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RESEARCH PAPER

Study of Optimum Conditions and Characterization for Green Synthesis of Silver Nanoparticles Using *Zamioculcas Zamiifolia* Leaves Extract and Using Its Against Bacterial Pathogens

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ABSTRACT

This study discusses green synthesis of silver nanoparticle (AgNP) mediated with an aqueous solvent of Zamioculcas zamiifolia leaves. Silver was chosen because of its unique physicochemical characteristics; thus it is very accommodative to biosynthesis. The fixed ratio of the plant extract to silver ions was used and the visual sign of nanoparticle formation was the unusual color change. UVFirst, visible spectrophotometric analysis was used to obtain the optimum conditions of the synthesis. Fourier-transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), transmission electron microscopy (TEM) and scanning electron microscopy (SEM) were used to study structural and morphological properties of the resulting AgNPs. The sizes of synthesized nanoparticles were 23-65 nm. Their antibacterial activity was determined by the well diffusion method which showed that they have notable inhibitory effects against Acinetobacter baumannii and Escherichia coli.

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INTRODUCTION

Nanoparticles represent the foundational elements of numerous nanotechnology-based innovations. Together with nanostructured materials, they are gaining significant attention in scientific research, development sectors, and even in daily consumer products due to the rising commercial availability of nanomaterial-based goods. Nanotechnology focuses on materials whose structural features are within the nanometer scale (typically less than 100 nm), a range at which materials exhibit unique physical and chemical behaviors compared to their bulk counterparts or isolated atoms and molecules [1]

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The nanoscale dimension plays a pivotal role in altering the properties of substances, leading to transformative applications that are already being realized in some areas. Two primary driving forces behind nanotechnology advancement are: first, the fact that most biological molecules and structures naturally exist at the nanoscale; and second, the potential this scale offers for studying and manipulating biological systems and their interactions with materials [2]. Additionally, the relentless push for miniaturization in the semiconductor industry has also propelled progress into the nanoscale domain.

Nanomaterials in nanotechnology include

individual nanoparticles, aggregated forms, thin films with nanoscale thickness, and nanocomposites that integrate components with nanoscale dimensions [3]. The transformation in physical, chemical, and biological behavior of nanoparticles compared to both atomic-scale and bulk materials is significant [4]. These nanoparticles can assume various morphologies, such as spheres, rods, tubes, or platelets [5], and their surfaces can be chemically modified to fit specific application needs. This structural and chemical versatility arises from their diverse shapes, compositions, and morphological feature [6].

Among the prominent examples of nanoparticles, silver nanoparticles stand out for their widespread utility. A significant area of ongoing research focuses on developing standardized production techniques for silver nanoparticles [7]. Their exceptional optical, thermal, and electrical characteristics make them suitable for applications in photovoltaics, sensors (biological and chemical), conductive inks, and fillers due to their high conductivity. Moreover, their optical traits make them particularly useful in molecular diagnostics. An important application includes their role as antimicrobial agents in coatings, with their integration in textiles, wound dressings, and medical devices enabling the sustained release of silver ions to provide long-term microbial protection [8].

MATERIALS AND METHODS

Materials

The silver nitrate ($AgNO_3$) that was used was prepared at Sigma-Aldrich and it was used without purification. In the lab the deionized water was made. Any glassware that was utilized in the experiment procedures was well cleaned by dilute nitric acid (HNO_3) and then rinsed using distilled water and then dried in hot air oven. The extraction of the plant was done by boiling 3.0 grams of the *Zamioculcas zamiifolia* leaves in the water of 15 minutes. The resultant mixture was stored in a filter and the filtrate volume was made to read 100 mL to get the final leaf extract as shown in Fig. 1. Natural reducing agent filtrate Additionally, this filtrate was kept in the dark in cold 8 0 C within seven days. Preparation of a 10 M solution of the silver nitrate under study was made through the dissolution of 0.34 grams of silver nitrate into 100 mL of demineralized water.

Green approaches of Ag NPs

Using a 1 mL *Zamioculcas zamiifolia* leaf extract with 2 mL of a silver nitrate stock solution prepared

Fig. 1 *Zamioculcas Zamiifolia* leaves and leaves extract.

earlier, silver nanoparticles were produced. The mixture volume was then adjusted to 20 mL with demineralized water. This combination was mixed very well and heated at 70 o C in order to take 30 minutes. The appropriate reaction solution was subjected to 24 hours of the dark to aid in full reduction of silver ions, after this, a thermal treatment ensued. Signs of development of reddish brown color, as presented in Fig. 2, contributed towards visual evidence of the generation of elemental silver (Ag0) nanoparticles. Various parameters affected the process of synthesis such as the concentration of the plant extract and silver nitrate, pH, temperature, and time taken to carry out the reaction. After synthesis, dehydration in order to isolate the silver nanoparticles was carried out and then they were further characterized [9].

AgNPs used as Anti- microbial activity

The antibacterial activity of synthesized silver nanoparticle (AgNPs) prepared by WDM technique was examined against Escherichia coli and Acinetobacter species by using a well diffusion method (WDM). It was able to produce bacterial cultures through the process of spread plate and it was subculture of microorganisms through nutrient broth. These cultures were incubated at 37oc temperature, 24 hours as revealed in Fig. 3. The testing medium was a Mueller-Hinton agar plate that has been inoculated previously with target bacterial strains. On each agar plate, sterile paper discs of 5 mm in diameter were mounted to obtain the surface. One disc would be loaded with naked silver nanoparitcles and the other impregnated with solution of *Zamioculcas zamiifolia* plant

Fig. 2. AgNPs have been prepared by the reduction of aqueous solution $AgNO_3$ using *Zamioculcas Zamiifolia* leaves extract.

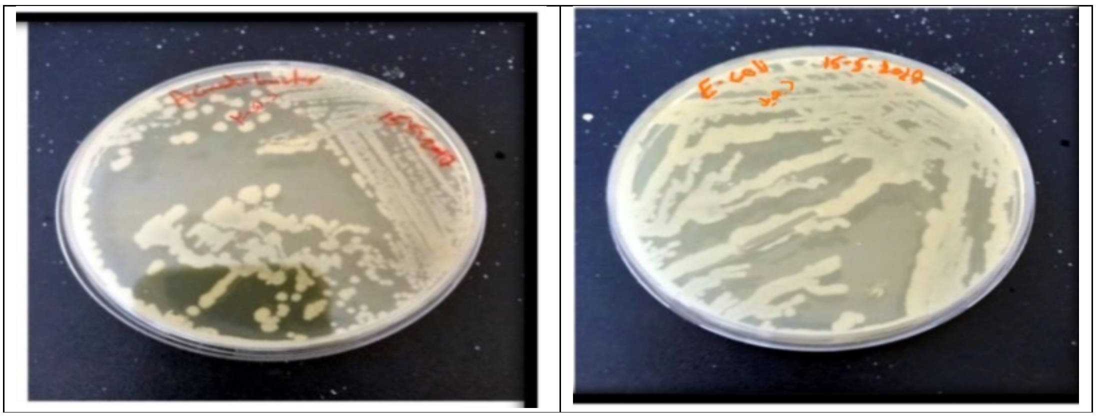

Fig. 3. *E.coli* and Acinetobacrer agar.

extract to act as the control. The plates were incubated again under 37 C for another 24-hour period. The measure of zones of inhibition around the discs was taken to determine the antimicrobial actions of the silver nanoparticles [10, 11].

RESULTS AND DISCUSSION

Optimum conditions study

Effect of concentrations of Zamioculcas Zamiifolia leaf extract

To observe the formation of silver nanoparticles, UV-vis absorption spectra were recorded in room temperature using a Shimadzu UV-1800 Spectrophotometer. The formed nanoparticles maximum absorption wavelength (lambda max) was determined. The UV-vis frequencies of silver nitrate solution of a constant concentration (10-3 M) with different proportions of *Zamioculcas zamiifolia* extract in solution was monitored after 24 hours of incubation at the room temperature as shown in Fig. 4. It was noted that the color changed gradually with an intensity to that of yellow and then reddish-brown showing that the silver nanoparticles were being formed in the solution. This color change is explained to be due to the excitation of surface plasmon resonance (SPR) connected with formation of nanoparticles [12]. SPRs of various concentrations of the plant extract were found between 410-442 nm. The peak sharpness and blue shift characteristic at 426 nm indicate the reduction of average nanoparticle size and the generation of spherical type nanoparticle with improved size distribution [13,14]. On the analysis of the results, it was found that a higher concentration extract (2 mL) was highly effective in high-efficiency production of silver nanoparticle.

Effect of silver nitrate concentration

The synthesis of silver nanoparticles (AgNPs) was examined by UV-visible spectroscopy after 24 hours, as shown in Fig. 5. The influence of different concentrations of silver ions in the nanoparticle was investigated in the experiment, keeping the volume of *Zamioculcas zamiifolia* Leaf extract constant (2 mL). When the extract was added to solutions of silver nitrate of 0.5 to 3.0 mL concentration, there was a clear change in color (Fig. 2) of which indicates that silver ions reduction to elemental silver occurred. The typical surface plasmon resonance (SPR) peaks were observed in the ranges of 420 to 437 nm in the corresponding UVvis absorption spectra. At an increased concentration of silver ions 2.5 mL, it was found there is a more distinct absorption peak but with a slight blue shift of about 430 nm. This ghost-like activity is an indication that high concentrations of silver ions accelerate nucleation process and increase the formation of larger silver

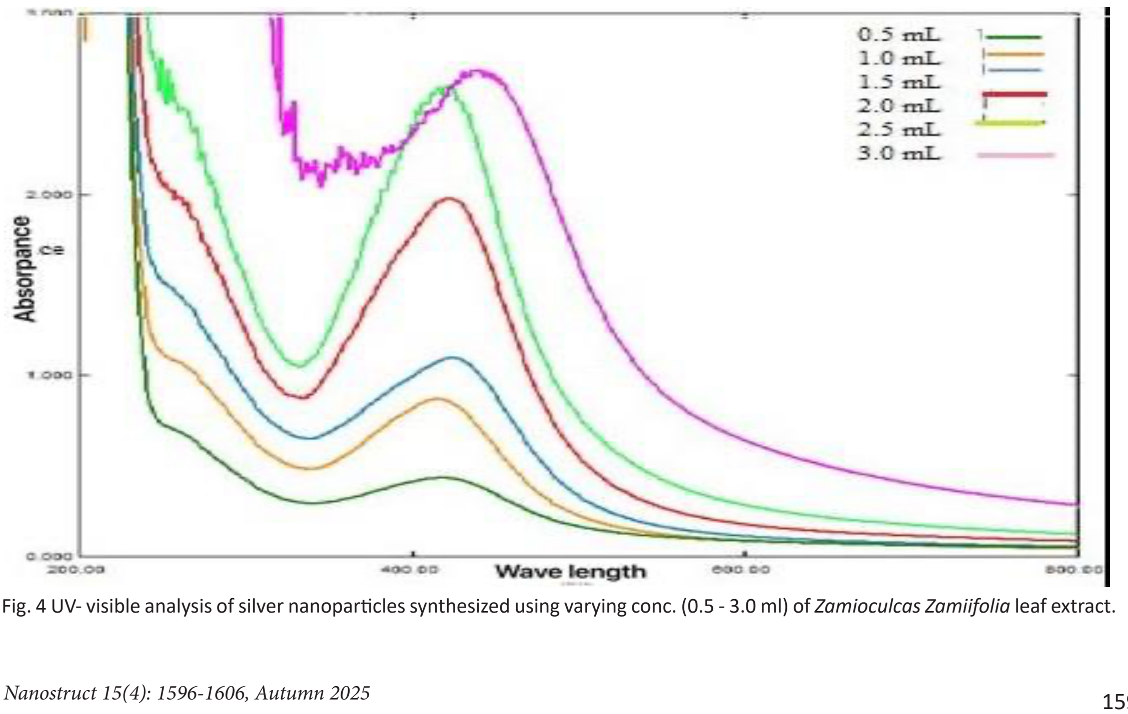

Fig. 4 UV- visible analysis of silver nanoparticles synthesized using varying conc. (0.5 - 3.0 ml) of *Zamioculcas Zamiifolia* leaf extract.

nanoparticles [15,16].

Effect of pH

The size and shape of silver nanoparticles (AgNPs) depends heavily upon the pH of the reaction medium. The major way that pH achieves this is by changing the surface charge of the biomolecules that is the direct link with its power to stabilize and cap the nanoparticles and thus regulate their nucleation and expansion. The effect of pH was investigated within the range of values (1.48, 2.10, 5.21, 7.00, and 9.33) as shown in Fig. 6. At room temperature, the pH was specially balanced using 0.1 M of H_3PO_4 and Na O H. The findings indicated that absorbance was higher at pH (1.48-7.00) and decreased at 23.00 and 25.00 in higher pH. The highest absorbance value and a significant blue shift in UV - visible spectrum of the sample prepared at pH 7.0 was at around 431 nm pointing to the best conditions of the AgNP formation at neutral pH [17,18].

Effect of temperature

The relevance of temperature during the synthesis of the silver nanoparticles (AgNPs) can be considered as significant regarding both the kinetics of the reduction and the characteristics of obtained particles. The UV visual absorption spectrum of the synthesis of AgNPs by the *Zamioculcas zamiifolia* extract at various temperatures, 30 o C, 40 o C, 50 o C and 60 o C, is illustrated in Fig. 7. The absorption maxima occurred at the wavelengths of 439-440 nm and there was a steep rise of the absorbance quantity with increasing temperature. The increase in absorbance is explained by a higher rate of reduction of silver ion in higher temperature thus one is supposedly able to have more efficient and uniform nucleation and atomization of the nanoparticles [19].

Effect of contact time

UV-vis spectroscopy was used to test the influence of contact time on the process of developing silver nanoparticles (AgNPs) by the *Zamioculcas zamiifolia* leaf extract. Fig. 8 shows how spectra were obtained in the range (231-445 nm). Measuring higher absorbance intensities with longer contact times showed that an ongoing formation of nanoparticles took place.

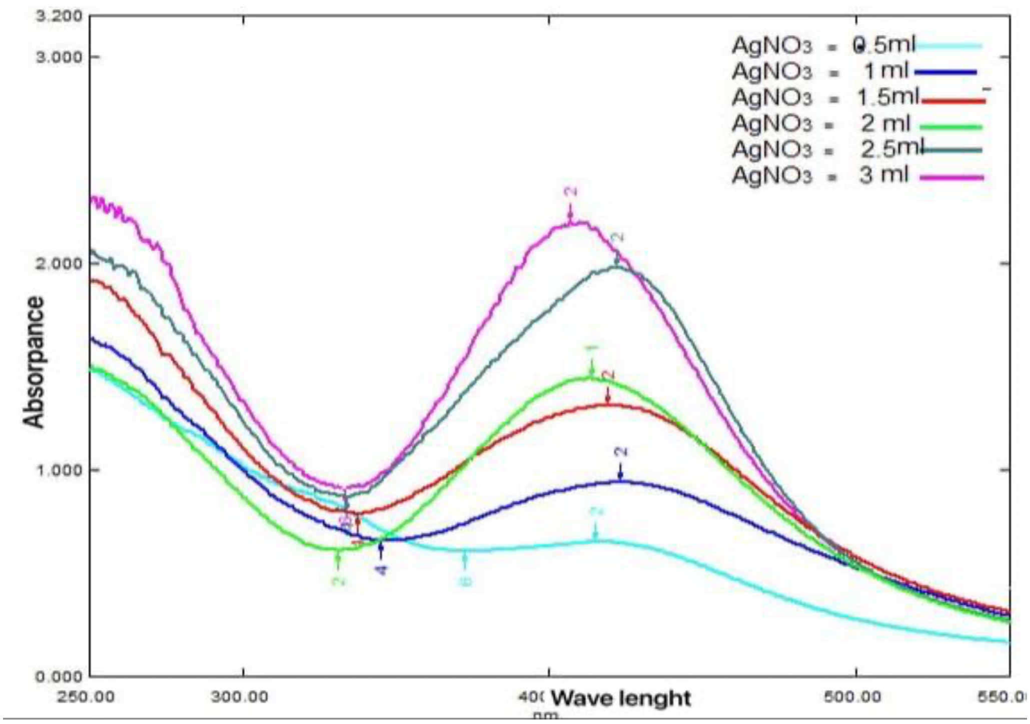

Fig. 5 UV-vis spectra of AgNPs prepared using a different amount of $AgNO_3$ (0.5, 1.0 and 3.0 mL) with the absorbance increment and SPR peak shift after increases in silver ion concentration.

Interestingly, when the reaction was ready and a sharp absorption peak started to develop at one hour and grew in intensity until 24 hours. A blue shift in this progression was observed as well as a formation of surface plasmon resonance (SPR) band indicating successful formation of spherical AgNPs [20].

Fourier transform infrared spectroscopy (FTIR)

The functional groups incorporated in the *Zamioculcas zamiifolia* leaf extract were identified using Fourier-transform infrared (FTIR) spectroscopy since it is believed that these functional groups help to reduce the silver ions (Ag^+) present and thus stabilize the consequent synthesis of silver nanoparticles (AgNPs). The FTIR spectra of the pure plant extract (A) and the synthesized AgNPs (B) is represented in Fig. 9. Comparison of the two spectra indicated that the peaks of characteristics of amine groups, initially

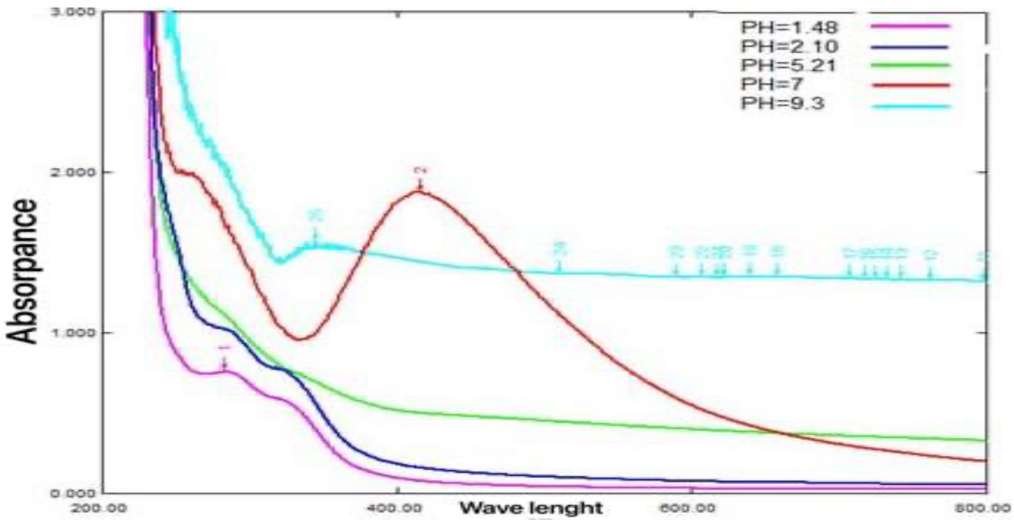

Fig. 6 UV-visible analysis of silver nanoparticles at varying value of pH.

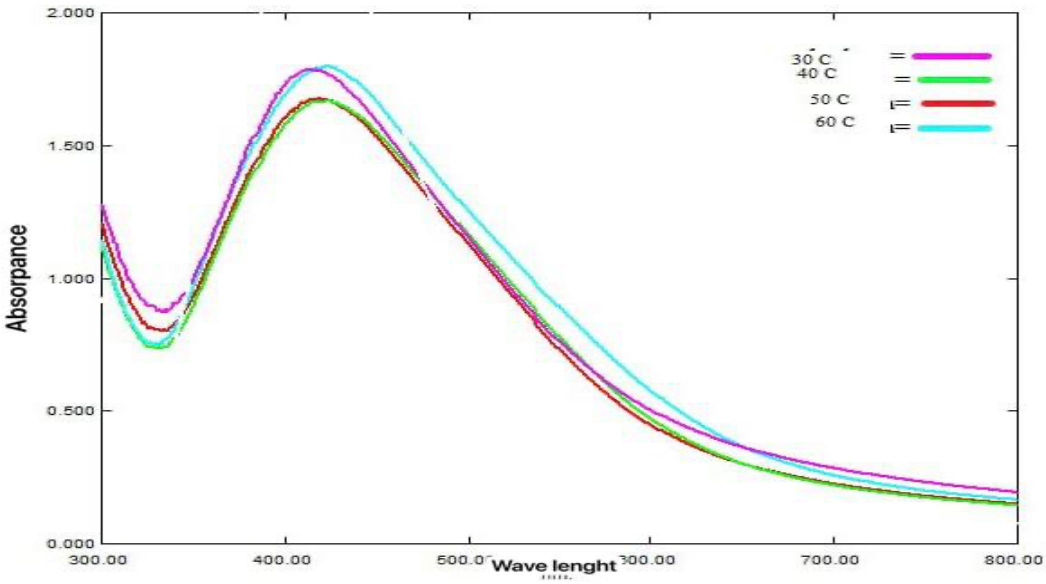

Fig. 7 UV-visible analysis of silver nanoparticles at varying Temperature.

seen as 3446 cm-1 and 3375 cm-1, changed and read 3406 cm-1 and 3361 cm-1 respectively after production of the nanoparticles. Also, there was a slight shift of carbonyl stretching band at 1610 cm -1 to 1606 cm -1, and the intensity of the peak was also changed. There was also the shift of peaks at 1051 cm 1 and 1089 cm 1 to 1068 cm 1, which was probably due to the ether, alcohol or ester contents. Such spectral modifications imply the positive role of hydroxyl, carboxylate, and amide functional groups in the chemical reduction and capping of Ag^+ ions, the development of AgNPs and their stabilization [21,22].

X-Ray diffraction (XRD)

The X-ray diffraction (XRD) pattern exhibits prominent peak broadening, a characteristic feature indicative of nanoparticle formation. The crystallite size of the synthesized silver nanoparticles can be estimated using the Debye–Scherrer equation [23,24].

$$D = k\lambda/(\beta \cos\Theta)$$

The synthesized silver nanoparticles were estimated using Scherrer equation to calculate the average crystallite size of the synthesized particles. This has to do with the X-ray diffraction (XRD) measurements of the width of the XRD peak with particle size. In these units, D is the average crystallite size (or the diameter of the nanoparticle), β is the full width half maximum (FWHM) of the diffraction peak measured in radians, θ is the Bragg diffraction angle and λ is the wavelength of the X-ray source. The peaks in the diffraction pattern are broad and this is mainly due to the nanometer scale size of the particles, the scherrer formula gives a good method of estimating the size of the particle using the XRD pattern [25].

Fig. 10 represents the X-ray diffraction (XRD) spectrum of the synthesized silver nanoparticles (AgNPs). The significant diffraction lines 38.30, 44.46 and 77.50 degree can be identified to (111), (200) and (311) crystallographic planes characteristic of metallic silver, in face-centered cubic (FCC) crystallographic structure. The crystallite of the AgNPs was estimated to be about 17.8 nm, therefore, confirming that the AgNPs are nanocrystalline in nature, using the Scherrer equation [26,27].

Scanning Electron Microscopy (SEM)

Scanning Electron Microscopy (SEM) was used in analysis of morphology, size, and distribution of the biosynthesized silver nanoparticles. Fig. 11 shows that the particles were very spherical in shape with a diameter between about 35-75 nm.

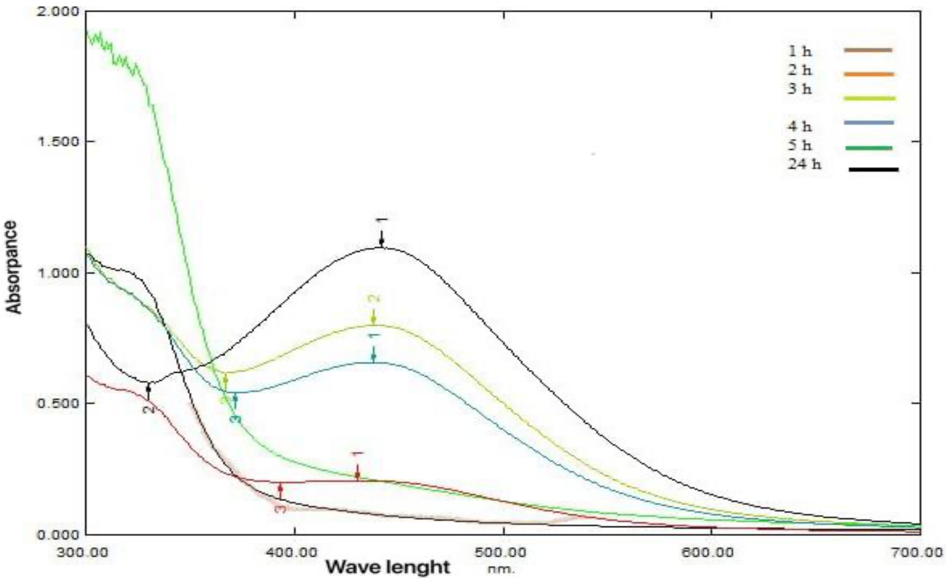

Fig. 8 UV-visible analysis of silver nanoparticles at varying contact time.

Although a great part of the nanoparticle was well-dispersed, there was some form of aggregation that led to larger clusters. Such agglomeration is probably caused by a combination of the nanoparticles with phytochemical components of the plant extract, which are natural surface-capping additions [28,29].

The silver nanoparticles (AgNPs) were also

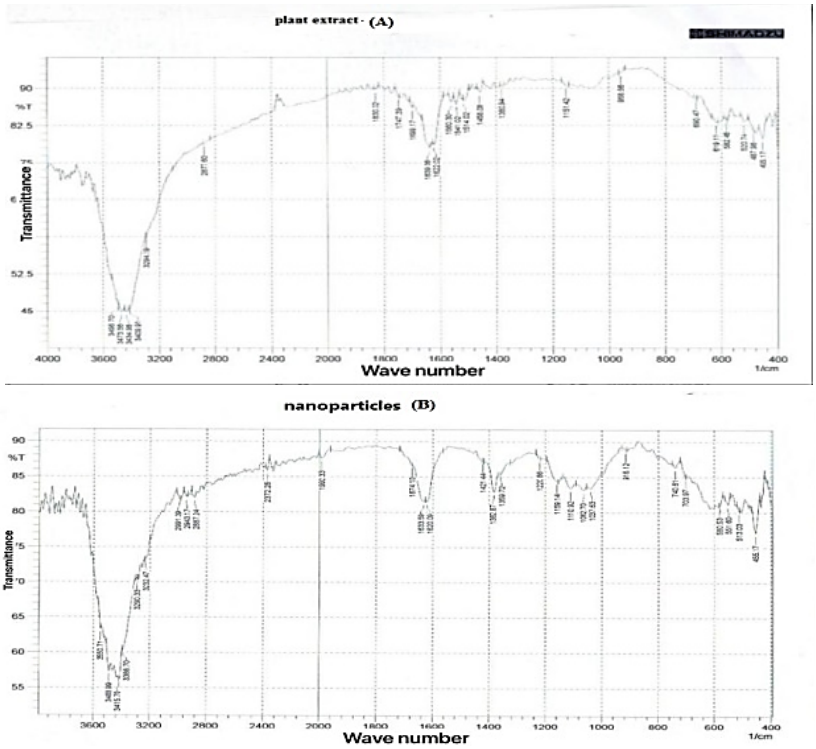

Fig. 9 Spectral analysis FTIR silver nanoparticles coated with *Zamioculcas zamiifolia* leaf extract.

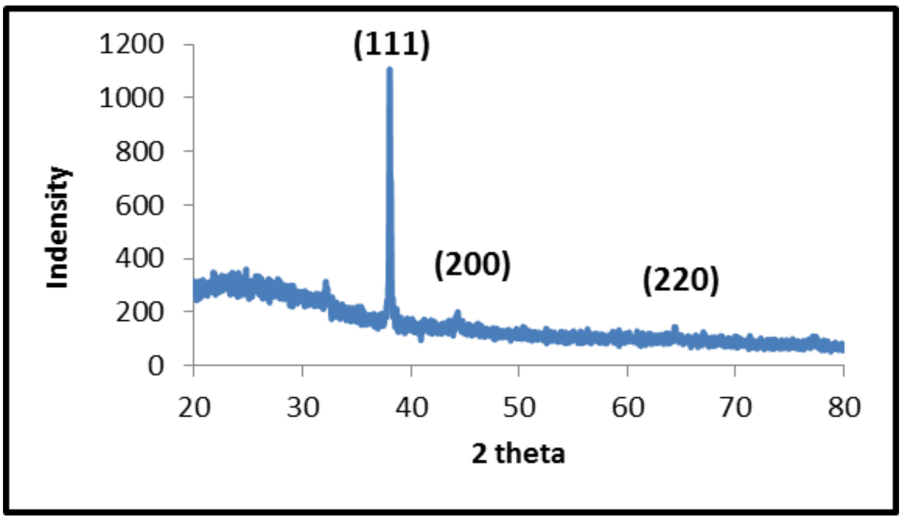

Fig. 10 X-ray diffraction pattern of silver nanoparticles synthesized using *Zamioculcas Zamiifolia* leaf extract.

characterized at Transmission Electron Microscopy (TEM) after synthesized using *Zamioculcas zamiifolia* leaf extract. According to Fig. 12, in TEM images, mainly spherical nanoparticles could be observed in sizes between 17 and 46 nm. This was done using high resolutions methods, where it reported structural, size-distribution information, which supported the homogeneous morphology and nanometer-sized dimensions of the biosynthesized AgNPs [30].

Anti-bacterial assay

The antibacterial efficiency of the synthesized silver nanoparticles was tested against Gram-negative bacteria such as, Escherichia coli and Acinetobacter and zones of inhibition evaluated (Fig. 13 and Table 1). The methodology of the experiment described the testing of the AgNPs

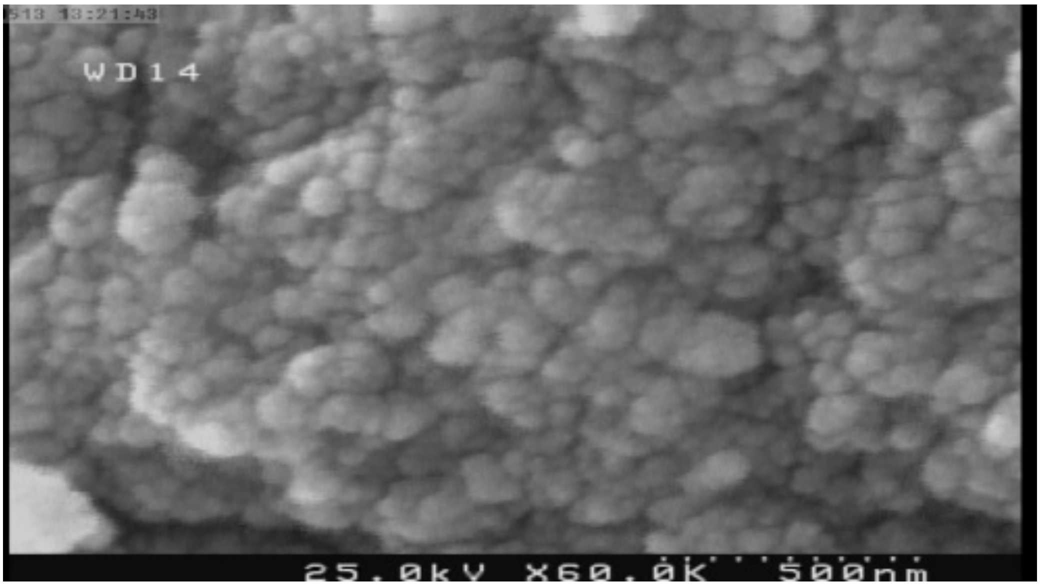

Fig. 11 SEM image of silver nanoparticles synthesized using *Zamioculcas Zamiifolia* leaf extract.

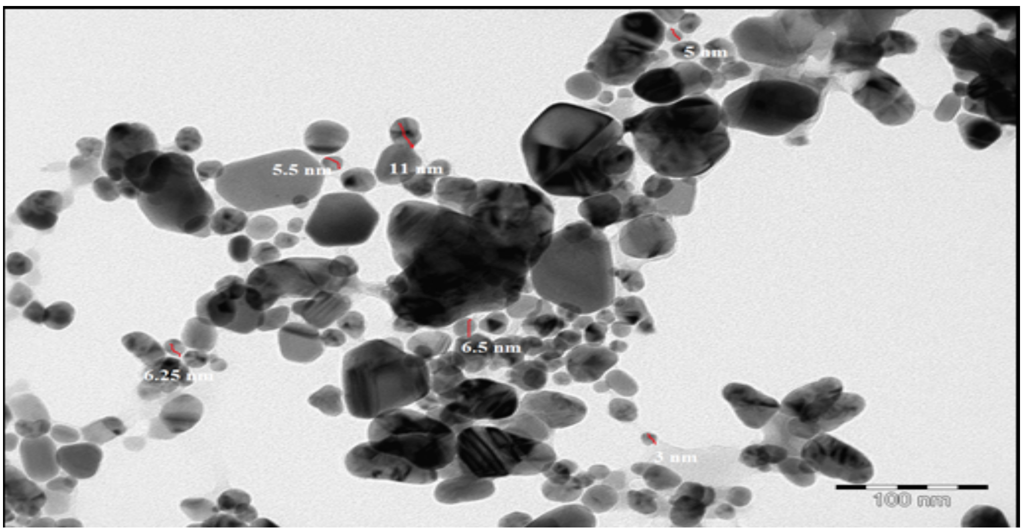

Fig. 12 TEM image of silver nanoparticles synthesized using *Zamioculcas Zamiifolia* leaf extract.

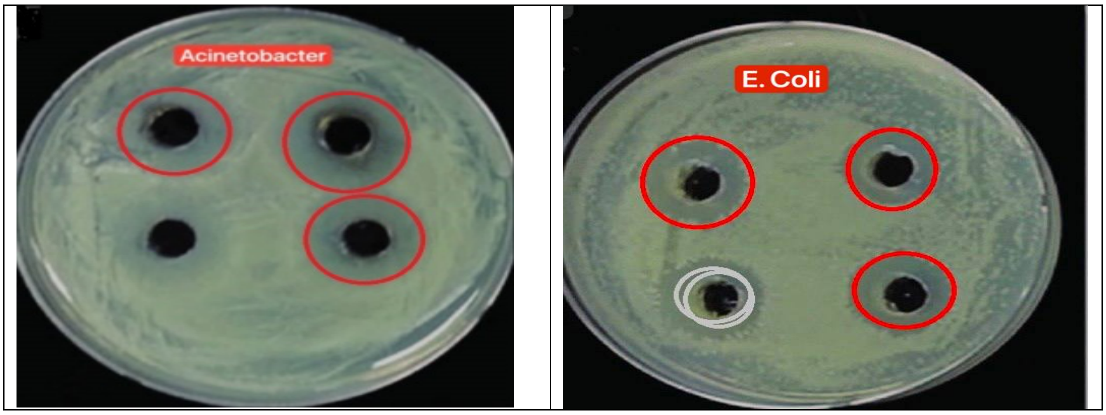

Fig. 13Antibacterial activity *Acinetobacter* and *E. coli.*

Table 1: Inhibition Area (mm).

Name of the organism	Silver nanoparticles 1.5 ml	Silver nanoparticles 3.5 ml	Silver nanoparticles 5.5 ml	*Zamioculcas Zamiifolia* leaf extract.
Acinetobacter	5.5 mm	7. 4 mm	11 mm	0 mm
E.coli	8.9 mm	9.2 mm	13.4 mm	0 mm

together with the plant extract. The findings revealed that the silver nanoparticles had high antibacterial activities on both the *E. coli* and Acinetobacter although, antibacterial activities were dependent on the concentration of the silver nanoparticles used. All this can be attributed to the fact that such findings are in line with other research conducted previously, which indicated that the antibacterial mechanism of AgNPs implicates its interaction with the cell walls of bacteria-especially the sulfur containing proteins-breaking its respiratory processes and ultimately killing the bacteria cells [31-33].

CONCLUSION

Ag NPs have effectively been synthesized utilizing the leaf extract of *Zamioculcas zamiifolia* as both a reducing and capping agent in the present study. The AgNPs biosynthesized were found to have good effect in killing the pathogenic bacteria E. coli and Acinetobacter. Different parameters of the synthesis process such as extract concentration, pH and reaction conditions were optimized in order to achieve a controlled size of the average size of the AgNPs. Out of them, pH 7 turned out to be the most generous, probably because of the increased activity of the bioactive compounds contained within the plant extract.

UV-Visible spectroscopy, FTIR, AFM, SEM and TEM were the various analytical techniques to characterize the synthesized silver nanoparticle. The green synthesis technique formulated in this study has several merits that make it better in comparison to the other traditional techniques that include advanced safety, low-cost, environmental-friendly, and non-toxicity elements.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

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