Research Article

A green synthesis strategy of silver nanoparticles using aqueous extract of waste banana pseudo-stem and its anti-MRSA activity

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Abstract

A green organic approach was employed to employ discarded banana plant stems as both a reducing agent and a capping agent for synthesising silver nanoparticles, followed by analytical characterization. The synthesised nanoparticles were analysed by UV-visible spectroscopy, FT-IR, XRD, and TEM. Preliminary UV-visible analysis warranted the presence of silver nanoparticles, with maximum absorption reported at 428 nm. The transmission electron microscopy (TEM) study showed the presence of spherical nanoparticles that were black in colour with an average size of 200 nm. The FT-IR test also revealed that some secondary compounds have functional groups that, through bio-reduction, change silver nitrate into metallic nanosilver. The growth kinetics, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) of Methicillin-Resistant Staphylococcus aureus (MRSA), a gramme-positive bacteria, were assessed to identify the impact of AgNPs on MRSA. The maximum zone of inhibition identified for MRSA was 15±2 mm. The minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and MBC/MIC ratio of silver nanoparticles against methicillin-resistant Staphylococcus aureus (MRSA) were found to be 81 mg/mL, 162 mg/mL, and 2.0, respectively.

Keywords: Musa paradisiaca, silver nanoparticles, anti-bacterial, Staphylococcus aureus, MIC, MBC.

Introduction

Self-assembling nanotechnology is revolutionising multiple industries like connectivity, sensing probes, medical device manufacturing, and sustainable research. Since ancient times, these nanoparticles have undergone restructuring and refinement through many approaches (Yadav et al 2020). The distinctiveness and methods for material synthesis of modern nanoscience and technology are evidence that they have their roots in ancient human origins (Savrikar and Ravishankar, 2011). Nanomedicine, an area of study that integrates therapeutics and medical diagnostics, proposes a forward-thinking pathway towards contemporary drug delivery systems (Ali et al 2023a). Scientists have been motivated to re-examine and advance the field of nanoformulations due to their wide-ranging potential in medicine, treatment, regulated drug release, regenerative therapy, and disease prevention. Medical nanotechnology is reorienting its focus in the direction of novel therapeutic avenues, such as transporting therapeutic cargoes and diagnostics. Metallic nanoparticles, a crucial element in these breakthroughs, have proved substantial utility in several sectors. The distinctive characteristics of nanoparticles have generated considerable enthusiasm for their use in several industries (Kumar et al 2023).

Nanoparticles are synthesized by various methods and from various sources but each process has its own limitations. Nanoparticles from biological source or synthesized from biological methods are demanding due to its eco-friendly nature, limited toxicity and moreover cost-effective compared to chemical method of synthesis. Biological systems has inbuilt catalytic agents, participating in the bio-reduction of metallic ions into metallic nanoparticles (Guilger-Casagrande and Lima, 2019).

Microbes are a highly promising biological resource for the synthesis of silver nanoparticles (Iravani et al 2014). Microbes can synthesise silver nanoparticles by employing an intracellular or extracellular process. Within the intracellular process, the organism's cells begin manufacturing while silver accumulates within it, whereas in the extracellular process, extracellular secretions are separated from bacterial populations. Microorganisms commonly exhibit resistance to silver ions, but they are capable of efficiently generating silver nanoparticles (Guilger-Casagrande and Lima, 2019). The resilience process differs among microorganisms. Fungi, along with bacteria, can

be utilised for nanoparticle synthesis due to their copious abundance of enzymatic agents, protein molecules, and reducing components on their cell surface (Iravani et al 2014).

The sustainable production of silver nanoparticles is a straightforward, practical, cost-effective, and eco-friendly technique. This procedure employs plant extracts as reducing agents, which typically consist of elements such as sugars, fatty acids, enzymes, flavonoids, terpenoids, polyphenols, and alkaloids (Klančnik et al 2010). Recently, numerous environmentally friendly methods have been identified and documented for the rapid manufacturing of silver nanoparticles. These methods involve utilising water-based extracts derived from various plant components, such as leaves, bark, and roots (Das and Velusamy, 2013). Silver nanoparticles synthesised using environmentally friendly methods have promising promise as antibacterial agents (Devaraj Bharathi et al 2014). The increasing popularity of this synthesis approach can be attributed to its environmentally friendly nature and the nanoparticles' ability to interact well with biological systems (Soltani et al 2024).

The banana plant, which is the biggest herbaceous flowering plant, plays a significant role in generating 88 million metric tonnes of food across more than 100 nations. Bananas, with a cultivated land area of 10 million hectares, serve as a crucial solution to the problem of food scarcity during periods between harvests. They greatly improve the stability of both the food supply and revenue for individuals engaged in their cultivation and commerce, particularly in developing nations (Onyema et al 2016). Substantial initiatives have been conducted to harvest banana pseudostem (BPS) extract. Banana stem extracts are considered a value-added product due to the presence of a plethora of secondary metabolites that are easily accessible, cost-effective, and environmentally friendly (Islam et al 2022). The present investigation assesses the role of secondary metabolites in banana pseudostem extract in the green synthesis of metal nanoparticles, followed by molecular characterization and assessing the anti-microbial activity of synthesized silver nanoparticles.

Materials and methods Phytochemical analysis

Phytochemicals analysis was performed as per standard protocols and procedures (Onyema et al 2016). The presence of sterols, terpenoids, alkaloids, carbohydrates, flavonoids, tannins, saponin and glycosides were analysed as procedure (Mondal et al 2021).

Bacterial strains

The methicillin-resistant Staphylococcus aureus (MRSA) organism was obtained from the Dr.M.G.R. Educational and Research Institute University in Porur, Chennai. The test bacterial cultures were sub-cultured intermittently at 37°C at room temperature and subsequently preserved at 4°C on nutrient agar for the following experiments.

Molecular characterization

The synthesized AgNPs was characterized using X-ray diffraction (XRD), Fourier transform infrared (FTIR) spectroscopy and scanning electron microscopy (SEM) using standard procedure. These instruments working on different principles help us to know the size, shape, stability, elemental nature and functional groups of the synthesized nanoparticles.

The powder X-ray diffraction (XRD) methodology was used to analyse the phase purity and molecular framework of synthesised silver nanoparticles using banana stem extract. Silver nanoparticles in dried form were then placed in the specimen holder's cavity and analysed using Cu-Kα1 radiation with a wavelength of 1.5406 Å. A nickel monochromator was used to filter the wave, with a tube voltage of 40 kV and a tube current of 30 mA. The scan was conducted between 20° and 70° at a scanning speed of 0.02°/min (Using Burker D8 Advance, panalytical xpert3 Instrument). FTIR is an essential technique used to identify the potential functional groups present on the external layer of newly synthesised AgNPs. It assists in validating the synthesis, capping, and molecular characteristics of the synthesised nanomaterials. The AgNPs solution was treated with potassium bromide (KBr) at a ratio of 1:100 to generate a pellet. The analysis was conducted using the Cavy 630 Agilent FT-IR spectrometer in diffuse reflectance mode, ranging from 650 to 4000 cm-1 at a resolution of 4 cm-1. The electromagnetic spectrum of transmittance was

documented (Reference from the intetrnet). The crystal structure of the AgNPs was visualised using transmission electron microscopy (TEM). Imaging specimens were generated by depositing a single drop of synthesised AgNPs (aqueous) on a copper-coated grid and then imaged using a high-resolution transmission electron microscope (HR-TEM) (Model: FEI-TTECNAI G2-20 TWIN) with operating voltages of 200 kV(Approximately 3,00,000X Magnification).

Antibacterial activity

The disc-diffusion method is widely employed to investigate the antimicrobial activity of silver nanoparticles using Muller-Hinton Agar (MHA) medium (Balouiri et al., 2016). The antibacterial activity of AgNPs was carried out by the agar-well difusion method as per standard procedure (Devaraj Bharathi et al 2014). 6 mm, four wells were punctured using sterile gel puncture on the prepared solidified Muller-Hinton agar plate. The bacterial strain at a concentration of 1×105 CFU/mL was swabbed uniformly on the prepared individual petri plates using sterile cotton swabs. 100 µL of banana pseudostem extract, 100 µL of silver nitrate solution (324 mg/ml), XXX gentamycin (1 mg/ml), and XXX silver nitrate solution acting as positive and negative controls were loaded in four wells on the plates, respectively. After incubation for 24 h at 37 °C under standard sterile conditions, the plates were examined and the zone of incubation (mm) was measured.

Preparation of Resazurin Solution

The preparation of the resazurin solution involved dissolving 0.002 g of resazurin powder in 10 mL of sterile distilled water in a sterilised beaker (Sarker et al 2007, Khalifa et al 2013). An aseptic vortex mixer was employed to agitate the mixture for 1 hour to achieve uniformity. The entire mixture was subjected to filtration using a Millipore membrane filter (0.2 µm pore size). All of the preparation procedures were carried out in a dark chamber, and the resazurin solution was subsequently stored in a brown bottle to protect it from light sources.

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) Evaluation

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of AgNPs synthesised using green methods were determined following the procedure recommended in the CLSI guideline (Ohiro et al 2019). The Minimum Inhibitory Concentration (MIC) test was conducted using standard broth microdilution techniques in a 96-well round-bottom microtiter plate (Klančnik et al 2010). On the other hand, the Minimum Bactericidal Concentration (MBC) test was carried out on Muller-Hinton agar (MHA) plates (Chakansin et al 2022). The concentration of the bacterial inoculum was adjusted to a fixed value of 1x105 CFU/mL. In the MIC test, 100 µL of the synthesised AgNPs stock solution (324 mg/mL) was added and diluted by twofold with the bacterial inoculums in 100 µL of Muller-Hinton Broth (MHB) (Column 3–12). Column 3 has the highest AgNp concentration, whereas column 12 represents the column with the lowest AgNp concentration. MHB acts as a negative control (Column 1), and MHB and inoculum act as positive controls (Column 2). After inoculation, the microtiter plate was placed in an incubator set at a temperature of 37°C for a duration of 24 hours. The minimum bactericidal concentration (MBC) was calculated using the spot method after 24 hours of incubation, where a loop of bacterial growth from the well was spotted on an MHA plate and incubated for 24 hours. The minimum inhibitory concentration (MIC) was established by adding 30 µL of a 0.002% resazurin solution to each well and incubating for 4 hours. After a duration of 4 hours, no alterations in colour were detected. The absence of bacterial growth was shown by a blue or purple colour, while bacterial growth was indicated by a pink or colourless colour.

Growth curve analysis

The effect of AgNPs on the growth kinetics of MRSA isolates was monitored by optical density measurements was carried out using standard protocol (Qais et al 2019). A 96-well microtitre plate was used to investigate the growth curve patterns. 100µL of the overnight-grown culture (~105 CFU/ml) was inoculated in each well containing varying concentrations of AgNPs (324mg/ml-0.632mg/ml). The control group consisted of wells containing only media (Column 1), while other wells consisted of media and silver nitrate, respectively (Columns 2-12). The rate of growth of the test bacterium was monitored using optical density measurements. The OD620nm was measured after 4 hours of incubation using a microtitre plate.

Results and discussion Phytochemical analysis

Phytochemical analysis revealed presence of terpenoids, alkaloids, flavonoids, saponin and glycosides. Literature report that presence of alkaloids, flavonoids, saponin, and terpenoids present in the banana pseudostem extract acted as reducing and capping agents for bio-reduction of silver ions into silver nanoparticles (Jini et al 2022). Similarly, glycosides participate in bio-reduction process (Usmani et al 2018).

UV-Visible spectroscopy

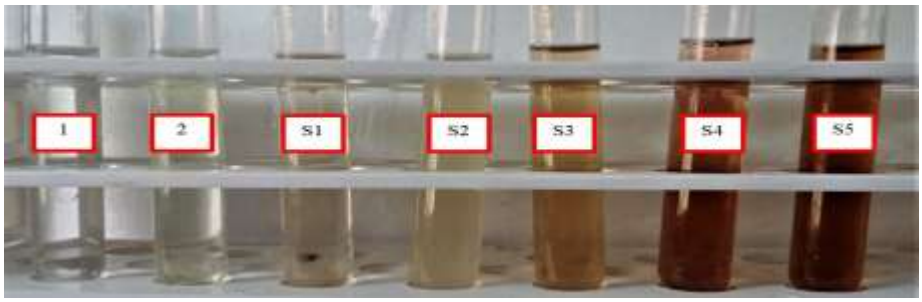
Figure 1.1 represents the various stages involved in extraction of aqueous banana pseudo-stem extract. Figure 1.2 various combinations of silver nitrate with banana stem extracts. Sample 01 represents silver nitrate solution (1mM) and sample 02 represents banana stem extract alone.

S1-S5 represents various proportions of banana stem extracts. Figure 1.3 (A-E) represents the UV-Visible spectral analysis of Banana Stem Extract mediated Silver Nanoparticle synthesis at various Silver Nitrate concentration. The mechanism for generating AgNPs in a water-based solution has been investigated through the analysis of its absorption spectrum within the range of 300–700 nm (Fig. 1.3). The incorporation of BSE extract into the silver nitrate solution resulted in a noticeable dark brown colour change, indicating the synthesis of AgNPs. In contrast, no change in colour was detected when the BSE was not present. A distinct and wide surface plasmon resonance (SPR) peak at 414 nm has been identified in the UV-Vis spectra, confirming the synthesis of AgNPs. The typical surface plasmon resonance (SPR) peaks for silver nanoparticles made by BSE were seen between 410 and 450 nm in wavelength. However, the BSE alone did not exhibit any SPR peaks for the entire course of the experiment (Figure 1.3 (A)). Nanoparticle synthesis is directly correlated with the quantity of the proteins, enzymes, and secondary metabolites participating in the metal reduction process (Ohiduzzaman et al 2024). Higher concentrations of BSE led to a gradual expansion and shifting of the SPR peaks towards longer wavelengths, ultimately leading to maximum absorbance (Jyoti et al 2016) (Figure 1.3 (E)). Literature have previously reported that silver nanoparticles (AgNPs) have a peak in their surface plasmon resonance (SPR) spectrum at wavelengths between 410 and 450 nm, attributing to presence of nanoparticles (Zaheer and Rafiuddin, 2012). Increased nanoparticle size results in increased absorption bands attributed to surface plasmon resonance. The absorption strength was found to be directly proportional to time, with the maximum absorption peak occurring at 428 nm (de Jesús Ruíz-Baltazar et al 2017). Various parameters, such as shape, dimension, morphological characteristics, solvent type, and concentration, affect the wavelength range of absorption (Ahmed et al 2016).



(A) Banana Stem (B) Boiling Process (C) Banana Stem Extract (BSE)

Figure 1.1: The various stages involved in extraction of aqueous banana pseudo-stem extract.



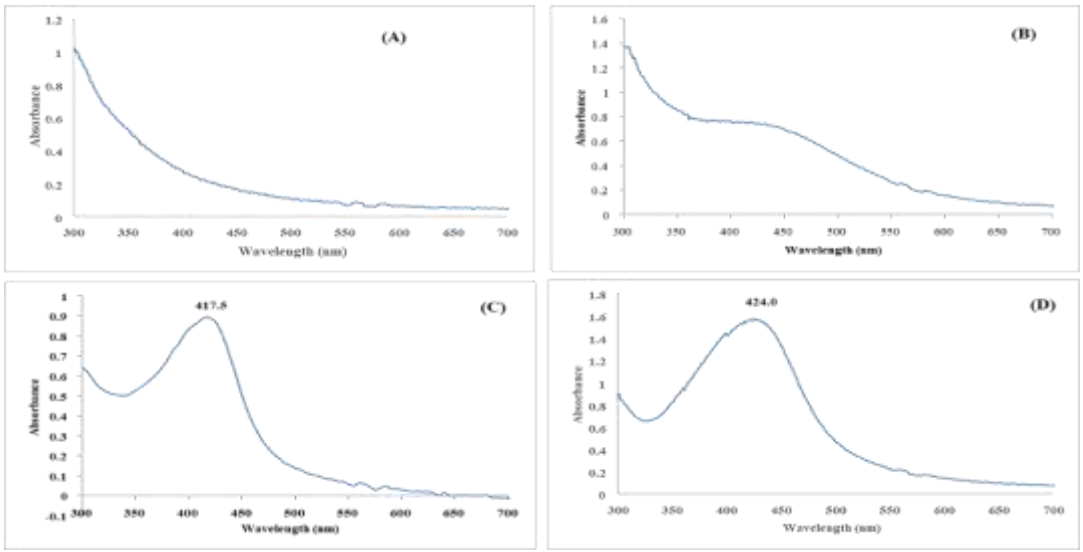


Figure 1.2: Various combinations of silver nitrate with banana stem extracts.

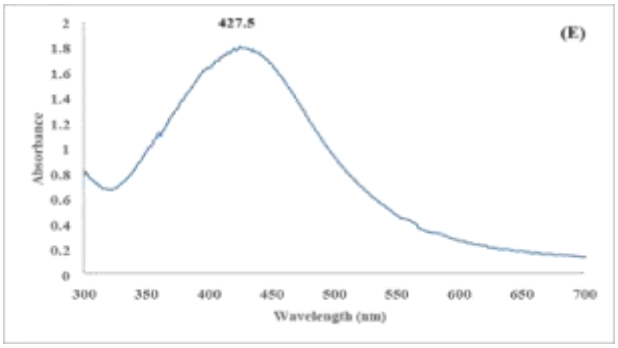


Figure 1.3 (A-E): UV-Visible spectral analysis of Banana Stem Extract mediated Silver Nanoparticle synthesis at various Silver Nitrate concentration.

FTIR

Spectra showed the presence of a strong absorption band for intermolecular bonded hydroxyl (O–H) groups at around 3800–3300 cm-1. The appearance of very intense and broad absorption was a clear indication of many hydroxyl groups associated with the fibre (Cellulose and hemi-cellulose) (Pereira et al 2014). The peaks around 2852 cm-1 is attributed to the asymmetric and symmetrical stretching modes of aliphatic methylene groups present in cellulose and hemicellulose (Das et al 2018). A strong shoulder peak at 2725 cm-1 represents a strong positive band of aldehydes (Judge et al 2008). 1460 cm-1, 1587 cm-1 is attributed to methylene stretching (-C=C-) and the band at 1373 cm-1 represents lignin (Pereira et al 2014). The short band at 1155 cm−1 is attributed to C–C stretching of the glycosidic bonds of cellulose & hemicellulose (Wei et al 2017). The bands below 1000 cm-1 (723 cm-1 and 457 cm-1) may be attributed to presence of alkyl halides. Literature have reported presence of few proteins and secondary molecules in the banana stem extract (Das and Velusamy, 2013). Literature related to FTIR studies further warrant that reducing and capping agents such as proteins and metabolites (Phenolic derivatives) plays a vital role in synthesis of silver nanoparticles (Jyoti et al 2016). Figure 1.4 represents FTIR analysis of synthesized Silver Nanoparticles.

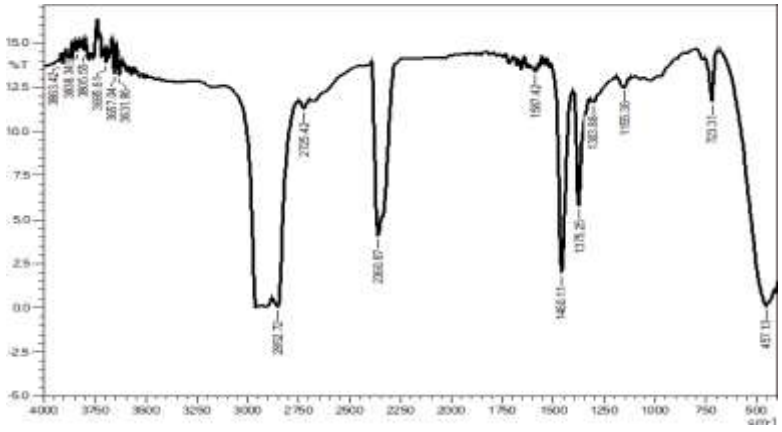


Figure 1.4 FTIR analysis of synthesized Silver Nanoparticles.

XRD

The peak shape suggests a complex overlapped with a sharp and a broader peak, which indicating the existence of very fine Ag nanoparticles. The observed diffractions were matched with JCPDS, File No. 04-0783 (Joint Committee on Powder Diffraction Standards) values for metallic silver. The sharp peak indicates the presence of a polycrystalline phase in the silver nanoparticles, validating the formation of face-centred cubic (FCC) crystalline silver nanoparticles (Ali et al 2023b). The increased size of silver nanoparticles is demonstrated by the broadening of their diffraction patterns compared to bulk silver. Diffraction peaks have been observed at 38.07, 44.29, 64.44, and 77.34 places in the XRD pattern, which correspond to 111, 200, 220, and 311 peaks, respectively (Ali et al 2023b). Moreover, XRD analysis indicated presence of crystalline silver chloride, closer to other scientific reports (Doan et al 2021). Figure 1.5 represents XRD analysis of synthesized Silver Nanoparticles.

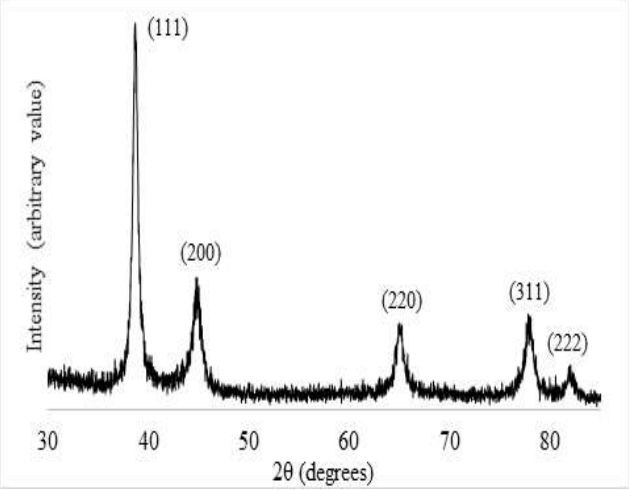


Figure 1.5 XRD analysis of synthesized Silver Nanoparticles.

TEM analysis

The transmission electron microscopy (TEM) picture shows silver nanoparticles (AgNPs) that are shaped like spheres and have some microscopic grains and nanoparticles spread out (Ohiduzzaman et al 2024). Figure 1.6 represents the TEM analysis of the synthesized Silver nitrate particles. Based on image analysis the particle size of synthesized nanoparticles was found to be 200nm (Approximately 3,00,000X). The silver nanoparticles were found to be at black spherical shape with uniform distribution against a bright background (Soltani et al 2024). Smaller diameter nanoparticles displayed greater activity because the surface area to volume ratio was inversely proportional to particle size (Sayed et al 2022). The spherical shape of Ag nanoparticles facilitates enhanced interaction with bacterial cells, leading to disruption of cell wall integrity and subsequent cell demise (Swolana et al 2020). It may be hypothesized that the metabolites banana pseudo-stem extract may act as a capping agent to inhibit excessive aggregation and ensure stability (Rautela et al 2019). Literature reported that spherical structure of the synthesized silver nanoparticles is warranted by Face-Centered Cubic (FCC) crystalline structure diffraction pattern of XRD analysis (Ali et al 2023b).

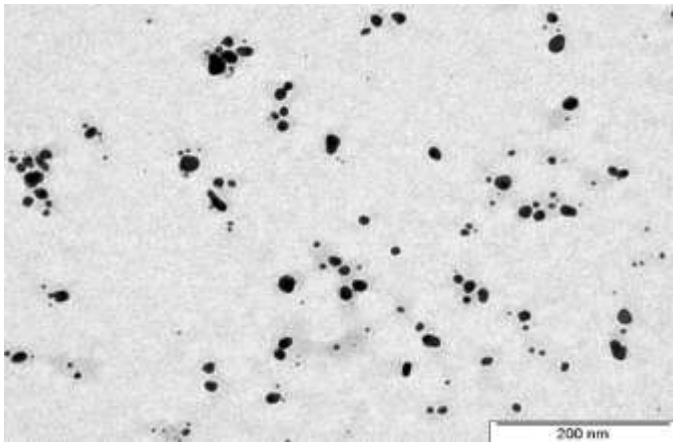


Figure 1.6 TEM analysis of the synthesized Silver nitrate particles.

Antibacterial activity

Antimicrobial activity was found of the biosynthesized AgNPs (100 µL) effectively inhibited MRSA (15±2mm). There was no substantial inhibition by the banana stem-extract and the negative control, while the positive control reported highest anti-microbial activity (20±2mm). Silver nanoparticles efficiently inhibit microbial activity in MRSA by altering the outer membrane structure and the functionality of binding sites through their interactions with the cell membrane (Leng et al 2020). The host environment may experience an increase in pH and osmotic pressure in the presence of AgNP nanoparticles (Wang et al 2017). This may lead to an osmotic imbalance, the release of ions, and a disruption in the membrane potential, which can damage the cell membrane and allow cytoplasm to leak out (Mikhailova, 2020). Figure 1.7 represents the anti-bacterial activity of Silver nanoparticles in MHA.



Figure 1.7: The anti-bacterial activity of Silver nanoparticles in MHA.

Growth curve analysis

Bacterial growth kinetics comprise three distinct stages: the lag stage, the exponential stage, and the stabilisation stage. Concentrations of nanoparticles with a concentration higher than
20.25 mg/mL induce a significant decrease in the number of live bacterial cells within 4–8 hours. On the other hand, nanoparticles with concentrations lower than 10.125 mg/mL demonstrate a decrease in the growth rate (between 8 and 16 hours), followed by a stabilisation phase. Silver nanoparticles (AgNPs) were reported to have a direct impact on the bacterial growth rate as they engage in electrostatic interactions with the cell membrane of microorganisms (Govarthanan et al 2016). This interaction results in changes to the structure of the cell, including a constricted cytoplasm and detached membrane, ultimately leading to the disruption of the cell wall (Leng et al 2020). The effect of synthesized silver nanoparticles on the growth kinetics of MRSA. Figure 1.8 represents the influence of silver nitrate nanoparticles on growth curve of MRSA.

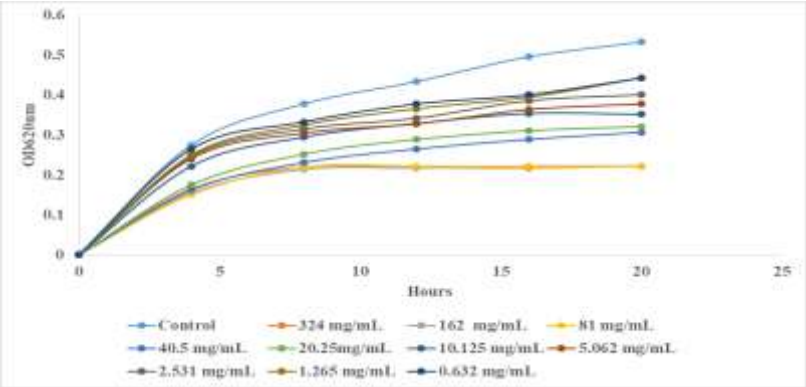


Figure 1.8: The influence of silver nitrate nanoparticles on growth curve of MRSA.

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) Evaluation

The MIC test is a technique employed to assess the antibacterial efficacy of a substance against particular bacteria. The MIC test using the microtitre plate dilution technique, is employed to identify metabolites that enhance antibiotic-induced death (Tao et al 2022). The minimum bacterial concentration (MBC) assessment approach, which is more cost-effective and convenient than the traditional Minimum Inhibitory Concentration (MIC) assessment, has been modified to evaluate metabolites that enhance antibiotic effectiveness. MBC is a vital parameter for evaluating the efficacy of antibiotics. The minimal bacterial concentration (MBC) of an antibacterial agent is determined by counting the bacteria on a plate following the MIC analysis. It significantly reduces the overall survival rate of the initial bacterial population by over 99.9%, and MBC is the minimum dose of an antibiotic substance that can decrease the bacterial count to below 10,000 CFU per well (Parvekar et al 2020). Literatures have reported that the S.aureus was effectively inhibited at silver nitrate nanoparticle concentration of 0.625 mg/mL and was assessed by MBC (Parvekar et al 2020). Silver nanoparticles have demonstrated efficacy against bacterial strains (Mateo and Jiménez, 2022). In addition to their antibacterial properties, they are capable of preventing bacterial and fungal infections and biofilm formation caused by a variety of bacteria and fungi (Prabhu and Poulose, 2012).

Methicillin-resistant Staphylococcus aureus (MRSA) has become a global phenomenon, with its prevalence spreading in both medical facilities and civilian environments (Okwu et al 2019). The absence of bacterial growth is represented by a blue or purple colour, while bacterial growth is indicated by a pink or colourless colour. The minimum inhibitory

concentration (MIC) was determined as the lowest concentration of antibacterial drugs that effectively suppressed bacterial growth, as indicated by the colour remaining blue. The minimum inhibitory concentration (MIC) of silver nanoparticles was observed at 81 mg/mL, whereas the minimum bactericidal concentration (MBC) was determined to be 162 mg/mL of silver nanoparticle concentration. The MBC/MIC ratio was found to be 2.0. Literatures report similar MBC/MIC values ≤4 for MRSA (Pazli et al 2024) and non-MRSA (Ayala-Núñez et al 2009). Experimental studies warrant that silver nanoparticles was found to be effective in inhibiting the microbial activity of MRSA. Literature have reported that silver ions exhibit a strong attraction to electron-donating groups found in cell membranes and proteins, causing changes in their structure and inhibiting the functioning of binding sites, thus inhibiting the growth of bacteria (Leng et al 2020). It was hypothesized that direct interaction with silver nanoparticles can result in cell membrane disruption, leading to detrimental effects on the cell membrane in in bacteria (Mikhailova, 2020). AgNPs nanoparticles can cause an increase in pH and osmotic pressure, leading to the release of ions and altering the membrane potential, resulting in membrane damage and the leaking of cytoplasm (Wang et al 2017). Reactive oxygen species (ROS), such as AgNPs, can generate ROS that harm internal cell membranes and regulate bacterial signalling pathways (Swolana and Wojtcyzka, 2022). Figure 1.9 represents the MBC and MIC assessment using 96 well microtitre plate assay.



Figure 1.9 MBC and MIC assessment using 96 well microtitre plate assay.

Conclusion

A bio-reduction reaction combining thrown-away banana peel extract (BPE) and silver nitrate solution effectively conducted the synthesis of silver nanoparticles. UV-visible spectroscopy, FT-IR, XRD, and TEM analyses were carried out to characterise and validate the formation of silver nanoparticles. Preliminary UV-visible examination indicated the existence of silver nanoparticles (428 nm). The BSE extract played a vital role in bio-reducing the silver nitrate into metallic silver, as observed from spectral analysis. It was observed that higher BSE concentrations resulted in a noticeable surface plasmon resonance (SPR) peak in the UV-Vis spectra. The transmission electron microscopy (TEM) experiment revealed the presence of black spherical nanoparticles with an average diameter of 200 nm. The FT-IR test showed that functional groups (Phenolic derivatives) make it easier for bio-reduction to turn silver nitrate into metallic nanosilver. XRD analysis indicated presence of crystalline silver chloride (~44°). An assessment was conducted to determine the growth kinetics, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) of AgNPs against Methicillin-Resistant Staphylococcus aureus (MRSA). We determined the minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and MBC/MIC ratio of silver nanoparticles against methicillin-resistant Staphylococcus aureus (MRSA) to be 81 mg/mL, 162 mg/mL, and 2.0,

respectively.

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