNPs

The distinctive color change and spectroscopic characterization of the reaction media and isolated nanoparticles proved that the examined plant extract was effective in producing AgNPs. In fact, a clear color shift was seen after an hour of swirling the AgNO₃ and plant extract mixture. From yellowish green to brown, the reaction mixture's hue altered, indicating the transition of colloidal silver (AgNPs) from ionic silver (Ag) to metallic silver (Ag).

Elemental analysis

The elemental composition of the synthesized nanoparticles with respect to 4% and 5% concentration is presented in Table 1, Table 2, and Fig. 1. It was observed that the 4% sample contained Pb, Al, Na, Mg, K, Si, Ca, P, Cl, S, and Ti having their atomic concentration of 62.05, 10.37, 10.90, 4.65, 2.78, 3.62, 2.36, 2.27, 0.99, 0.00, and 0.00 respectively. On the other hand, the 4% sample containing Pb, Al, K, Mg, Si, Ca, P, Na, Cl, and S having atomic concentrations of 76.74, 7.59, 2.33, 3.72, 2.82, 1.85, 1.79, 2.25, 0.90, and 0.00 respectively.

Table 1: Elemental composition of 4% sample

Element Number	Element Symbol	Element Name	Atomic Conc.	Weight Conc.
82	Pb	Lead	62.05	92.42
13	Al	Aluminium	10.37	2.01
11	Na	Sodium	10.90	1.80
12	Mg	Magnesium	4.65	0.81
19	K	Potassium	2.78	0.78
14	Si	Silicon	3.62	0.73
20	Ca	Calcium	2.36	0.68
15	P	Phosphorus	2.27	0.51
17	Cl	Chlorine	0.99	0.25
16	S	Sulfur	0.00	0.00
22	Ti	Titanium	0.00	0.00

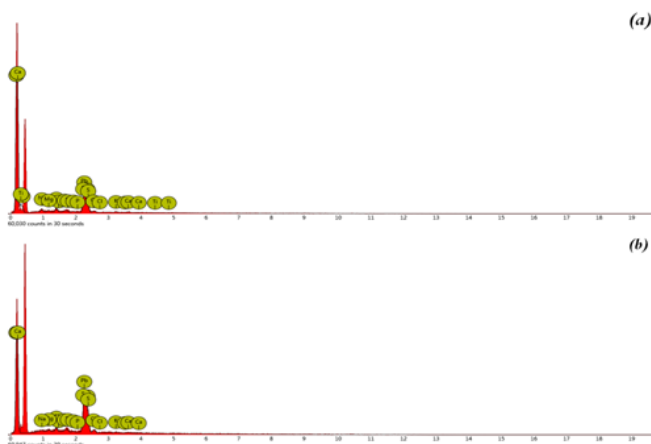


Fig. 1. Elemental analysis of (a) 4% and (b) 5%

Table 2: Elemental composition of 5% sample

Element number 0.5%	Element symbol	Element name	Atomic conc.	Weight conc.
82	Pb	Lead	76.74	95.90
13	Al	Aluminium	7.59	1.24
19	K	Potassium	2.33	0.55
12	Mg	Magnesium	3.72	0.55
14	Si	Silicon	2.82	0.48
20	Ca	Calcium	1.85	0.45
15	P	Phosphorus	1.79	0.33
11	Na	Sodium	2.25	0.31
17	Cl	Chlorine	0.90	0.19
16	S	Sulfur	0.00	0.00

UV-vis spectroscopy

UV-Vis spectroscopy is a common technique used to characterize the optical properties of materials, including silver nanoparticles. When interpreting UV-Vis spectroscopy results for silver nanoparticles, you can gain information about their size, shape, and surface plasmon resonance (SPR) behavior. The position, intensity, and shape of the absorption peak are the key parameters to consider when interpreting UV-Vis spectra of silver nanoparticles. These insights can be crucial for optimizing nanoparticle synthesis and understanding their properties for various applications. UV-Vis spectroscopy confirmed the formation of AgNPs by exhibiting a characteristic surface plasmon resonance peak at around 401 and 420 nm. Similar results have been also reported by other researchers supporting our findings of the UV-Vis absorption spectra presented herewith (Mollick *et al.*, 2019). The most critical information obtained from UV-Vis spectroscopy of silver nanoparticles is the position of the absorption peak. Typically, silver nanoparticles exhibit a distinct absorption peak in the UV-Vis spectrum due to surface plasmon resonance. The absorption peak of silver nanoparticles is usually located in the visible to near-UV region (approximately 380-450 nm for spherical nanoparticles) (Mollick *et al.*, 2019). The exact position of the peak depends on

the size, shape, and dielectric environment of the nanoparticles. It was observed in this study that, the nanoparticles synthesized presented dissimilar sizes for 4% and 5% samples as can be observed in Fig. 2. A shift in the absorption peak position can indicate changes in the size or shape of the nanoparticles. Larger nanoparticles generally have absorption peaks at longer wavelengths. The smaller particle sized of the 5% sample is also noticeable in the UV spectrum with the less-broader absorbance peak while the 4% sample is seen to possess a broader absorbance peak.

It was equally observed from the UV-vis results that the peak intensity of the 5% sample was more than that of 4% sample. It is well known that the intensity of the absorption peak is related to the concentration of silver nanoparticles in the sample and the extent of light absorption (Mollick *et al.*, 2019). Higher absorption peak intensities indicate a higher concentration of nanoparticles or a higher degree of light absorption, which is

often associated with larger or more concentrated nanoparticles as seen herewith. Again, seeing that the shape and width of the absorption peak can provide information about the distribution of nanoparticle sizes. Narrow and symmetrical peaks here in our study suggest a relatively uniform size distribution in 5% sample, while broad peak in 4% indicate a wider range of sizes as also supported by the TEM results.

In this case study, it was observed that the shoulder peaks on the absorption spectrum of both 4% and 5% samples. These features could indicate the presence of larger or irregularly shaped nanoparticles, aggregates, or surface modifications. The absorption peak in the UV-Vis spectrum of silver nanoparticles is attributed to the collective oscillation of free electrons on the nanoparticle's surface when excited by incident light. This phenomenon is known as surface plasmon resonance (SPR) (Adegboyega *et al.*, 2013; Kushwaha *et al.*, 2018).

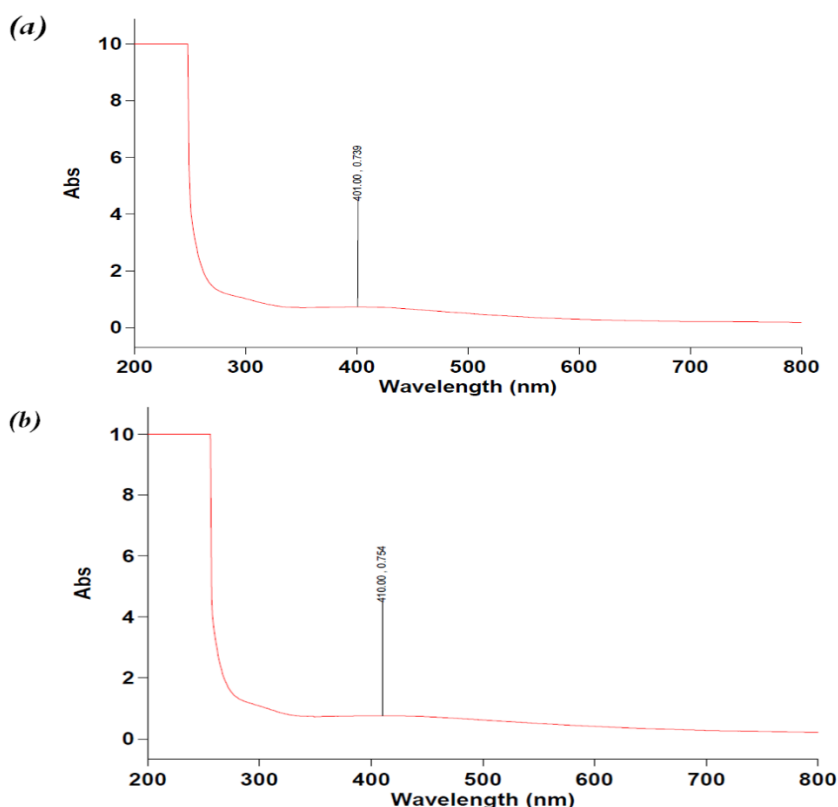


Fig. 2. UV-vis spectroscopy of (a) 4% and (b) 5%

Morphological Analysis

Transmission Electron Microscopy (TEM) is a powerful imaging technique used to visualize the morphology and measure the particle size of silver nanoparticles at the nanoscale. TEM images were used to reveal the shape of silver nanoparticles which was primarily spherical similar to previous literature by Bankura *et al.* (2012); Mollick *et al.* (2019) though other literatures have reported rod-like, triangular, hexagonal, and irregular shapes for nanosilver sourced from diverse materials. The shape of the

nanoparticles can provide valuable insights into their synthesis method and properties (Bankura *et al.*, 2012; Mollick *et al.*, 2014; Ghosh *et al.*, 2020).

TEM images of the synthesized AgNPs showed a range of particle sizes as presented in Fig. 3 by means of Image J image analysis software to measure the dimensions of individual nanoparticles. The 4% sample presented a standard deviation and mean particle size of ~494 and ~600 nm while 5% sample presented a standard deviation and mean particle size of ~157 and ~316 nm. The smaller

particle sized of the 5% sample is also noticeable in the UV spectrum with the maximum absorbance peak at ~410 nm while the 4% sample produced maximum absorbance peak at ~401 nm. It was also observed from the TEM images that the nanoparticles were well-dispersed in the 5% but tend to aggregate in the 4%

samples. It's essential to consider the synthesized AgNPs revealed polydispersity, meaning they have a range of sizes. Therefore, we reported the mean size along with the size distribution to provide a comprehensive understanding of the nanoparticle population.

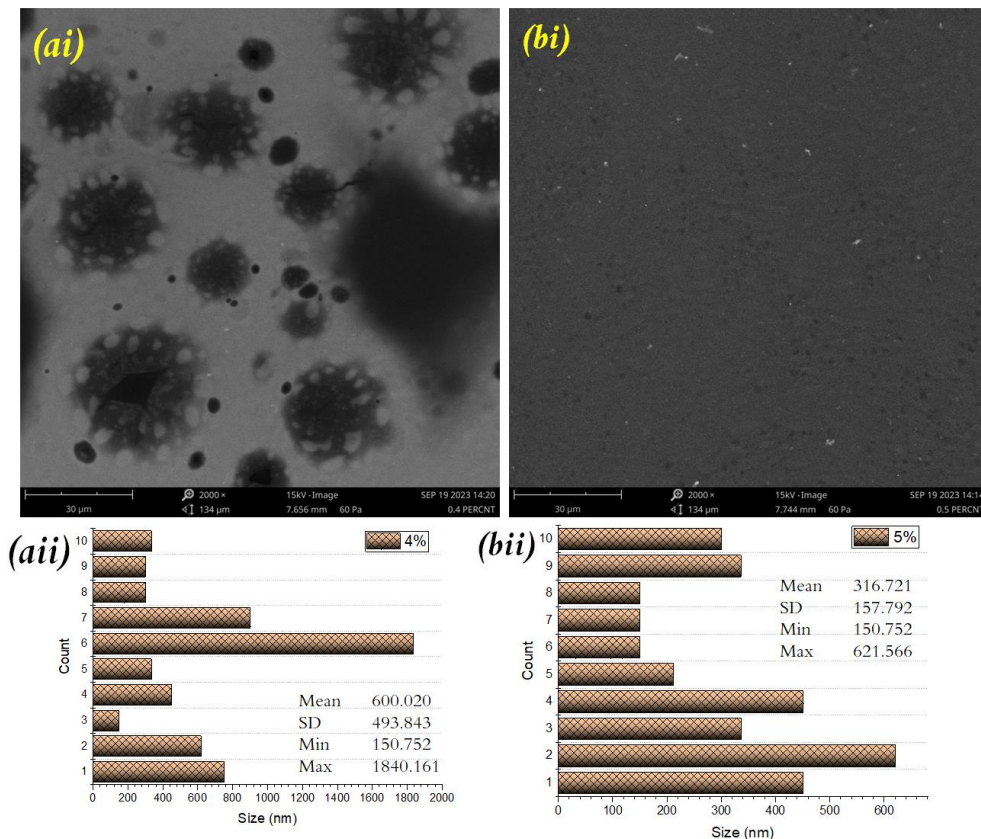


Fig. 3. TEM morphology and particle size distribution of (a) 4% and (b) 5% synthesized silver nanoparticles

X-ray diffraction (XRD) analysis

X-ray diffraction (XRD) is a powerful technique used to characterize the crystallographic structure of materials, including silver nanoparticles. Upon interpreting XRD patterns of silver nanoparticles, we gained valuable information about their size, shape, and crystallinity.

The position of diffraction peaks in the XRD pattern corresponds to the interatomic spacing (d-spacing) between planes of atoms in the crystal lattice. The intensity of diffraction peaks relates to the number of atoms in the crystal planes and the arrangement of atoms in the lattice. Sharp, intense peaks indicate a highly

crystalline structure, while broad, less intense peaks suggest a more disordered or amorphous structure. The 5% sample presented a sharper and more intense peak in comparison to the 4% sample.

The existence of elemental silver in the samples (4% and 5%) was indicated by the recording of four silver peaks at around 38.2, 44.6, 64.3, and 76.9 at $2\theta^\circ$, which correspond to levels (111), (200), (220), and (311) as presented in Fig. 4. Due to the sample's several washings, the silver nitrate was cleaned in a way that the XRD results belonged to SNPs and verified the presence of these kinds of nanoparticles in the sample (Pourali *et al.*, 2023).

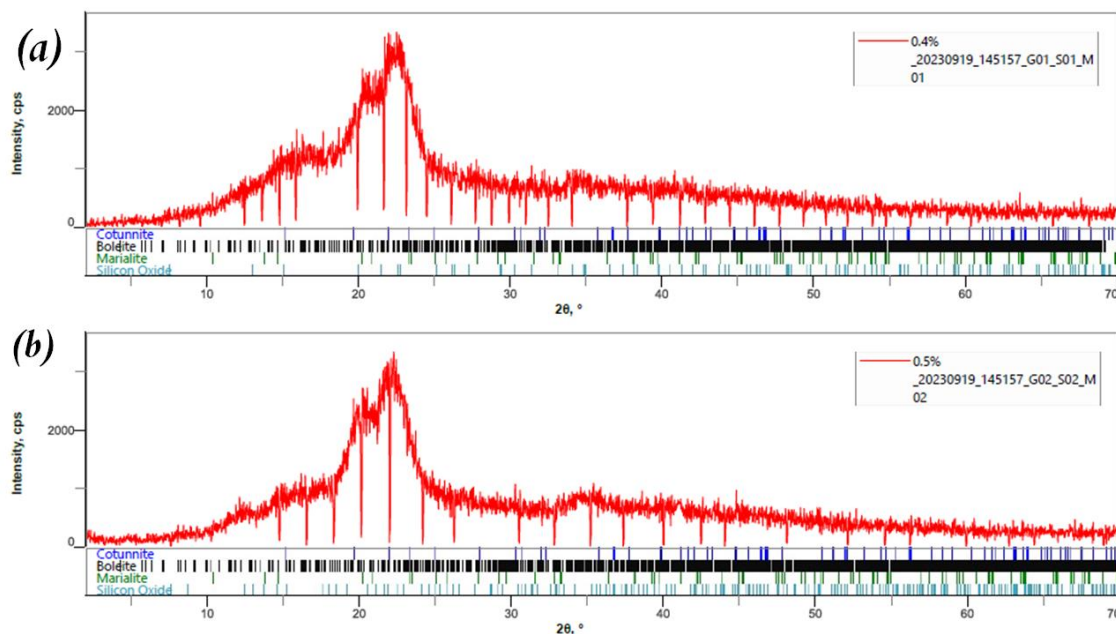


Fig. 4. XRD spectra of (a) 4% and (b) 5%

Fourier-Transform Infrared Spectroscopy (FTIR)

Fourier-Transform Infrared Spectroscopy (FTIR) is a valuable analytical technique used to study the chemical composition and functional groups present on the surface of materials, including silver nanoparticles. Herewith, we have analysed the FTIR results of silver nanoparticles in order to obtain information about the chemical characteristics and potential surface modifications of the nanoparticles. FTIR spectra typically covered a broad range of wavelengths (wavenumbers), usually from 4000 to 400 cm^{-1} where different regions of the spectrum correspond to different vibrational modes of chemical bonds as presented in Fig. 5.

Silver nanoparticles possess the ability to form metal-oxygen (M-O) bonds. Silver nanoparticles may show characteristic peaks related to metal-oxygen bonds, indicating the presence of stabilizing ligands or capping agents. Seeing that our synthesized nanoparticles are functionalized or capped with organic molecules, peaks related to C-H, C=O, C-O, and other organic functional groups are present.

In particular, the moieties of carbohydrate, lipid, protein, and polyphenols in plant extract were observed in the spectrum of the 4% and 5% samples, respectively. These bands between the wavenumbers of 1,800 and 750 cm^{-1} (fingerprint regions) of the spectrum for the control reflected the biochemical compositions. Complex vibrational modes that are highly specialized to the

chemical functional groups in nanosilver can be found in this region. Peaks in this area can be analyzed to learn more about the chemical makeup of the nanoparticle surface. Also, OH stretching around 3700-3000 cm^{-1} was observed in the spectrum of the 4% and 5% samples, respectively. Peaks in this region could be said to be associated with O-H stretching vibrations, indicating the presence of hydroxyl groups. C-H stretching was observed around 3000-2800 cm^{-1} which is related to C-H stretching vibrations, typically found in organic molecules such as plant extracts as used in this study (Abboud *et al.*,2013). C=O stretching peaks were observed in the 1625 cm^{-1} range corresponding to aliphatic amines carbonyl (C=O) stretching vibrations both in 4% and 5% FTIR spectrum. Amido III (random coil) C-H bonds for protein in the plant extract are responsible for the minor band at 1,304 cm^{-1} . The functional groups in the wavenumber range between 1,200 and 820 cm^{-1} are primarily carbohydrate-derived. For instance, the C-OH stretching band of the oligosaccharide residue is responsible for the "shoulder" peak at 1,104 cm^{-1} , as well as the bands at 1,028 cm^{-1} (4%) and 1025 cm^{-1} (5%) wavenumbers (Khatami *et al.*,2015). According to the FTIR analysis, the primary players in the reduction of Ag^+ ions to Ag^0 nanoparticles in seed exudates are most likely the carboxyl (-C=O), hydroxyl (-OH), and amine (N-H) groups.

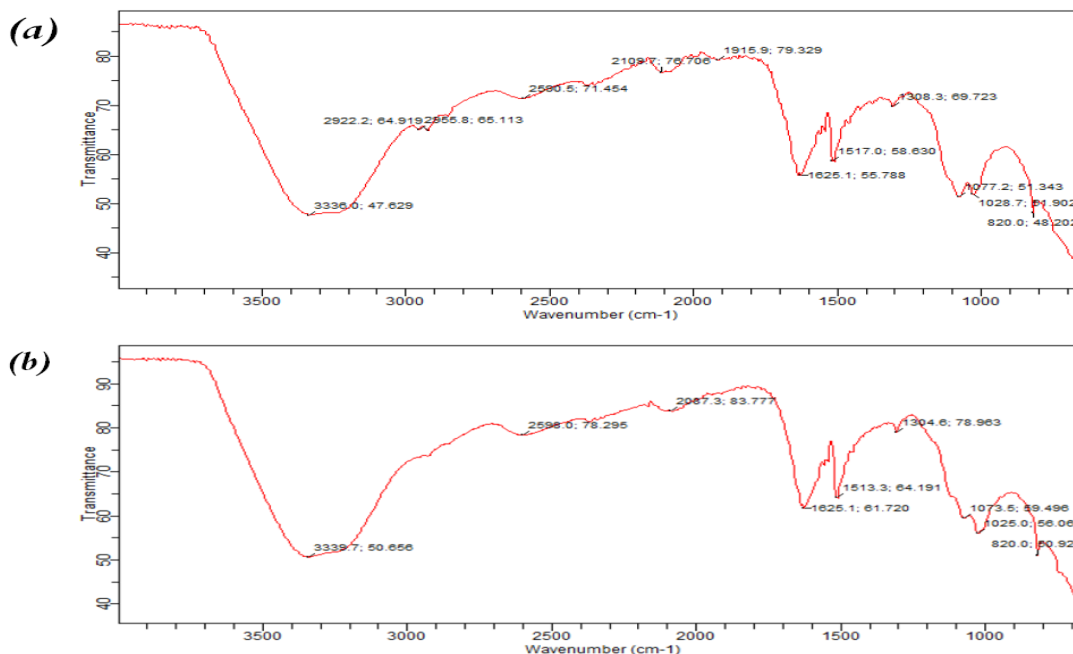


Fig. 5. FTIR analysis of (a) 4% and (b) 5%

Bioassay Findings

The antimicrobial activity of AgNPs was investigated against *E. coli*, *Pseudomonas aeruginosa*, *Salmonells typhi* and *S. aureus* using the agar well diffusion assay. All the bacterial isolates were sensitive to the AgNPs. This result agrees with that of Bruna *et al.* (2021) who reported antibacterial activity of AgNPs against *S. aureus*, *S.typhimurium*, *E. coli* and *P. aeruginosa*. Among the tested bacteria, *S. aureus* was found to be most resistant, while *E. coli* was found to be the most sensitive. This agrees with the report of Yin *et al.* (2020) who report that Gram-negative bacteria are more susceptible to silver nanoparticles. The cellular wall of gram-negative bacteria is narrower than that of gram-positive strains. The thick cellular wall may reduce the penetration of nanoparticles into cells. The different antibacterial effects of silver nanoparticles on gram-negative and gram-positive bacteria suggest that the

uptake of silver nanoparticles is important to the antibacterial effect. It is commonly acknowledged that silver nanoparticles smaller than 10 nm can directly alter cell permeability, enter bacterial cells, and cause cell damage (Ahmad *et al.*, 2020). The enhanced antibacterial activity of AgNPs is attributed to their large surface area that provides more surface contact with microorganisms. Another important reason of the enhanced antibacterial activity of AgNPs as documented in the literature is the synergistic effect between particles and natural compounds. The mechanism of action of the antibacterial activity of AgNPs is attacking the respiratory chain and cell division which ultimately leads to cell death. The silver nanoparticles have also been reported to release silver ions inside the bacterial cells, further enhancing their bactericidal activity (Qais *et al.*, 2019; Crisan *et al.*, 2021).

Table 3: Mean zone of Inhibition of the Silver Nano Particles against Some Bacterial Isolates

Isolates	Concentration (%)			
	0.3%	0.4%	0.5%	Gentamicin 80mg
	Mean zone of inhibition ± SD			
<i>Staphylococcus aureus</i>	6.0±0.00 ^a	6.0±0.00 ^a	12.8±0.29 ^b	35.0±0.00 ^{ab}
<i>Salmonells typhi</i>	6.0±0.00 ^a	17.0±0.50 ^b	19.2±0.29 ^c	33.0±0.00 ^{bc}
<i>Pseudomonas aeruginosa</i>	6.0±0.00 ^a	6.0±0.00 ^a	16.2±0.76 ^b	28.0±0.00 ^c
<i>Escherichia coli</i>	6.0±0.00 ^a	6.0±0.00 ^a	19.8±0.29 ^c	43.0±0.00 ^{ac}

The data are presented as mean ± SD n=3 the values with different superscript differ significantly (p≤0.05)

Conclusion

In conclusion, this study successfully demonstrated the green synthesis of silver nanoparticles (AgNPs) using a biocompatible and environmentally friendly approach. The characterization of these nanoparticles through various analytical techniques, including UV-Vis spectroscopy, X-ray diffraction (XRD), and transmission electron microscopy (TEM), provided valuable insights into their size, shape, and crystalline structure. The results revealed that the green-synthesized AgNPs possessed unique morphological characteristics and exhibited excellent antibacterial properties.

The antibacterial activity of the synthesized AgNPs was evaluated against a panel of bacterial isolates, including both Gram-positive and Gram-negative strains. The AgNPs exhibited significant inhibitory effects on bacterial growth, indicating their potential as effective antibacterial agents. Importantly, the green synthesis method employed in this study not only yielded nanoparticles with antibacterial properties but also reduced the environmental impact associated with traditional chemical synthesis methods.

Furthermore, the biocompatibility of these AgNPs makes them promising candidates for various biomedical applications, such as wound healing, drug delivery, and medical device coatings. The results suggest that AgNPs can be explored as alternatives to conventional antibiotics, especially in the context of antibiotic-resistant bacterial strains.

It is worth noting that the green synthesis approach, utilizing plant extracts or natural compounds as reducing and stabilizing agents, aligns with the principles of sustainability and eco-friendliness. This study contributes to the growing body of research on green nanotechnology, highlighting the potential of harnessing natural resources for the synthesis of nanoparticles with valuable properties.

While this research has provided valuable insights into the green synthesis, characterization, and antibacterial activity of silver nanoparticles, further investigations are needed to explore their mechanisms of action, toxicity profiles, and potential applications in a broader spectrum of healthcare and environmental settings. Additionally, efforts to optimize the synthesis process and scale up production should be pursued to make these nanoparticles more accessible for practical use.

Thus, the findings of this study emphasize the promising prospects of green-synthesized silver nanoparticles as effective antibacterial agents with a reduced environmental footprint. These nanoparticles hold great potential for addressing pressing challenges in healthcare and beyond, underscoring the importance of continued research in this field.

REFERENCES

Abboud, Y., Eddahbi, A., El Bouari, A., Aitenneite, H., Brouzi, K. and Mouslim, J. (2013). Microwave-assisted Approach for Rapid and Green Phytosynthesis of Silver Nanoparticles using Aqueous Onion (*Allium cepa*) Extract and their Antibacterial Activity. *Journal of Nanostructure in Chemistry*, 3, 1-7.

Adegboyega, N. F., Sharma, V. K., Siskova, K., Zboril, R., Sohn, M., Schultz, B. J. and Banerjee, S. (2013). Interactions of Aqueous Ag⁺ with Fulvic Acids: Mechanisms of Silver Nanoparticle Formation and Investigation of

Stability. *Environmental science and technology*, 47(2), 757-764.

- Ahmad, S. A., Das, S. S., Khatoon, A., Ansari, M. T., Afzal, M., Hasnain, M. S. and Nayak, A. K. (2020). Bactericidal Activity of Silver Nanoparticles: A Mechanistic Review. *Materials Science for Energy Technologies*, 3, 756-769.
- Akinpelu, D.A. and Onokoya, T.M. (2006). Antimicrobial Activities of Medicinal Plants Used in Folklore Remedies in South-Western Africa. *Journal of Biotechnology*, 5:1078-1081.
- Bankura, K. P., Maity, D., Mollick, M. M., Mondal, D., Bhowmick, B., Bain, M. K. and Chattopadhyay, D. (2012). Synthesis, Characterization and Antimicrobial Activity of Dextran Stabilized Silver Nanoparticles in Aqueous Medium. *Carbohydrate Polymers*, 89(4): 1159-1165.
- Baran, M. F. (2019). Green Synthesis, Characterization and Antimicrobial Activity of Silver Nanoparticles (AgNPs) from Maize (*Zea mays* L.). *Dergi*.
- Bruna, T., Maldonado-Bravo, F., Jara, P. and Caro, N. (2021). Silver Nanoparticles and their Antibacterial Applications. *International Journal of Molecular Sciences*, 22(13): 7202.
- Burduşel, A. C., Gherasim, O., Grumezescu, A. M., Mogoantă, L., Ficai, A. and Andronescu, E. (2018). Biomedical Applications of Silver Nanoparticles: An Up-to-date Overview. *Nanomaterials*, 8(9): 681.
- Crisan, C. M., Mocan, T., Manolea, M., Lasca, L. I., Tăbăran, F. A. and Mocan, L. (2021). Review on Silver Nanoparticles As a Novel Class of Antibacterial Solutions. *Applied Sciences*, 11(3): 1120.
- de Lacerda Coriolano, D., de Souza, J. B., Bueno, E. V., Medeiros, S. M. D. F. R. D. S., Cavalcanti, I. D. L. and Cavalcanti, I. M. F. (2021). Antibacterial and Antibiofilm Potential of Silver Nanoparticles against Antibiotic-sensitive and Multidrug-resistant *Pseudomonas aeruginosa* Strains. *Brazilian Journal of Microbiology*, 52: 267-278.
- Ghaffari-Moghaddam, M., Hadi-Dabanlou, R., Khajeh, M., Rakhshanipour, M. and Shamel, K. (2014). Green Synthesis of Silver Nanoparticles using Plant Extracts. *Korean Journal of Chemical Engineering*, 31: 548-557.
- Ghosh, T., Chattopadhyay, A., Mandal, A. C., Pramanik, S. and Kuri, P. K. (2020). Optical, Structural, and Antibacterial Properties of Biosynthesized Ag Nanoparticles at Room Temperature using *Azadirachta indica* Leaf Extract. *Chinese Journal of Physics*, 68: 835-848.
- Gorup, L. F., Neto, F. N. S., Kubo, A. M., Souza, J. A. S., Fernandes, R. A., Fernandes, G. L. and Camargo, E. R. (2017). Nanostructured Functional Materials: Silver Nanoparticles in Polymer for the Generation of Antimicrobial Characteristics. *Recent Advances in Complex Functional Materials: From Design to Application*, 271-292.
- Hamad, A., Khashan, K. S. and Hadi, A. (2020). Silver Nanoparticles and Silver Ions as Potential Antibacterial Agents. *Journal of Inorganic and Organometallic Polymers and Materials*, 30(12): 4811-4828.
- Jain, S. and Mehata, M. S. (2017). Medicinal Plant Leaf Extract and Pure Flavonoid Mediated Green Synthesis of Silver Nanoparticles and their Enhanced Antibacterial Property. *Scientific Reports*, 7(1): 15867.
- Kędzierska, M. and Miłowska, K. (2021). Silver Nanoparticles—Possible Applications and Threats. *Acta Universitatis Lodzianis. Folia Biologica et Oecologica*, 17: 14-31.

- Khatami, M., Pourseyedi, S., Khatami, M., Hamidi, H., Zaeifi, M. and Soltani, L. (2015). Synthesis of Silver Nanoparticles Using Seed Exudates of *Sinapis arvensis* as A Novel Bioresource, and Evaluation of their Antifungal Activity. *Bioresources and Bioprocessing*, 2: 1-7.
- Konop, M., Damps, T., Misicka, A. and Rudnicka, L. (2016). Certain Aspects of Silver and Silver Nanoparticles in Wound Care: A Minireview. *Journal of Nanomaterials*, 2016: 47-47.
- Kushwaha, A. S., Kumar, A., Kumar, R. and Srivastava, S. K. (2018). A Study of Surface Plasmon Resonance (SPR) Based Biosensor with Improved Sensitivity. *Photonics and Nanostructures-Fundamentals and Applications*, 31: 99-106.
- Losasso, C., Belluco, S., Cibir, V., Zavagnin, P., Mičetić, I., Gallochio, F. and Ricci, A. (2014). Antibacterial Activity of Silver Nanoparticles: Sensitivity of Different *Salmonella* serovars. *Frontiers in Microbiology*, 5: 227.
- Mohammadi, F., Yousefi, M. and Ghahremanzadeh, R. (2019). Green Synthesis, Characterization and Antimicrobial Activity of Silver Nanoparticles (AgNPs) Using Leaves and Stems Extract of Some Plants. *Advanced Journal of Chemistry-section A*, 2(4): 266-275.
- Mollick, M. M. R., Bhowmick, B., Maity, D., Mondal, D., Roy, I., Sarkar, J. and Chattopadhyay, D. (2014). Green Synthesis of Silver Nanoparticles-based Nanofluids and Investigation of their Antimicrobial Activities. *Microfluidics and Nanofluidics*, 16: 541-551.
- Mollick, M. M. R., Rana, D., Dash, S. K., Chattopadhyay, S., Bhowmick, B., Maity, D. and Chattopadhyay, D. (2019). Studies on Green Synthesized Silver Nanoparticles Using *Abelmoschus esculentus* (L.) Pulp Extract having Anticancer (in vitro) and Antimicrobial Applications. *Arabian Journal of Chemistry*, 12(8): 2572-2584.