Heliyon 8 (2022) e09920

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Green synthesis of silver nanoparticles using *Cinnamomum tamala* (Tejpata) leaf and their potential application to control multidrug resistant *Pseudomonas aeruginosa* isolated from hospital drainage water

Md. Abdullah Al Mashud [a,c], Md. Moinuzzaman [a,c], Md. Shamim Hossain [b,e], Sabbir Ahmed [b,e], Galib Ahsan [b], Abu Reza [b,e], Robayet Bin Anwar Ratul [b,e], Md. Helal Uddin [d], Md. Abdul Momin [a], Mohammad Abu Hena Mostofa Jamal [b,e,*]

[a] Department of Electrical and Electronic Engineering, Faculty of Engineering and Technology, Islamic University, Kushtia 7003, Bangladesh
[b] Department Biotechnology and Genetic Engineering, Faculty of Biological Sciences, Islamic University, Kushtia 7003, Bangladesh
[c] Biophysics and Biomedicine Research Lab, Faculty of Engineering and Technology, Islamic University, Kushtia 7003, Bangladesh
[d] Department of Applied Chemistry and Chemical Engineering, Faculty of Engineering and Technology, Islamic University, Kushtia 7003, Bangladesh
[e] Laboratory of Environmental and Clinical Microbiology, Department Biotechnology and Genetic Engineering, Islamic University, Kushtia 7003, Bangladesh

ARTICLE INFO

Keywords:
Green synthesis
Silver nanoparticles
Leaf extract
Antibiotic resistance
Antibacterial activity

ABSTRACT

Green Synthesis of Metal Nanoparticles is becoming a more common method for producing nanoparticles with a diameter of 1–100 nm that may be employed in a variety of medical applications. The antibacterial efficacy of silver nanoparticles (AgNPs) derived from *Cinnamomum tamala* (Tejpata) leaf extract against antibiotic-resistant *Pseudomonas aeruginosa* is investigated in this study. Green AgNP synthesis is safe, cost-effective, and ecologically friendly. The biosynthesized AgNPs were studied using UV-Visible spectroscopy, Fourier Transform Infrared Spectroscopy (FTIR), Dynamic Light Scattering (DLS), X-ray Diffraction (XRD), and Transmission Electron Microscopy (TEM). The AgNPs were virtually spherical, with an average size of 25–30 nm, according to TEM observations. Biochemical and molecular identification were used to isolate multidrug-resistant *P. aeruginosa* from the hospital's drainage water. The antibacterial potential of AgNPs against *P. aeruginosa* is determined using the agar diffusion method. Silver nanoparticles produced from *Cinnamomum tamala* (Tejpata) leaf extract were shown to be effective in inhibiting four strains of *P. aeruginosa*. According to the agar disc diffusion method, AgNPs had the largest inhibition zone of 17.67 ± 0.577 mm, while aqueous extract had 5.67 ± 0.5777 mm, indicating that AgNPs had antibacterial activity. This study on AgNPs might assist with managing multidrug resistant pathogenic bacteria and be a possible source of medicinal application due to its potential antibacterial effect.

1. Introduction

Because of its broad variety of applications in adsorption, optical sensors, chemistry, water management, and drug administration, the green syntheisis of silver nanoparticles (AgNPs) has emerged as one of the most promising areas of prospective therapeutic research [1, 2]. Nanomedicine is a branch of nanotechnology that includes biological, physical, and material sciences and has a major impact on therapeutic healthcare because they do not rely on high pressure, energy, temperature, or harmful chemicals, plant extract or green synthetic procedures for the production of silver nanoparticles [3]. They are also more cost-effective, eco-friendly, and easily scaled up for large-scale manufacturing than other established processes [4, 5]. Plant extracts may also be more advantageous for nanoparticle synthesis than microorganisms due to their simplicity of improvement, reduced biohazard, and more complex cell culture maintenance approach [6, 7]. Secondary metabolites and alkaloids are prevalent in plants and act as antimicrobials, insect repellents, and herbivore defenses [8, 9]. The green synthesis of AgNps biological extract converts silver salts (Ag^{+}) to metallic silver (Ag^{0}). Biological molecules may function as both reducing agents and metal salt reducers. This coating reduces harmful effects by reducing nanoparticle aggregation [10]. Biological chemicals found in *Cinnamomum tamala* leaf extract include α-Pinene, β-Pinene, Camphene, Benzaldehyde, Cymene, phellandrene, Terpeneol, Cinamyl Acetene,

* Corresponding author.
E-mail address: jamalbtg@gmail.com (M.A. Hena Mostofa Jamal).

https://doi.org/10.1016/j.heliyon.2022.e09920
Received 7 February 2022; Received in revised form 25 April 2022; Accepted 6 July 2022

Cinnamaldehyde, *Bornyl acetate* [11]. As a result, it has the potential to be employed as a treatment. Silver nanoparticles have been shown to have anti-inflammatory, antifungal, and antiviral properties [12]. Because of their antibacterial properties, AgNPs are gaining much interest. They are expected to be classified as an antibacterial agent in the future. Silver nanoparticles have been shown to have antibacterial action against gram-positive and gram-negative bacteria, as well as antibiotic-resistant species [13]. Although the mechanism underlying silver N.P.s' antibacterial effect is still being researched, it has yet to be identified. Several facts about the antibacterial action of silver nanoparticles in microbes have been revealed [14]. Silver nanoparticles may bind to and enter the bacterial cell wall, causing structural alterations in the cell membrane and bacterial death. Nanoparticles accumulate on the cell's surface, creating bumps [15, 16]. Since the cell wall contains sulfur protein, the silver nanoparticle may continuously release silver ion, which has an electrostatic attraction to sulfur and sticks to the cell wall and plasma membrane. Silver nanoparticles may cause cell rupture by increasing the permeability of the cell wall. When silver nanoparticles reach the cytoplasm and disrupt ATP and DNA production, respiratory enzymes are blocked and reactive oxygen is created. Silver nanoparticles end the life cycles of microbes in this way. By inactivating ribosomes, silver ions may also inhibit protein production. During the green manufacturing of silver nanoparticles, a biological extract capped the silver ion; capping agents may change the surfaces of silver nanoparticles, modifying their dissolving capabilities to destroy the microbial cell (Figure 1). A synergistic effect of silver nanoparticles and capped molecules may play a role in antimicrobial activity in addition to the antibacterial capabilities of these coating agents [17].

In this study, *C. tamala* is used to synthesize AgNPs, and the disc diffusion technique is used to investigate the activity of the produced AgNPs against *Pseudomonas aeruginosa* that has been isolated from hospital drainage water. *Pseudomonas aeruginosa* is a gram-negative aerobic bacterium that does not produce spores and may thrive in a variety of environments [18]. The opportunistic pathogen *Pseudomonas aeruginosa* may be found in aqueous solutions. Bacterial illness may spread in both hospitals and communities [19, 20]. The bacteria *Pseudomonas aeruginosa* has been recognized as a major source of hospital outbreaks [21]. The bulk of these outbreaks has been linked to environmental factors, primarily the water system [22]. The purpose of this study is to identify multidrug-resistant *Pseudomonas* species from the drainage water of several medical institutions in Kushtia and Jhenaidah, Bangladesh, and control them using biologically produced silver nanoparticles derived from *Cinnamomum tamala* (Tejpata) leaf. The plant was obtained in Bangladesh's Kushtia and Jhenaidah.

2. Materials and methods

2.1. Preparation of leaf extract

To eliminate dust particles, *Cinnamomum tamala* (Tejpata) leaves are gently rinsed with water before being cleansed in ethanol-mixed water. 5 grams of green leaves are put in a 250 ml beaker after they have been separated and dried. The leaves are cooked for 15 min in 100 ml of double-distilled water [23, 24]. Once the leaf extract has cooled, it is filtered using Whatman No.1 filter paper. In a conical flask, the extract is maintained at 4 °C [15].

2.2. Synthesis of silver nanoparticles

A magnetic stirrer machine was used to whirl the leaf extract and 1mM Silver Nitrate in 1:1, 1:2, 1:3, 1:4, and 1:5 ratios for a specified time (0–120) minutes. After stirring, the colorless silver nitrate solution became a deep brown hue, indicating the formation of AgNPs [24].

2.3. Silver nanoparticles characterization

2.3.1. UV-Vis spectrophotometric analysis

To study the SPR (Surface Plasmon Resonance), UV-Vis Spectrophotometer is frequently employed. The concentration of $AgNO_3$ and the leaf extract-to-$AgNO_3$ solution ratio determine the rate and size of

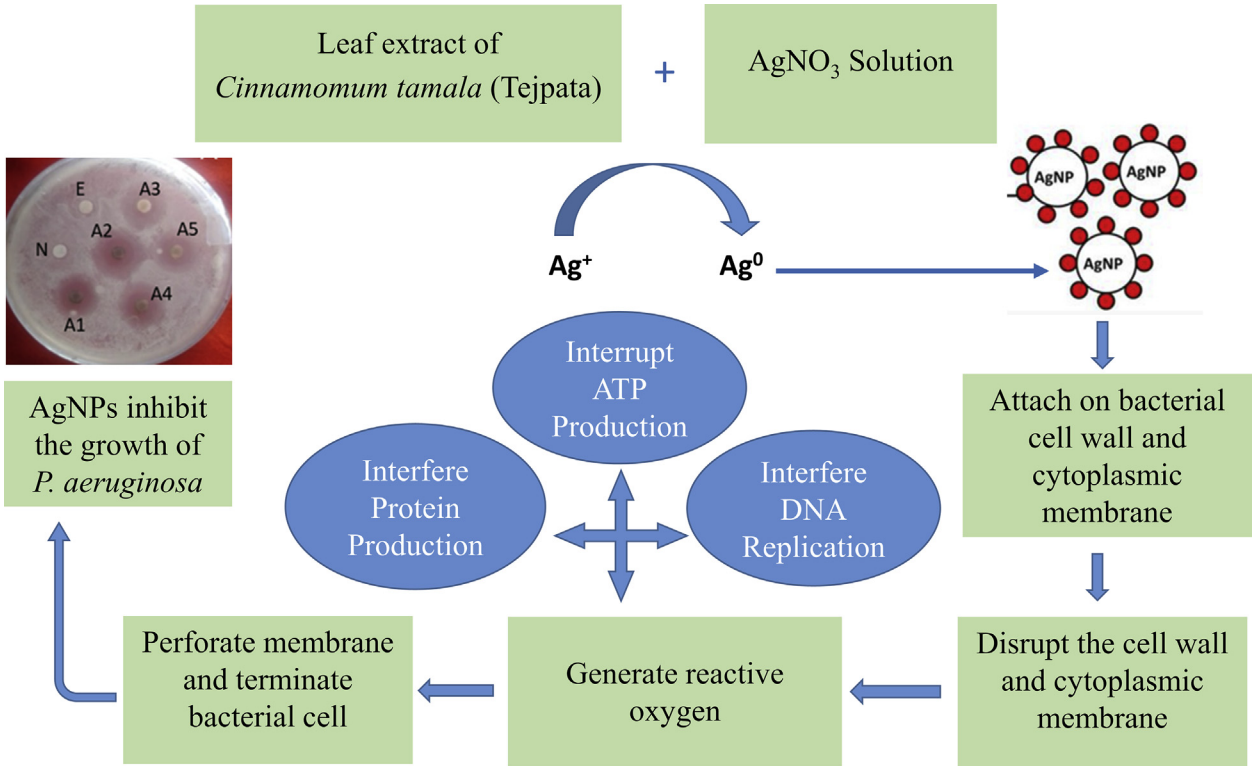

Figure 1. The mechanism of green synthesis of AgNP and its antibacterial action is depicted schematically.

nanoparticle production. UV-Vis spectrophotometer is used to evaluate the optical properties of AgNPs (SHIMADZU-1700, in a materials science lab, Dept. of EEE of Islamic University).

After adding $AgNO_3$ to the plant extract, the spectra are obtained in various ratios (1:2, 1:3, 1:4, and 1:5) at different time intervals up to 60 min between 300 nm and 700 nm [16]. UV-Vis Spectrophotometer is widely used to investigate SPR (Surface Plasmon Resonance). The pace and size of nanoparticle generation are determined by the concentration of $AgNO_3$ and the leaf extract-to-$AgNO_3$ solution ratio. The optical characteristics of AgNPs are assessed using a UV-Vis spectrophotometer (SHIMADZU-1700, in a materials science lab, Dept. of EEE of Islamic University). The spectra are obtained in various ratios (1:2, 1:3, 1:4, and 1:5) after adding $AgNO_3$ to the plant extract at varying time intervals up to 60 min between 300 nm and 700 nm [16].

2.3.2. FTIR spectroscopic analysis

The presence of biomolecules involved in nanoparticle synthesis is routinely detected using Fourier-Transform Infrared Spectroscopy (FTIR), which is becoming more significant in academic and industry research. Furthermore, for identifying the role of biological molecules in the conversion of silver nitrate to silver, FTIR is an appropriate, helpful, cost-effective, and straightforward method [16]. The chemical composition of the generated silver nanoparticles was studied using an FTIR spectrometer (Perkin-Elmer LS-55-Luminescence spectrometer, class: laser product, BSEN-60852-1:2007; IEC-60852-1:2007) at the atomic energy commission's materials science lab in Bangladesh. The answers may be found in the frequency ranges of (400–4000) cm^{-1}.

2.3.3. DLS (Dynamic Light Scattering) analysis

DLS is a non-destructive method for estimating the average diameter of nanoparticles dispersed in liquid. The size distribution and stability of AgNPs were determined using DLS [13]. Using a computer-controlled particle size analyzer, the dispersion of particles in liquid was investigated at the materials science laboratory of the atomic energy commission in Bangladesh (ZETA sizer Nano series; Malvern instrument Nano Zs; model: ZEN3600; serial no: MAL-1181676).

2.3.4. XRD (X-ray diffraction) analysis

At the atomic level, XRD is a frequently used method for identifying the crystalline state of substances. X-ray diffraction spectroscopy was used to determine the phase angle and crystal size of the produced silver nanoparticles. At BSCIR's Thin Film Lab, the created silver nanoparticles are dried in a hot air oven in the form of a pellet for XRD analysis, and a small nanoparticle film is placed on the XRD grid (Bruker D8 Advanced XRD Spectroscopy). The Debey Scherrer equation, which is shown below, is used to compute the particle size of the processed samples.

$$D = 0.9\lambda/\beta\cos\theta \quad (1)$$

Where D represents the crystal size, λ represents the X-ray wavelength, θ represents the Braggs angle in radians, and β represents the whole width at half the maximum of the peak in radians.

2.3.5. TEM (transmission electronic microscopy) analysis

For analyzing the morphology of nanomaterials, the transmission electron microscope (TEM) is an appropriate and widely used tool. The morphological pictures of the nanomaterials were examined using transmission electron microscopy (Talos f200x; Materials Science Lab of Atomic Energy Commission in Bangladesh). It has the potential to provide a high-resolution image; as a consequence, sample preparation is critical for the best outcomes [14].

2.4. Isolation of Pseudomonas species from Hospital's drainage water

Drainage water was collected from 20 different medical facilities in Kushtia and Jhenaidah, Bangladesh. The samples were disseminated using *Pseudomonas* Cetrimide Agar Base selective supplement (Thermofisher SR0102) [25].

2.5. Antibiotic susceptibility test

Antibiotic resistance in bacteria must be assessed as part of the therapy of bacterial illnesses. In research, Kirby and Bauer's disc diffusion method is a well-established and helpful alternative to broth dilution processes [26]. Kirby-Bauer disc diffusion technique is used to determine whether pathogenic bacteria are susceptible to or resistant to certain antibiotics. In this procedure, nutrient agar media with Petri plates were produced, and 0.01 ml of each sample's direct primary broth culture was put on the surface of the plate's media. Following the spread, each of the four antibiotic discs (Ciprofloxacin, Gentamicin, Erythromycin, Streptomycin, Tetracycline, Amoxycillin, Metronidazole, Levofloxacin, Azithromycin, Doxycycline) was placed on the surface of the medium at a set distance from the others. After a 24-hour incubation period at 37 °C, the dishes were examined and the clear zone surrounding the discs was quantified for each sample [27].

2.6. Biochemical characterization

The isolated bacteria were identified using a variety of biochemical assays, including the KOH test [28], the Motility Test, Oxidase Test, Catalase Test, [29], the Nitrate Reduction Test, Citrate Utilization Test, Indole Production Test, and the MR-VP Test [30].

2.7. Molecular identification of isolates

2.7.1. Genomic DNA extraction

Pseudomonas sp. liquid culture in 10 ml test tubes was incubated overnight at 37 °C in a shaking incubator. Turbidity was detected in bacterial broth culture, indicating that enough bacterial cells had grown. In a 2 ml Eppendorf tube, put 1 ml overnight broth culture and centrifuged for 5 min at 10,000 rpm (rotation per minute), then discarded the supernatant. The pellet did not appear to be enough. Then this operation was repeated to achieve a big bacterial pellet and the supernatant was discarded once again (upper liquid part).

To resuspend the particle, we pipetted it multiple times in 467 μl T.E. buffer. 30 μl (10% SDS) and 3 μl (20 mg/ml proteinase K) were combined in a water bath and incubated at 37 °C for 30 min to 1 h. By inverting, an almost comparable amount of 500 μl was added and thoroughly but gently mixed. For 10 min, we centrifuged at 12,000 rpm. Then, in a fresh microcentrifuge tube (2 ml), we added an equal amount of phenol, chloroform, and isoamyl alcohol to the top aqueous, viscous supernatant (about 450 μl), mixed well, and microcentrifuged at 10000 rpm for 5 min. The supernatant (about 400 μl) was transferred to a fresh tube. 1/10 volume of 3M sodium acetate was added and mixed. In order to precipitate the nucleic acid, we added 6/10 volume of isopropanol. We centrifuged for 15 min at 13,500 rpm after 10 min on ice. For 5 min, we cleaned the pellet in 1 ml of 95% ethanol. After that, we centrifuged for 10 min at 12,000 rpm. The supernatant was then decanted. We I dried the pellet thoroughly for approximately 20 min, making sure there was no alcohol present. The pellet was resuspended in 100 μl of T.E. buffer (with 3 μl RNase added since the DNA concentration ratio was 2.00). In the end, we kept it at -20 °C for a long period. The concentration of DNA was determined using a spectrophotometer from Thermo Scientific called the Nanodrop 2000. (Nanodrop 2000 software).

2.7.2. PCR amplification of 16S rRNA gene

A 50 μl reaction vessel comprising nuclease-free water, 4 × 1.25 ml Dream Taq PCR Master Mix (2X), which contains Dream Taq DNA Polymerase, 2X Dream Taq buffer, dNTPs, and 4mM $MgCl_2$, DNA template (sample), primer 27F (5′-AGAGTTTGATCCTGGCTCAG-3′), and primer 1492R (5′-GGTTACCTTGTTACGACTT-3′). In a heat cycler, the PCR tubes were put (Gene atlas, Model G02, Japan). A 5-minute

denaturation stage at 95 °C was followed by 30 cycles of denaturing at 95 °C for 30 s, annealing for 1 min at 57 °C, extension at 72 °C for 2 min, and a final extension phase of 10 min at 72 °C. The PCR tubes were then kept at -20 °C.

2.7.3. Sequencing of the PCR amplicons

The amplicon was excised from the agarose gel after electrophoresis, purified with Thermo Fisher Scientific's PureLink® PCR Purification Kit, and partly sequenced on a 3500 Genetic Analyzer automated DNA sequencer (Applied Biosystems, USA). CodonCode Aligner was used to assemble and align the sequences, and nucleotide BLAST (https://blast.ncbi.nlm.nih.gov) was used to identify the species and strains. To get accession numbers, all of the sequences were submitted to the National Center for Biotechnology Information (NCBI) database.

2.7.4. Phylogenetic tree construction

The online tool Phylogeny.fr (https://www.phylogeny.fr/) was used to create the phylogenetic tree [31].

2.8. Antibacterial activity studies

Because of their high surface-to-volume ratios and crystalline surface structure, AgNPs have emerged as potential antibacterial agents [32]. The antibacterial activity of extract, silver nitrate, and silver nanoparticles are evaluated using the disc diffusion method [33]. *Pseudomonas sp*. with multidrug resistance were identified from the drainage water of several medical institutions. These bacteria are grown for 24 h at 37 °C in L.B. broth before being disseminated on L.B. agar plates using a sterilized glass spreader. After that, sterile paper discs (3mm diameter) were placed on infected plates, and 10L AgNP suspension was fed onto each disc at the following stirring times: 0 min (A1), 5 min (A2), 15 min (A3), 30 min (A4), and 60 min (A5). The antibacterial activity is measured by measuring the zone of inhibition created around the disc after all plates have been incubated for 24 h at 37 °C. Negative control (N) was a disc with solvent, while positive control (P) was Plant extract (E) [4].

3. Results

3.1. Biosynthesis and characterization of the synthesized nanoparticles

The UV-Vis absorbance of silver nanoparticles was evaluated at different ratios and time intervals in the *Cinnamomum tamala* (Tejpata) leaf extract and 0.001M silver nitrate (1:2, 1:3, 1:4, and 1:5) for 5, 15, 30, and 60 min to standardize the nanoparticles production. A comparably low absorption band at 416 nm is visible when the ratio of the Tejpata leaf extract to 0.001M $AgNO_3$ solution is 1:2, suggesting that the particle size obtained by using Eq. (1) is smaller than the other samples (Figure 2A). When the ratio is 1:4, the absorption peak is likewise found to be extended and relocated to a higher wavelength of 426 nm [34]. The absorption peak rises when the ratio reaches 1:5, but it changes to a higher wavelength of 429 nm, showing that the particles are increased in the 1:4 and 1:5 samples. When the ratio is raised to 1:3, on the other hand, the absorption peak is greatly enlarged, and the wavelength shifts to 424 nm.

Furthermore, the time interval spectra (Figure 2B) reveal that at various times, the absorption spectra of the leaf extract and 0.001M $AgNO_3$ solution had a 1:10 ratio. The absorption peak at a wavelength of 425 nm is low after 5 min of stirring on a magnetic stirrer, as seen in the picture.

Figure 2B further demonstrates that after 15 and 30 min, the materials tested had a broad absorption peak about 425 nm. The peak for the material examined became sharper after 1 h, shifting the peak position towards high absorbance. As a result, Figure 2B shows that the Surface Plasmon Resonance (SPR) of silver nanoparticles formed a peak at 425 nm [35]. The presence of the functional group in the solution used in silver nanoparticle capping agents may be determined via FTIR analysis (Figure 2C). Alcohols and phenols are present because the particles show a strong peak at 3339 cm^{-1}, which corresponds to O–H stretching vibrations. A prominent peak of 1638 cm^{-1} related to C–N, which displays proteins, is also seen. The silver nanoparticles are clearly capped by several phytochemicals that contain distinct functional groups by their individual peaks, according to the FTIR investigation. Figure 2D shows a histogram of particle size distribution based on dynamic light dispersion (DLS).

The size of AgNPs formed from *Cinnamomum tamala* aqueous leaf extract is measured using dynamic light dispersal techniques. The silver nanoparticles used in the silver nitrate XRD patterns are derived from a *Cinnamomum tamala* combination. Figure 2E shows four strong diffraction peaks at angles of 38, 44.1, 64.42, and 77.46, respectively, corresponding to the (1 1 1), (2 0 0), (2 2 0), and (3 1 1) planes. This suggests that suggesting that the produced silver nanoparticles are face-centered cubic (FCC) and crystalline in nature. The bio-organic phase crystallization on nanoparticle surfaces might explain the X-ray diffraction pattern [36]. The encapsulation of Ag atoms in the plant extract by these chemical compounds was confirmed in our study. The Joint Committee on Powder Diffraction Standards (JCPDS file 04-0783) data has been verified. *Cinnamomum tamala* (Tejpata) leaf extract was combined with a 0.001 *M silver* nitrate solution to create green manufactured silver nanoparticles. The TEM examination is used to determine the form and structure of the produced AgNPs. Figure 3 displays TEM images at different magnifications. The particles are virtually spherical, with an average size of roughly 25 nm, according to imaging software employed in TEM equipment [37]. The crystalline nature of biosynthesized silver nanoparticles could be observed clearly in TEM images. Particle sizes of (A) 20 nm, (B) 50 nm, and (C) 100 nm, as well as the crystalline nature of the particles created in this study, were indicted by the circular spot.

3.2. Identification of Pseudomonas sp. from medical wastewater

Drainage water from 20 medical facilities in Kushtia and Jhenaidah, Bangladesh, was cultured on Cetrimide Agar Base selective supplement (Figure 4A) and morphological properties were examined (Figure 4B). The isolates were motile bacteria since they were transported in semi-solid nutrient agar colonies. All isolates were cultivated, and an antibiotic sensitivity test was performed [38]. Four isolates were found to show multiple antibiotic resistance in this investigation (Table 1 and Figure 4 C-F). Ciprofloxacin, Gentamicin, Erythromycin, Streptomycin, Tetracycline, Amoxycillin, Metronidazole, Levofloxacin, Azithromycin, and Doxycycline resistance was found in those samples. The isolated bacteria were identified using a biochemical assay. *Pseudomonas* sp. was identified as the presumptive bacterium in all biochemical tests. The Oxidase, Catalase, Citrate Utilization, and Nitrate Reduction tests were passed by four isolate samples, however the Indole Production, MR, and VP tests were not. The isolates were gram-negative bacteria, as shown by a positive KOH test result. (See Figure 5 and Table 2).

The 16S rRNA gene was amplified by PCR from four isolate samples (DW1, DW2, DW3, DW4). *Pseudomonas* is suspected in all four samples, which gave PCR results of around 1400bp. The 16S rRNA gene sequences of known *Pseudomonas aeruginosa* were shown to have a high degree of similarity (98–99%) with those of unknown *Pseudomonas aeruginosa* (Figure 5F). Four isolates exhibited 99% similarity to *Pseudomonas* strains, and all sequences were submitted to the National Center for Biotechnology Information (NCBI) database (Table 3) and assigned accession numbers. In addition, the phylogenetic connection is shown Figure 5G.

3.3. Control of multidrug resistance Pseudomonas aeruginosa by synthesized silver nanoparticles using Cinnamomum tamala (Tejpata) leaf

Pseudomonas aeruginosa with multidrug resistance might pose a serious hazard to human health. As a result, different medications are

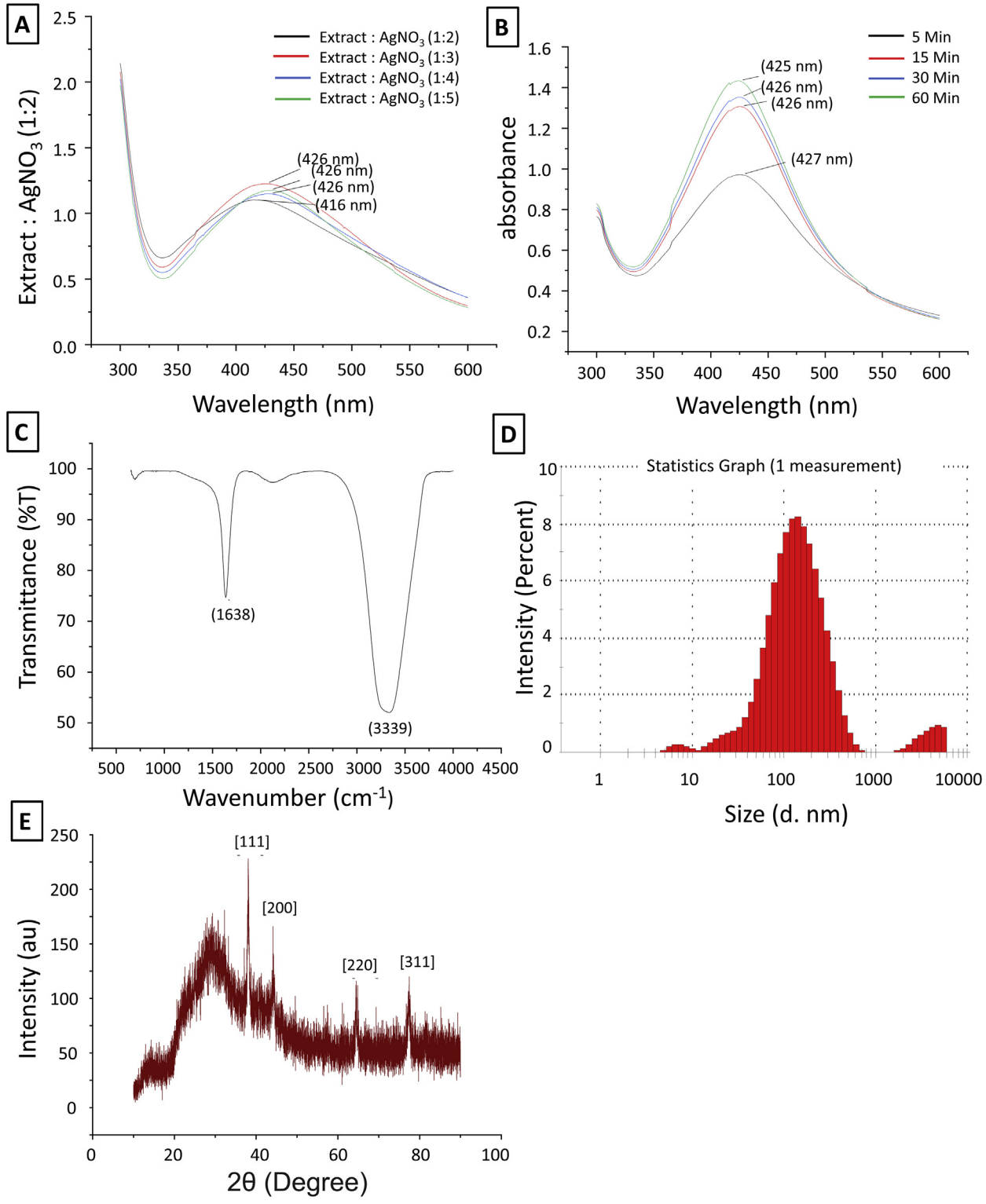

Figure 2. Characterization of Synthesized Silver Nanoparticles (A) UV-Vis spectra of Ag nanoparticles produced using *Cinnamomum tamala* (Tejpata) leaf extract and 0.001M $AgNO_3$ at various ratios (1:2, 1:3, 1:4 and 1:5) (B) UV-Vis Ag nanoparticles made from *Cinnamomum tamala* (Tejpata) and 0.001M silver nitrate at a rate of 1:10 for 5, 15, 30, and 60 min (1 ml of Tejpata leaf extract and 10 ml of $AgNO_3$ aqueous solution). (C) FTIR spectra of Silver nanoparticles produced in a 1:3 ratio by *Cinnamomum tamala* (Tejpata) leaf extract. (D) *Cinnamomum tamala* leaf synthesized particle size and intensity DLS (Dynamic Light Scattering) spectrum (%). (E) XRD patterns of AgNPs made from *Cinnamomum tamala* (Tejpata) leaf extract and $AgNO_3$ (Numbers in parentheses represent the face-center cubic planes of AgNPs).

desperately needed to combat this infection and natural resources may be an option. *Cinnamomum tamala* aqueous leaf extract was used as the control. Silver nanoparticles made through biogenic synthesis may also be more efficient in combating multidrug-resistant bacteria. The lowest inhibitory concentration of 5% aqueous extract and AgNP was found to be 128 µl/ml. We employed a blank disc as a negative control (N), 128 µl/ml aqueous extract (E), and 128 µl/ml AgNP suspension as positive controls (P) at stirring durations of 0 min (A1), 5 min (A2), 15 min (A3),

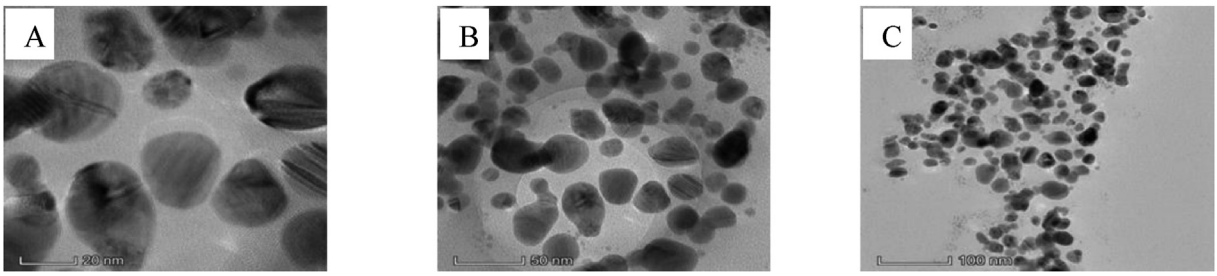

Figure 3. TEM pictures of AgNPs at various magnifications (500 kx.tif, 245 kx.tif, 120 kx.tif) revealing particle sizes of (A) 20 nm, (B) 50 nm, and (C) 100 nm.

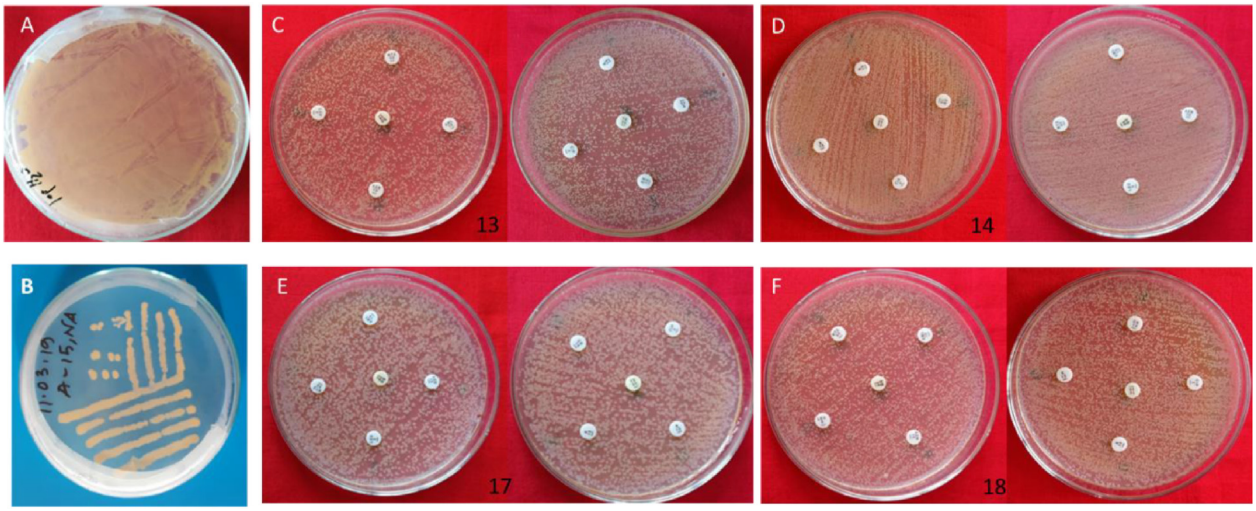

Figure 4. Isolate culture on Cetrimide Agar Base Selective Supplement (A); pure culture and morphological characterization (B). Antibiotic susceptibility testing of drainage water from a medical institution (C–F). Antibiotic resistance isolates 13,14,17,18 were designated as DW1, DW2, DW3, and DW4 for a subsequent experiment.

30 min (A4), and 60 min (A5). It exhibited antimicrobial efficacy against *Pseudomonas aeruginosa* with multidrug resistance (Figure 6).

Pseudomonas aeruginosa with multidrug resistance demonstrated greater inhibitory action when silver nanoparticles were generated between 15 and 30 min. To reduce multidrug resistance in *Pseudomonas aeruginosa* DW4, a 30-minute stirring interval was used to manufacture silver nanoparticles. It had a 17.67 ± 0.577 mm inhibition zone, whereas the aqueous extract had a 5.67 ± 0.5777 mm inhibition zone. AgNP

Table 1. Antibiotic susceptibility test of medical center drainage water.

Sample number	Ciprofloxacin	Gentamicin	Erythromycin	Streptomycin	Tetracyclin	Amoxycillin	Metronidazole	Levofloxacin	Azithromycin	Doxycycline
1	R	S	R	S	R	R	R	S	S	R
2	R	S	R	R	R	R	R	R	R	S
3	R	S	R	R	R	R	R	R	R	R
4	R	R	S	R	R	R	R	R	R	S
5	R	S	R	S	R	R	R	S	S	R
6	R	S	R	R	R	R	S	R	R	S
7	R	R	S	R	S	R	R	R	R	R
8	S	S	R	S	S	R	R	S	S	S
9	R	S	R	S	R	R	R	R	R	R
10	S	S	R	S	S	R	R	S	S	S
11	R	R	S	S	S	R	R	R	S	R
12	R	R	S	R	R	R	S	R	R	R
13	R	R	R	R	R	R	R	R	R	R
14	R	R	R	R	R	R	R	R	R	R
15	R	S	R	S	R	R	S	R	R	R
16	R	R	R	S	R	R	R	R	R	S
17	R	R	R	R	R	R	R	R	R	R
18	R	R	R	R	R	R	R	R	R	R
19	R	R	S	S	R	R	R	R	R	S
20	R	R	S	R	R	S	R	R	R	S

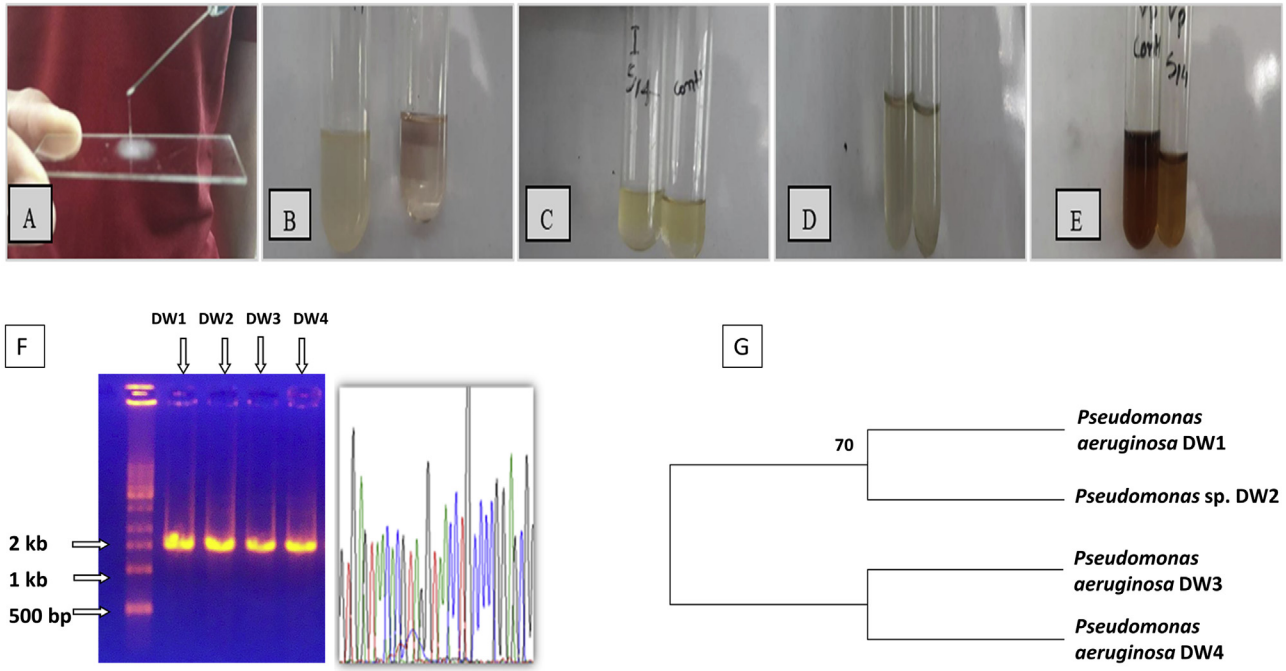

Figure 5. (A) Positive result KOH test, (B) Nitrate reduction test positive result, (C) Negative Indole test, (D & E) MR-VP test result, (F) Agarose gel electrophoresis of 16s rRNA sequencing PCR product and electrogram of sequencing alignment, (G) Phylogenetic relationship of isolates.

Table 2. The biochemical results of four isolated samples.

Isolate samples	KOH Test (A)	Motility Test	Oxidase Test	Catalase Test	Citrate Utilization Test	Nitrate reduction Test (B)	Indole Production Test (C)	MR Test (D)	VP Test (E)
DW-1	(-ve)	(+ve)	(+ve)	(+ve)	(+ve)	(+ve)	(-ve)	(-ve)	(-ve)
DW-2	(-ve)	(+ve)	(+ve)	(+ve)	(+ve)	(+ve)	(-ve)	(-ve)	(-ve)
DW-3	(-ve)	(+ve)	(+ve)	(+ve)	(+ve)	(+ve)	(-ve)	(-ve)	(-ve)
DW-4	(-ve)	(+ve)	(+ve)	(+ve)	(+ve)	(+ve)	(-ve)	(-ve)	(-ve)

suspension had higher inhibitory efficacy than aqueous extract of aqueous extract in all cases (Figure 7). As a result, biogenic production of silver nanoparticles is possible. The leaf of *Cinnamomum tamala* (Tejpata) may be used to suppress the development of multidrug-resistant bacteria.

4. Discussion

The natural product-based green production of AgNPs has potential therapeutic usages such as antibacterial, antioxidant, and anticancer properties [39]. Antibiotics are the most effective means of preventing infection. However, a large number of microorganisms have developed resistance to the antibiotic in recent years. Multidrug-resistant microorganisms have become a public health concern making alternative treatment for multidrug-resistant microorganisms is a hot topic among scientists [40]. The green manufacturing of silver nanoparticles has the potential to be a new source of medication. *Pseudomonas aeruginosa*, a multidrug-resistant microbe, was found in the hospital's drainage water in this investigation. The leaves of *Cinnamomum tamala* offer therapeutic properties [41]. Silver nanoparticles produced from *Cinnamomum tamala*

Table 3. *Pseudomonas aeruginosa* bacterial strain identified by 16S rRNA gene.

Sample Name	Bacteria Name with strain	Accession Number
DW-1	*Pseudomonas aeruginosa* strain DW1	OK355436
DW-2	*Pseudomonas aeruginosa* strain DW2	OK355437
DW-3	*Pseudomonas aeruginosa* strain DW3	OK355438
DW-4	*Pseudomonas aeruginosa* strain DW4	OK355439

were shown to be more effective than the plant extract alone in this investigation. The green synthesis and characterisation of AgNPs from *Cinnamomum tamala* leaf extract are described in this study, and their antibacterial effectiveness against antibiotic-resistant bacteria is established. Because silver nanoparticles are benign yet efficient antibacterial agents, the biosynthesis of silver nanoparticles from plant extract is a beautiful technique for therapeutic advances [42]. It was discovered that introducing silver nitrate to *Cinnamomum tamala* leaf extract at varied ratios and stirring times resulted in the formation of AgNPs. The production of nanoparticles caused a change in the absorption spectra of the leaf extract, which showed an absorption peak at 425 nm. This result was most likely generated by $AgNO_3$ reduction and was linked to the quantity of extract and the stirring time. During the biosynthesis of *Cinnamomum tamala* aqueous leaf extract, silver ions (Ag^+) were transformed to AgNPs (Ag^0) [43]. The existence of a functional group was confirmed by FTIR spectrum analysis, revealing that silver nanoparticles were capped by multiple phytochemicals [44]. The XRD pattern revealed the presence of AgNPs with varied face-centered cubic (FCC) silver planes with two theta values [45]. This discovery also suggests that these organic compounds encapsulate Ag atoms in the plant extract. The form and shape of AgNP were determined using electron microscopy. The production of silver nanoparticles was observed in this work using TEM.

Hospital's drainage is a Reservoirs of *Pseudomonas aeruginosa*, in this study 4 antibiotic resistance *Pseudomonas aeruginosa* strains were identified [46]. *Cinnamomum tamala* leaf extract likewise inhibits the development of such multidrug-resistant bacteria, although its inhibitory efficacy was increased by the addition of AgNP after stirring. The antibacterial activity was amplified by increasing the stirring time from 15 to

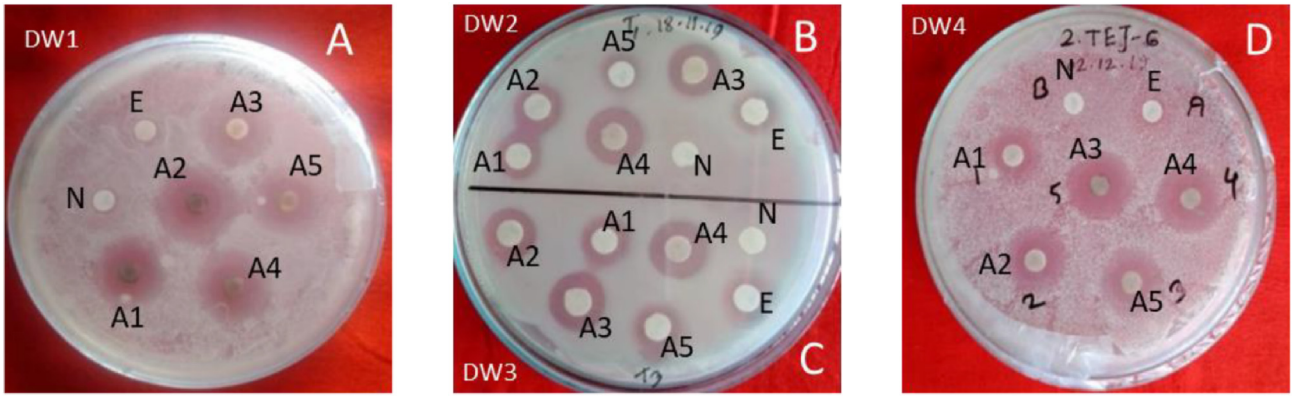

Figure 6. Antibacterial activity of Silver Nanoparticles Using *Cinnamomum tamala* against multidrug resistance *Pseudomonas aeruginosa* strain DW1 (A), *Pseudomonas aeruginosa* strain DW2 (B), *Pseudomonas aeruginosa* strain DW3 (C), *Pseudomonas aeruginosa* strain DW4 (D).

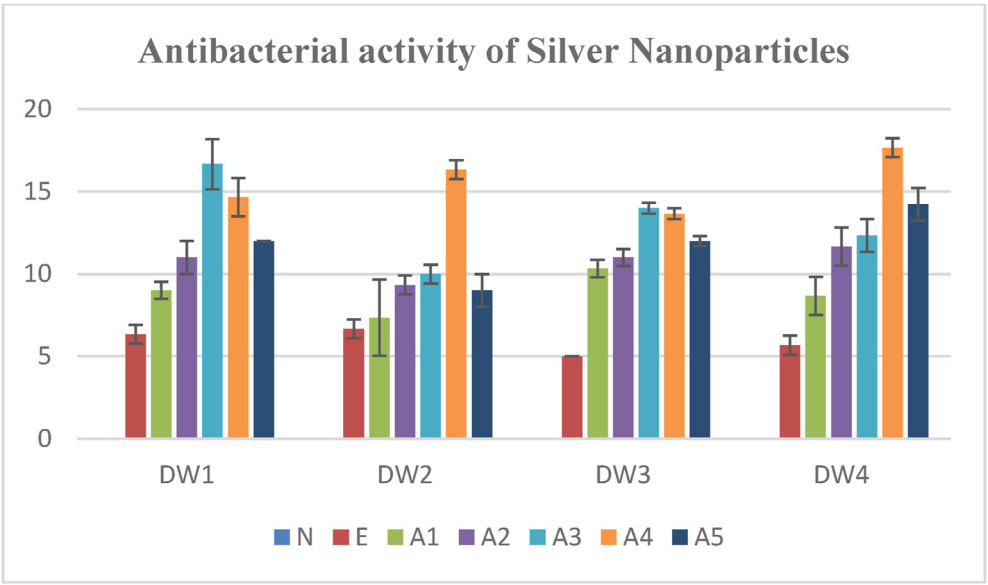

Figure 7. Silver nanoparticles' antibacterial efficacy against multidrug resistance using *Cinnamomum tamala Pseudomonas aeruginosa* strain DW1, *Pseudomonas aeruginosa* strain DW2, *Pseudomonas aeruginosa* strain DW3, and *Pseudomonas aeruginosa* strain DW4 at various control or starring times as negative control (N), aqueous extract (E), and AgNP suspension at various stirring times of 0 min (A1), 5 min ((A5).

30 min. This research also revealed that nanoparticles were generated throughout this time period linked to the characterization of AgNP. As a result, the antibacterial activity of the extract enhanced by AgNP synthesis also supported the inhibitory action of the extract. Due to the presence of carboxyl, phosphate, and amino groups in the bacterial cell walls [47], a positive charge produced electrostatic interaction between the cell membrane and AgNP [48, 49]. As a consequence, it is clear that silver nanoparticles have antibacterial properties. Furthermore, the AgNP has been shown to be a therapeutic target for the control of multidrug-resistant bacteria.

5. Conclusion

One of the most serious risks to public health is antibiotic resistance. Four antibiotic-resistant *Pseudomonas aeruginosa* strains were discovered in the hospital's waste. Controlling various drug-resistant diseases is one of the scientist's major issues. Silver nanoparticles were made from *Cinnamomum tamala* (Tejpata) leaf extracts and analyzed by UV–Vis spectrophotometer, FTIR, DLS, XRD, and TEM. The disc diffusion test on ager Petri plates revealed zones of inhibition against *P. aeruginosa*, demonstrating that the AgNPs synthesized in this approach were efficient antibacterial agents against the multidrug-resistant bacterium. This study might aid researchers in the development of new therapeutic medicines to combat antibiotic-resistant microorganisms. Green nanoparticle production may be a key research tool for finding medicinal components.

Declarations

Author contribution statement

Md. Abdullah Al Mashud, Mohammad Abu Hena Mostofa Jamal: Conceived and designed the experiments; Wrote the paper.

Md. Moinuzzaman, Md. Shamim Hossain: Performed the experiments; Wrote the paper.

Sabbir Ahmed, Galib Ahsan: Analyzed and interpreted the data.

Abu Reza: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Robayet Bin Anwar Ratul: Contributed reagents, materials, analysis tools or data; Wrote the paper.

Md. Helal Uddin, Md. Abdul Momin: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Funding statement

This work was supported by the Ministry of Science and Technology, Government of the People's Republic of Bangladesh (Grant No: 39.00.0000.009.06.009.20-1331/BS-323 and 39.00.0000.009.14.019.21-745-MS-563/1310).

Data availability statement

Data associated with this study has been deposited at "NCBI DNA sequence database" under the accession number (OK355436; OK355437; OK355438; OK355439).

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

References

[1] A.G. Femi-Adepoju, et al., Green synthesis of silver nanoparticles using terrestrial fern (*Gleichenia Pectinata* (Willd.) C. Presl.): characterization and antimicrobial studies, Heliyon 5 (4) (2019), e01543.
[2] S. Some, et al., Effect of feed supplementation with biosynthesized silver nanoparticles using leaf extract of *Morus indica* L. V1 on *Bombyx mori* L. (Lepidoptera: Bombycidae), Sci. Rep. 9 (1) (2019), 14839.
[3] S.O. Aisida, et al., Biosynthesis of silver nanoparticles using bitter leave (*Veronica amygdalina*) for antibacterial activities, Surface. Interfac. 17 (2019), 100359.
[4] P. Anbu, et al., Temperature-dependent green biosynthesis and characterization of silver nanoparticles using balloon flower plants and their antibacterial potential, J. Mol. Struct. 1177 (2019) 302.
[5] N.A. Bouqellah, M.M. Mohamed, Y. Ibrahim, Synthesis of eco-friendly silver nanoparticles using *Allium* sp. and their antimicrobial potential on selected vaginal bacteria, Saudi J. Biol. Sci. 26 (7) (2019) 1789–1794.
[6] S. Ahmed, et al., A review on plants extract mediated synthesis of silver nanoparticles for antimicrobial applications: a green expertise, J. Adv. Res. 7 (1) (2016) 17–28.
[7] S. Some, et al., Biosynthesis of silver nanoparticles and their versatile antimicrobial properties, Mater. Res. Express 6 (1) (2018), 012001.
[8] M. Devi, et al., Green synthesis of silver nanoparticles using methanolic fruit extract of *Aegle marmelos* and their antimicrobial potential against human bacterial pathogens, J. Tradit. Compl. Med. 10 (2) (2019) 158–165.
[9] V. Ravichandran, et al., Green synthesis, characterization, antibacterial, antioxidant and photocatalytic activity of *Parkia speciosa* leaves extract mediated silver nanoparticles, Results Phys. 15 (2019), 102565.
[10] A. Roy, et al., Green synthesis of silver nanoparticles: biomolecule-nanoparticle organizations targeting antimicrobial activity, RSC Adv. 9 (5) (2019) 2673–2702.
[11] V. Sharma, L.J.M. Rao, An overview on chemical composition, Bioactivity and processing of leaves of Cinnamomum tamala, Crit. Rev. Food Sci. Nutr. 54 (4) (2014) 433–448.
[12] S. Ekrikaya, et al., Investigation of ellagic acid rich-berry extracts directed silver nanoparticles synthesis and their antimicrobial properties with potential mechanisms towards Enterococcus faecalis and Candida albicans, J. Biotechnol. 341 (2021) 155–162.
[13] A. Nurul Aini, et al., A new green method for the synthesis of silver nanoparticles and their antibacterial activities against gram-positive and gram-negative bacteria, J. Chin. Chem. Soc. 66 (7) (2019) 705–712.
[14] D. Kalpana, et al., Green biosynthesis of silver nanoparticles using Torreya nucifera and their antibacterial activity, Arab. J. Chem. 12 (7) (2019) 1722–1732.
[15] A. Ashraf, et al., Synthesis, characterization, and antibacterial potential of silver nanoparticles synthesized from *Coriandrum sativum* L, J. Infection Public Health 12 (2) (2019) 275–281.
[16] A.A. Alfuraydi, et al., Eco-friendly green synthesis of silver nanoparticles from the sesame oil cake and its potential anticancer and antimicrobial activities, J. Photochem. Photobiol., B 192 (2019) 83–89.
[17] I.X. Yin, et al., The antibacterial mechanism of silver nanoparticles and its application in dentistry, Int. J. Nanomed. 15 (2020) 2555–2562.
[18] A.E. LaBauve, M.J. Wargo, Growth and laboratory maintenance of Pseudomonas aeruginosa, Chapter 6, in: Current Protocols in Microbiology, 2012. Unit-6E.1.
[19] J.A. Driscoll, S.L. Brody, M.H. Kollef, The epidemiology, pathogenesis and treatment of Pseudomonas aeruginosa infections, Drugs 67 (3) (2007) 351–368.
[20] J. Yayan, B. Ghebremedhin, K. Rasche, Antibiotic resistance of Pseudomonas aeruginosa in pneumonia at a single university hospital center in Germany over a 10-year period, PLoS One 10 (10) (2015), e0139836.
[21] A.I. Hidron, et al., NHSN annual update: antimicrobial-resistant pathogens associated with healthcare-associated infections: annual summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2006-2007, Infect. Control Hosp. Epidemiol. 29 (11) (2008) 996–1011.
[22] C. Aumeran, et al., Pseudomonas aeruginosa and Pseudomonas putida outbreak associated with contaminated water outlets in an oncohaematology paediatric unit, J. Hosp. Infect. 65 (1) (2007) 47–53.
[23] V. Sunderam, et al., *In-vitro* antimicrobial and anticancer properties of green synthesized gold nanoparticles using *Anacardium occidentale* leaves extract, Saudi J. Biol. Sci. 26 (3) (2019) 455–459.
[24] R. Seifipour, M. Nozari, L. Pishkar, Green synthesis of silver nanoparticles using *Tragopogon collinus* leaf extract and study of their antibacterial effects, J. Inorg. Organomet. Polym. Mater. 30 (2020).
[25] R. Weiser, et al., Evaluation of five selective media for the detection of Pseudomonas aeruginosa using a strain panel from clinical, environmental and industrial sources, J. Microbiol. Methods 99 (2014) 8–14.
[26] L. Bužn-Durßn, R. Capita, C. Alonso-Calleja, Antibiotic susceptibility of methicillin-resistant staphylococci (MRS) of food origin: a comparison of agar disc diffusion method and a commercially available miniaturized test, Food Microbiol. 72 (2018) 220–224.
[27] M.S.M. Nassar, W.A. Hazzah, W.M.K. Bakr, Evaluation of antibiotic susceptibility test results: how guilty a laboratory could be? J. Egypt. Publ. Health Assoc. 94 (1) (2019) 4.
[28] C. Dash, R.J. Payyappilli, KOH string and Vancomycin susceptibility test as an alternative method to Gram staining, J. Int. Med. Dent. 3 (2) (2016) 88–90.
[29] K. Reiner, Catalase Test Protocol, American Society for Microbiology (ASM), 2010, pp. 1–6.
[30] F. Islam, N. Roy, Screening, purification and characterization of cellulase from cellulase producing bacteria in molasses, BMC Res. Notes 11 (1) (2018) 1–6.
[31] A. Dereeper, et al., Phylogeny.fr: robust phylogenetic analysis for the non-specialist, Nucleic Acids Res. 36 (Web Server issue) (2008) W465–W469.
[32] G. Franci, et al., Silver nanoparticles as potential antibacterial agents, Molecules 20 (5) (2015).
[33] K. Shameli, et al., Investigation of antibacterial properties silver nanoparticles prepared via green method, Chem. Cent. J. 6 (1) (2012) 73.
[34] A. Arya, V. Mishra, T.S. Chundawat, Green synthesis of silver nanoparticles from green algae (*Botryococcus braunii*) and its catalytic behavior for the synthesis of benzimidazoles, Chem. Data Collections 20 (2019), 100190.
[35] P. Das, et al., Green synthesis and characterization of silver nanoparticles using belladonna mother tincture and its efficacy as a potential antibacterial and anti-inflammatory agent, Mater. Chem. Phys. (2019).
[36] S. Ghojavand, M. Madani, J. Karimi, Green synthesis, characterization and antifungal activity of silver nanoparticles using stems and flowers of felty germander, J. Inorg. Organomet. Polym. Mater. 30 (8) (2020) 2987–2997.
[37] S. Hamedi, S.A. Shojaosadati, Rapid and green synthesis of silver nanoparticles using *Diospyros lotus* extract: evaluation of their biological and catalytic activities, Polyhedron (2019).
[38] L. Coorevits, J. Boelens, G. Claeys, Direct susceptibility testing by disk diffusion on clinical samples: a rapid and accurate tool for antibiotic stewardship, Eur. J. Clin. Microbiol. Infect. Dis. : Offi. Publ. European Soci. Clin. Microbiol. 34 (6) (2015) 1207–1212.
[39] J. Flieger, et al., Green synthesis of silver nanoparticles using natural extracts with proven antioxidant activity, Molecules (Basel, Switzerland) 26 (16) (2021) 4986.
[40] J. Tanwar, et al., Multidrug resistance: an emerging crisis, Interdiscipl. Perspect. Infect. Dis. 2014 (2014), 541340.
[41] R. Upadhyay, Therapeutic and pharmaceutical potential of *Cinnamomum tamala*, Res. Rev.: Pharm. Pharmaceut. Sci. 6 (2017) 18–28, e-ISSN: 2320-1215 p-ISSN: 2322-0112, www.rroij.com.
[42] V.K. Sharma, R.A. Yngard, Y. Lin, Silver nanoparticles: green synthesis and their antimicrobial activities, Adv. Colloid Interface Sci. 145 (1-2) (2009) 83–96.
[43] C.G. Kumar, S.K. Mamidyala, Extracellular synthesis of silver nanoparticles using culture supernatant of *Pseudomonas aeruginosa*, Colloids Surf. B Biointerfaces 84 (2) (2011) 462–466.
[44] B. Selvi, J. Madhavan, A. Santhanam, Cytotoxic effect of silver nanoparticles synthesized from *Padina tetrastromatica* on breast cancer cell line, Adv. Nat. Sci. Nanosci. Nanotechnol. 7 (2016), 035015.
[45] W.M. Mahmoud, T.S. Abdelmoneim, A.M. Elazzazy, The impact of silver nanoparticles produced by *Bacillus pumilus* as antimicrobial and nematicide, Front. Microbiol. 7 (2016).
[46] C. Lalancette, et al., Hospital drains as Reservoirs of *Pseudomonas aeruginosa*: multiple-locus variable-number of tandem repeats analysis genotypes recovered from faucets, sink surfaces and patients, Pathogens (Basel, Switzerland) 6 (3) (2017) 36.
[47] A. Antonoplis, et al., Vancomycin–arginine conjugate inhibits growth of carbapenem-resistant *E. coli* and targets cell-wall synthesis, ACS Chem. Biol. 14 (9) (2019) 2065–2070.
[48] M. Abbaszadegan, et al., Detection of enteroviruses in groundwater with the polymerase chain reaction, Appl. Environ. Microbiol. 59 (5) (1993) 1318–1324.
[49] E.R. Caudill, et al., Wall teichoic acids govern cationic gold nanoparticle interaction with Gram-positive bacterial cell walls, Chem. Sci. 11 (16) (2020) 4106–4118.