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Evaluation of *In vitro* antibacterial activity *Vibrio cholera* using silver nanoparticles synthesized from banana peel extract

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ABSTRACT

Recent advances in nanotechnology and the synthesis of nanoparticles through biosynthesis have increased the urge in scientists than for chemical or physical methods. The biosynthesis method is the most significant method than a conventional method because of its eco-friendly, low cost and rapid synthesizing process. The present study describes the antibacterial activity of silver nanoparticles (AgNPs) synthesized from banana peel extracts. The synthesis of AgNPs was confirmed by color change from light yellow to brown color. Further, the morphology of the biosynthesized nanoparticles, average size and presence of functional groups were characterized by UV – Visible spectroscopy (UV-Vis), X-Ray diffraction (XRD) and Fourier transform infrared spectroscopy, respectively. The UV spectra results show a strong resonance center and surface of silver nanoparticles at 450 nm. XRD studies revealed that the synthesized AgNPs show crystalline in shape. The FT-IR spectrum described the biological molecules which stabilize and form the silver nanoparticles in the aqueous medium. The antimicrobial property of AgNPs was tested against Vibrio cholerae, which showed maximum zones of inhibition of 32 mm at a concentration of 100 µL. Therefore, the biosynthesized AgNPs proved to have significant antibacterial activity.

Keywords: Silver nanoparticles, XRD, TEM, antimicrobial, Banana peel.

Introduction

Numerous scientific applications have been significantly impacted by the science of nanotechnology in recent decades. Nanoparticles, which are smaller particles with tens or hundreds of atoms and range in size from 1 to 100 nm, are the subject of nanotechnology (Madkour, 2019). Particular characteristics of nanoparticles (NPs) include their high reactivity, ultra-small size, and enormous surface area to mass ratio, which demonstrate a variety of altered behaviors (Khan et al., 2019; Jamkhande et al., 2019). Due to metal nanoparticles' numerous innovative uses in catalysis (Astruc, 2020), medicine (Rudramurthy and Swamy, 2018), pharmaceuticals (Mitchell et al., 2021), and agriculture (Alomar et al., 2020), a great deal of study has been conducted. Numerous types of nanoparticles, including solid, polymeric, inorganic/ metal, nanotube, and nanocrystal.

Metal nanoparticles can be synthesized using a variety of physical, chemical, and biological techniques, as shown in various literature. The physical approach for creating nanoparticles is top-down and involves reducing the size of the material using a variety of physical techniques, such as electrochemical methods (George et al., 2018). In comparison to physical and chemical approaches, which have caused numerous environmental stresses due to their toxic metabolites and rigorous procedures, the biological method is more efficient for the synthesis of nanoparticles with the advantages of fewer failure chances, eco-friendliness, cost-effectiveness, and ease of characterization (Gour and Jain, 2019; Kanchi and

Ahmed, 2018). Green synthesis uses plant extracts (El Shafey, 2020), vitamins (Hou et al., 2020), and amino acids (Xue et al., 2020).

Three stages are involved in the synthesis of nanoparticles from plant extracts: 1) the activation phase, which involves the reduction of metal ions and the nucleation of the reduced metal atoms; 2) the growth phase, which involves the spontaneous coalescence of small adjacent nanoparticles into larger particles; and 3) the termination phase, which determines the final shape of the nanoparticles (Punjabi et al., 2015). Terpenoids, flavonoids, polyphenols, sugars, alkaloids, phenolic acids, and proteins are among the many metabolites that have collected in the plant and are crucial to the bioreduction of metal ions, which produces nanoparticles (Makarov et al., 2014). Because of their potential for use in biological applications such as medicine, biomolecular detection and diagnostics, medication administration, food production, and agriculture, silver nanoparticles (AgNPs) have drawn particular attention among other NPs. According to recent research, AgNPs can effectively interact with a variety of pathogens and manage a number of plant diseases when compared to other compounds (Lamsal et al., 2011). The green synthesis of silver nanoparticles has drawn a lot of interest from young researchers in the current situation because of their distinct physicochemical characteristics and numerous uses. The plant extract (flower, leaf, root, etc.) used to create AgNPs is safe to handle, easy to approach, and has strong stability and antibacterial activity (Sadeghi and Gholamhoseinpoor, 2015; Kathiravan et al., 2015).

Thus, the goal of this study is to use a banana peel extract to create silver nanoparticles biologically. Fourier Transform InfraRed spectroscopy, X-Ray Diffraction, and UV-Visible spectroscopy were also used to investigate the produced nanoparticles' characteristics. Additionally, possible antibacterial efficacy against Vibrio cholerae's harmful gram-negative bacteria was examined.

Many fruits' peels have drawn interest as a natural source of antioxidants and phytochemicals, which are abundant in substances that have the ability to scavenge free radicals. Peels from bananas and plantains are important agricultural wastes that have been utilized for a variety of purposes, including medicine, animal feed, leather blackening, soap production, rubber fillers, and more (Arawande and Komolafe 2010). According to Someya et al. (2002), banana peel, an underappreciated source of phenolic compounds, is a good source of antioxidants for meals and functional foods that help prevent heart disease and cancer. Numerous antioxidant chemicals, including gallocatechin (Someya et al. 2002) and dopamine (Kanazawa and Sakakibara 2000), are found in the fruit's peel. The literature discusses a number of uses for banana peels, including: (1) using them for their medicinal qualities (Parmar and Kar 2008), (2) using them in the fermentation of ethanol (Tewari et al. 1986), (3) using them as a substrate to produce fungal biomass (Essien et al. 2005), (4) using them to make laccase (Osma et al. 2007), and (5) using them as a biosorbent to remove heavy metals (Annadurai et al. 2003). We reasoned that banana peels, which are naturally abundant in polymers like lignin, hemicellulose, and pectins (Happi Emaga et al. 2007), might be utilized to create silver nanoparticles based on the literature that is currently available.

Materials and Methods

Preparation of banana peel extract: Banana peel was repeatedly washed with fresh water to remove dust and soil particles and then dried to remove moisture content by direct sun rays. Dried leaves were grained with the help of a mixer grinder mechanically to make a fine powder. In 250 mL of the conical flask, 5 g of powdered banana peel were taken and added 100 mL of distilled water. The aqueous solution was mixed using a magnetic stirrer and then boiled for 10 min using a heating mantle. The leaf extract was filtered by a Whatman (No.1) filter paper and collected the filtrate, stored at 4°C for the biosynthesis of silver nanoparticles

Biosynthesis of silver nanoparticles: For the synthesis of AgNPs, 1 mM of silver nitrate solution was prepared and stored in an amber bottle. About 50 mL of 1 mM AgNO3 were added drop wise with constant stirring in 5 mL of banana peel extract taken in a conical flask. The aqueous solution was incubated at room temperature for 24 h in dark conditions to prevent photochemical reactions. The obtained solution was kept in an orbital shaker overnight to ensure the synthesis of silver nanoparticles. The light-yellow color of the banana peel extract was changed to dark brown which indicates that the silver nanoparticles were formed from the peels. The obtained mixture was added to deionized water and centrifuged (repeat three times) at 1000 rpm for 10 minutes to isolate pure AgNPs free from other bioorganic compounds. The synthesized NPs were kept in a hot air oven at 50°C for 3 hrs to get the powder form of AgNPs and then stored for further analysis (Lekshmanaswamy and

Anusiyadevi, 2020). The biosynthesis of silver nanoparticles by this method was optimized with different parameters such as pH, experiment was done in the CAC center / Baghdad

Characterization of synthesized silver nanoparticles: Characterization was performed using a variety of analytical techniques including: The X-ray diffraction (XRD) was used to determine phase identification, crystals structure, composition and physical properties of the synthesized materials silver nanoparticles (AgNPs) (Shwetha et al., 2020). The AFM was also used to determine surface topography of the silver nanoparticles by SPM-AA300 of angstrom advanced Inc. USA, using AFM contact mode (14). Transmission Electron Microscopy (TEM) analysis was performed to investigate the size, shape, and morphology (Srikar et al., 2016)., the experiments was done in the CAC center / Baghdad

In vitro inhibitory activity of silver nanoparticles against pathogenic bacteria (*Vibrio cholera*):

The antibacterial studies of biosynthesized silver nanoparticles were tested by a well diffusion method (Holder and Boyce, 1994; Gudikandula and Charya Maringanti, 2016) for the determination of antibacterial activity against pathogenic bacteria namely Vibrio cholerae. The selected pathogenic gram-negative bacterial strains were procured from Microbial Type Culture Collection and Genbank. About 25 mL of molten Mueller-Hinton agar was poured into a Petri plate. The plates were allowed to solidify, after which 18 h grown (OD adjusted to 0.6) 100 µL of microorganisms were transferred onto the plate and made culture lawn by using a sterile L-rod spreader. After five minutes of setting of the pathogenic samples, a sterile cork borer was used to make 6 mm in size (well) are made on prepared plates. The synthesized silver nanoparticles were loaded into wells with various concentration of 25, 50, 75 and 100 µL. The solvent saline loaded served as negative control and azithromycin (30 µL/well) served as a positive control. The antibacterial activity was determined by measuring the diameter of the inhibition zones were measured in millimeter (mm) around using an antibiotic zone scale. The plates were incubated at 37°C in an incubator for 24 hrs to examine the zone of inhibition. All the experiments were carried out in triplicates and the results were expressed as mean ± standard deviations.

Results and Discussion

Biosynthesis of silver nanoparticles: This study's biosynthesis of silver nanoparticles from banana peel extract demonstrated that the plant's renewable and ecologically friendly source may be utilized as a stabilizing and reducing agent to effectively produce AgNPs. This is the first test to generate AgNPs using banana peel. Figure 1 depicts the general schematic illustration of the AgNP biosynthesis process.

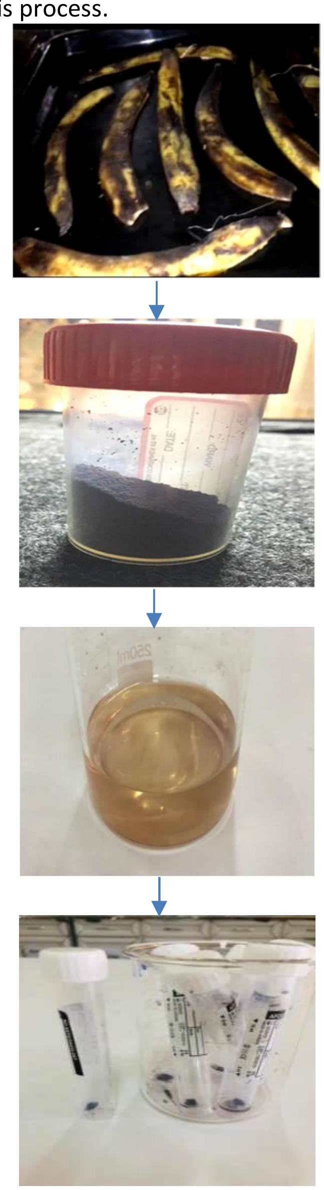

Figure (1): The overall schematic representation of the biosynthesize process for AgNPs.

The presence of silver nanoparticles was confirmed by mixing the extracts from banana peels with the aqueous solution of $AgNO_3$ at room temperature and then letting them sit for the entire night. The AgNO3 and banana peel extract solution changes color during the synthesis process, going from light yellow to orange to brown to dark brown (Figure 1). The reaction between the leaf extract and the silver nitrate solution, where the reductant from the plant extract directly converted Ag+ to Ag atoms (Ag0), is indicated by the obvious color change that was evident to the unaided eye. The production of silver nanoparticles, which is caused by the activation of surface plasmon resonance and characteristic of AgNPs, was tentatively validated by these color changes (Lekeshmanaswamy and Anusiyadevi, 2020; Mathew et al., 2020).

The reductive actions of banana peel may be attributed due to the presence of various phytochemicals and bioactive compounds such as flavonoids, coumarins, sterols, terpenoids, quinones and alkaloids (El-Ghonemy et al., 2019). Hence, the specific species of the Arecaceae family has a certain medicinal property like anticancer, antioxidant and hypolipidemic activity (Chiduruppa et al., 2018). Moreover, the hydroxyl groups present in biomolecules are responsible for the bioreduction of Ag+ ions as shown in the proposed mechanism. Flavonoids contain various functional groups, which have an enhanced ability reduce metal ions through the production of reactive hydrogen atom due to tautomeric transitions. During this transition, the enol-from is converted to keto-form, the process ensured by the reduction of metal ions into metal NPs (Singh et al., 2018). The bioreduction mechanism for the silver nanoparticles made from Phoenix dactylidera was disclosed by Zafar and Zafar (2019). Stabilization in an aqueous solution is made easier by proteins' potent capacity to form bonds with metal ions and their ability to surround the nanoparticles to prevent agglomeration. Both the capping and reducing mechanisms are essential to the synthesis of AgNPs (Ajitha et al., 2015). The color shift from pale yellow to dark brown, which resulted from the collective oscillation of free conduction electrons leading to surface plasmon resonance (SPR), served as the first visual confirmation of the NPs. The verified AgNPs were then admitted for antibacterial testing and characterization.

UV-Visible absorption studies: One of the most important investigations for determining the main appearance of metal nanoparticles in the plant aqueous media is UV-visible spectroscopy. UV-visible spectroscopy was used to observe the phyto-reduction of silver ions in the silver aqueous solution as it reacted with the biomolecules found in the extracts of banana peels. They can be differentiated from other nanoparticles including semiconductor quantum dots, polymeric, and magnetic nanoparticles due to their noble metal NPs (plasmonic). AgNPs' optical absorption spectra are dominated by SPR, with a shift toward the brown or yellow end contingent on the dielectric medium, particle size, shape, and aggregation state. The solution's hue changed from colorless to yellow brown, confirming the synthesis of AgNPs.The absorption peak is clearly visible in Figure 3 and correlates to the surface plasmon resonance band detected at 421 nm. When employing other plant extracts for silver nanoparticles, the findings are comparable (Mathew et al., 2020; Lekshmanswamy and Anusiyadevi, 2020). The simultaneous oscillation of the metal nanoparticles' electrons in resonance with lightwaves results in free electrons that reflect the SPR absorption band (Elangovan et al., 2015). Consequently, the produced silver nanoparticles are confirmed by the high absorption peak detected at wavelength 421 nm.

Fourier transform infrared analysis: To determine the main functional groups in the plant extract and how the produced silver nanoparticles might be involved, FTIR measurements were performed (Babu and Prabu, 2011). The presence of distinct functional groups at different places in the FTIR spectrum (Figure 4) demonstrates the complex character of the bioactive compounds, which are effective as both stabilizers and reducing agents. The stretching vibrations of the strong O-H bond of the phenol group, the C-H stretching of aromatic groups, and the C=O for amide group were attributed to the bands that appeared at 3268, 2962, and 1625 cm-1 in Figure 4, respectively. The C-I and C-Br stretching of halo compounds are represented by the bands seen at 528, 551, 598, and 670 cm-1. C–N stretch vibrations are represented by the strong peak at 1392 cm-1. The ether linkages are responsible for the bands at 1064 cm-1, which indicate the existence of flavanones adsorbed on the nanoparticle's surface. The capping ligands of the NPs are caused by the weaker band at 1239 cm-1, which corresponds to the C-O-C stretch. The capping ligands' primary function is to stabilize the NPs in order to stop them from growing and clumping together. The C≡C stretching is represented by the band seen at 2361 cm-1. The presence of phenolic compounds, flavonoids, and antioxidants, which are all constrained by these stretching vibrations, may be the cause of the instant reduction and capping of silver ions into silver nanoparticles (Lekshmanswamy and Anusiyadevi, 2020; Kalishwaralal et al., 2010).

The production of metallic nanoparticles is aided by the functional groups (hydroxyls, carbonyls, and aldehyde) found in flavonoids, coumarins, sterols, terpenoids, quinones, and alkaloids (Sheny et al., 2011). Figure 2 depicted the comprehensive synthesis process. Therefore, by oxidizing the molecules' aldehydic groups to carboxylic acids, terpenoids can aid in the reduction of metal ions (Ovais et al., 2018). The leaf extract's covering agents can actively cover the nanoparticles throughout the procedure, preventing their aggregation and resulting in stabilization (Shah et al., 2015). The existence of all functional groups, which correlates to the bioreduction of Ag+ to Ag0 ions, was therefore verified by the FTIR pattern.

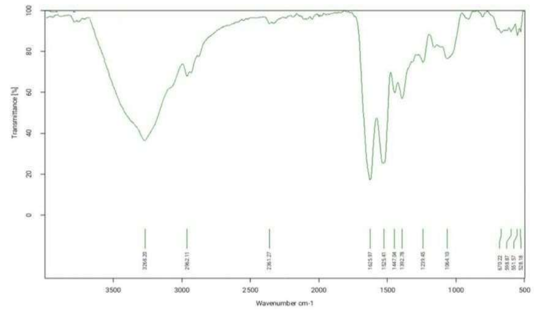

Figure (2): FTIR spectra of biosynthesized silver nanoparticles.

Atomic Force Microscope (AFM): Surface analysis (AFM) needs careful consideration due to variables like pollution that affect the results. AFM-SPM, which is displayed in table (1), was used to estimate the size of the silver nanoparticles. According to the results, AgNPs had an average size of 54 nm (Figure 3).

Table (1) Estimation size of AgNPs.

SPM Scanning Probe Microscope

Granularity Cumulation Distribution Report	
Sample: AA	**Code: Sample Code**
Line No.: lineno	**Grain No.:**241
Instrument: CSPM	**Date:**2021-10-06
Avg. Diameter:54.06 nm	**<=10% Diameter:0 nm**
<=50% Diameter:51.00 nm	**<=90% Diameter:60.00 nm**

Diameter (nm)<	Volume (%)	Cumulation (%)	Diameter (nm)<	Volume (%)	Cumulation (%)	Diameter (nm)<	Volume(%)	Cumulation (%)
46.00	14.48	14.48	56.00	23.70	65.54	66.00	13.77	100.00
51.00	29.37	42.84	61.00	23.70	88.23			

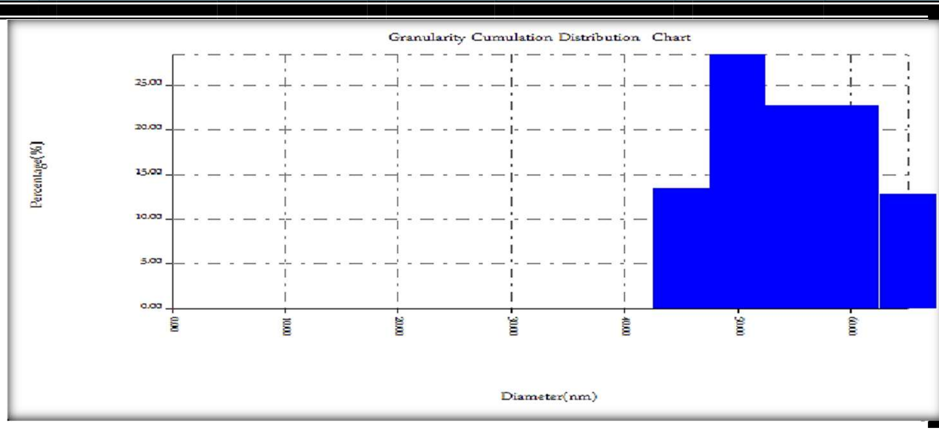

Figure (3): Average size of silver nanoparticles.

Transmission electron microscopy (TEM) analysis of silver nanoparticles (AgNPs): TEM is a useful instrument for examining the shape and size of nanoparticles. AgNPs' TEM images, as shown in figure (3), revealed dispersed spherical particles in a variety of sizes between 20 and 300 nm.

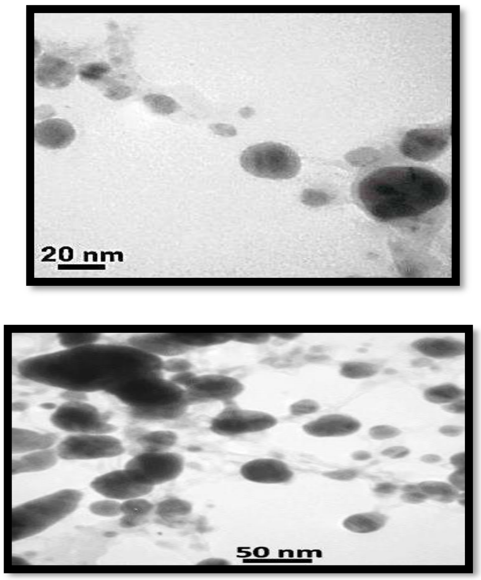

Figure (4): TEM image of silver nanoparticles prepared by banana peel extract.

Antibacterial studies of AgNPs: An agar well diffusion assay was used to investigate the biosynthesized silver nanoparticles' possible antibacterial efficacy against Vibrio cholerae, and the results are displayed in Figure 6. Agar wells are filled with several doses of aqueous silver nanoparticles (0, 25, 50, 75, and 100 µL), while a positive control of azithromycin is tested at a concentration of 30 µL/well. Figure 6 illustrates how the diameter of the inhibition zone progressively grew as the concentration of AgNPs for both harmful bacteria increased. Therefore, a dose-dependent analysis of the bactericidal trials has been conducted (Zafar and Zafar, 2019). When compared to the positive control, the azithromycin well's width almost closed at the designated concentration of the inhibitory zone. Table 2 lists all of the mean values for both samples as well as the results of the in vitro inhibitory activity of silver nanoparticles against harmful bacteria. As the concentration of AgNPs grew from 25 to 100 µL/well, the diameter of the inhibitory zone for the gram-negative bacteria of V. cholerae increased from 18 to 32 mm. At 100 µL/well, a maximal inhibition zone of 28 mm was noted for pathogenic *V. cholerae* strains. The observation results are almost identical to the 29 mm positive control values for V. cholerae that were obtained at 30 µL/well. The findings verify that when the concentration of nanoparticles against the bacterial strains increases, so does the inhibition zone.

Figure (5): Agar well diffusion assay of bactericidal activity against Vibrio cholera of synthesized AgNPs from banana peel extracted.

Against Vibrio cholerae, the current investigation demonstrated that the produced AgNPs exhibited the strongest antibacterial efficacy. The sample exhibited a wide range of antibacterial activity against Vibrio cholerae. Electrostatic attraction between the positively charged NPs and the negatively charged microbe cell membrane is most likely the source of antibacterial activity (Esmaielzadeh et al., 2015; Mushir et al., 2016). The primary causes of the zones of inhibition were the silver nanoparticles' depletion of intracellular ATP, the collapse of the plasma membrane, and the instability of the outer membrane (Morones et al., 2005). By releasing silver ions, it disrupts the replication of deoxyribonucleic acid, creates reactive oxygen species, and makes cell membranes more permeable. A moderate inhibitory zone against both pathogenic agents was observed at low AgNP concentrations. A distinct inhibitory zone was shown by the elevated AgNP concentration. As a result, as AgNP concentrations rise, so does the inhibition zone's diameter. Because Vibrio cholerae has a thin covering of peptidoglycan, this work demonstrated strong biocidal activity against the bacteria in green produced AgNPs (Bharathi et al., 2018).

Table (2): In-vitro inhibitory activity of silver nanoparticles against Vibrio

Bacterial Strains	Diameter of Zone of Inhibition (mm) of aqueous concentration of AgNPs			
	25 µL	50 µL	75 µL	100 µL
Vibrio cholerae	18±0.2	22±0.2	24±0.2	32±0.1

The phytocompounds that are responsive to the creation of Sterols, triterpenoids, glycosides, and flavanols are the alkaloids that make up silver nanoparticles (Dahibhate et al., 2020). Gram-negative bacteria are resistant to the effects of the studied extracts, according to the study's findings, and Lekeshmanaswamy and Anusiyadevi (2020) have reported this for the leaf extract of Perfularia daemia. Furthermore, compared to gram-positive bacteria, gram-negative bacteria are typically more resistant. This is because the cell wall of Gram-negative bacterial strains is impermeable to antimicrobial drugs due to the presence of an outer phospholipid membrane containing structural lipopolysaccharide components (Singhal et al., 2011; Elumalai et al., 2010). Hence, Banana peel extract-derived silver nanoparticles have the highest antibacterial activity against gram-negative pathogenic bacteria, according to antibacterial research, which shows that they have great potential antibacterial activity against Escherichia coli and Vibrio cholerae. This may be due to the release of reactive species oxygen that damages the membrane proteins (Azizi et al., 2017).

Conclusion

This study suggests using extracts from banana peels to create silver nanoparticles in a green way. UV-visible spectroscopy was used to characterize the produced nanoparticles' absorption peak. Using X-ray diffraction, the crystalline nature of silver nanoparticles was investigated, and the average size of the nanoparticles was determined to be 31 nm. According to the FTIR analysis, the synthesis, capping, and stability of silver nanoparticles (AgNPs) are caused by the presence of active biomolecules and phytomolecules. Tests of the synthesized AgNPs' strong antibacterial activity revealed that the biosynthesized AgNPs from the extract of banana peels are highly resistant to the gram-negative Vibrio cholerae bacteria. The inhibitory zone's diameter range demonstrated the pathogens' capability for antibacterial action. Consequently. Therefore, the present study concludes that biologically derived AgNPs using banana peel is eco- friendly, rapid, non-toxic and cost-effective approaches.

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