

Green Synthesis and Characterization of Silver Nanoparticles Using *Mimusops elengi* Flower Extract and Its Synergistic Antimicrobial Potential

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Authors' contributions

This work was carried out in collaboration of all the authors. Author JJ planned all experiments. Author PSP performed all the experiments. Authors SR and JJ designed, analyzed, interpreted data and wrote the whole manuscript. Author KK supported the chemical study. Authors PSP and KK supported the antibacterial activity study. Author YBAJ wrote the first draft of the manuscript and performed the correction of English throughout the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To express environment friendly method for the green synthesis of silver nanoparticles from *Mimusops elengi* flower extract and incorporation of AgNPs with selected antibiotics at distinct dose.

Study Design: Samples of *Mimusops elengi* flowers were collected in NMSSVN College garden. Silver Nitrate purchased from TCI Chemicals Chennai, Tamilnadu, INDIA. The procedure used is simple and sustainable making it suitable for economic production of SNPs.

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Place and Duration of Study: PG and Research Department of Chemistry, NMSSVN College, Madurai, Tamilnadu, INDIA, between December 2014 and September 2015.

Methodology: About 20 gm of finely cut flowers were kept in a beaker containing 200 mL double distilled water and boiled for 30 minutes. The extract was cooled down and filtrate was stored in refrigerator until the synthesis of silver nanoparticles was carried out. About 6-8 mL of the *Mimusops elengi* flower extract was added to 40 ml of 1 mM AgNO₃ solution for the reduction of Ag⁺ ions. The reduction of the Ag⁺ ions by *Mimusops elengi* flower extract in the solutions was monitored by UV-Vis spectrum further characterized by XRD, SEM-Eds and FTIR.

Results: This method shows that Aqueous Ag⁺ ions were reduced to AgNPs when added to the *Mimusops elengi* flower extract, this indicated by the color change from whitish yellow to brown and the control showed no color change. UV-Visible spectrophotometer showed absorbance peak in range of 431.96 nm. X-ray diffraction was employed to ascertain the crystalline nature and purity of the silver nanoparticles which implied the presence of (111) (200) and (220) lattice planes of the face centered cubic (fcc) structure of metallic silver.

Conclusion: AgNPs synthesis is a simple, clean which involves efficient method to reduce metal ions. *Mimusops elengi* flower extract was found to be an effective biological tool for the biosynthesis of AgNPs. The results confirmed that SNPs can act as a powerful antibacterial agent against various pathogenic bacteria.

Keywords: Green synthesis; silver nanoparticles; SEM; FTIR; antibacterial activity.

1. INTRODUCTION

Over the past few years, synthesis and characterization of nanoparticles has gained increasing momentum due to their large surface area to volume ratio because of which nanoparticles exhibit novel and new properties than their macroscopic counterparts. Nanoparticle research is inevitable today not because of only application and also by the way of synthesis [1]. Silver is a nontoxic, safe inorganic antibacterial agent that is capable of killing about 650 types of diseases causing microorganisms [2]. For the last two decades, the biosynthesis of noble metal nanoparticles (silver, gold, platinum, and palladium) has received considerable attention to develop environmentally sociable technologies in material synthesis with different chemical compositions, sizes, shapes, and controlled dispersities [3]. Applications of engineered Ag, Au, Fe, Pt, Zn, etc., and metal oxide NPs have evolved positively, influencing medicine, science, and many new agro-food industries [4]. The plant extract, which act as reducing and capping agents for nanoparticles synthesis, are more advantageous over other biological processes [5], because they eliminate the elaborated process of culturing and maintaining of the cell, and can also be scaled up for large-scale nanoparticle synthesis [6]. Incidentally, biological systems have long been known to reduce metal ions into nano-sized particles [7] and many researchers have recently reported the biogenic synthesis of silver and gold nanoparticles using a wide range of biological resources like bacteria

[8], fungi and plants [9,10]. Colloidal silver is of particular interest because of distinctive properties, such as good conductivity, chemical stability, catalytic and antibacterial activities [11]. Among the metal nanoparticles, silver nanoparticles (SNPs) have gained importance due to their applications in diverse areas such as catalysts, nanomedicine, biological labeling, sensing, solar cell surface coatings, electronics and surface-enhanced Raman scattering detection, staining pigments for glasses and ceramics and antimicrobial agents [12]. The AgNPs have several important applications in the field of antimicrobial agents, capable of purifying drinking water, degrading pesticides and killing human pathogenic bacteria. A literature survey shows that smaller AgNPs having a large surface area available for interaction would have a stronger antibacterial effect than larger AgNPs. It is also possible that AgNPs not only interact with the membrane surface, but may also penetrate inside bacteria. Antibacterial effects of AgNPs may be associated with the characteristics of certain bacterial species [13-16].

The present study aims to synthesize silver nanoparticles by a green biological route, using an extract derived from *Mimusops elengi* flower extract and characterization of the synthesized nanoparticles utilizing UV-visible spectroscopy, scanning electron microscope (SEM-Eds), X-ray diffraction (XRD) and Fourier transform infrared spectroscopy (FT-IR) analysis. Besides, their antimicrobial activity against representatives of human pathogenic microorganisms was investigated.

2. MATERIALS AND METHODS

2.1 Microorganisms

Representative microorganisms of Gram-positive bacteria (*Staphylococcus aureus*, *Bacillus cereus*) and Gram-negative bacteria (*Escherichia coli*, *Klebsiella pneumonia*) were used to evaluate the antimicrobial activity of prepared silver nanoparticles. Bacterial strains were maintained on nutrient agar slants and the yeast was maintained on potato-dextrose agar slants at 4°C. The different concentrations used (10, 20, 30 and 40 µL) for the identification of antimicrobial activity of the above bacterial species. All the plates were incubated at 37°C for 24 hours, and the zone of inhibition of bacteria was measured. The bactericidal activity is evaluated by the size of clear zone and greater the zone of inhibition greater the bactericidal activity.

2.2 Materials

Silver nitrate (AgNO₃) was obtained from TCI Chemicals, Chennai, Tamilnadu, India. Deionized water was used throughout the reactions. All glass wares were washed with dilute nitric acid HNO₃ and distilled water, then dried in hot air oven. A stock solution of AgNO₃ 2×10⁻² M was prepared by dissolving 0.34 g/100 ml de-ionized water.

2.3 Preparation of Flower Extract

Mimusops elengi flower extract was used to prepare silver nanoparticles on the basis of cost effectiveness, ease of availability and its medicinal property. Flowers were collected from NMSSVN College, Tamilnadu, India, campus in the month of April 2015. They were surface cleaned with running tap water to remove debris and other contaminated organic contents, followed by double distilled water and air dried at room temperature. About 20 gm of finely cut flowers were kept in a beaker containing 200 mL double distilled water and boiled for 30 minutes. The extract was cooled down and filtered with Whatman filter paper no. 1. Then the collected filtrate was stored in refrigerator until the synthesis of silver nanoparticles was carried out. This extract was used as reducing as well as stabilizing agent.

2.4 Synthesis of Silver Nanoparticles

Aqueous solution (1 mM) of silver nitrate (AgNO₃) was prepared and used for the

synthesis of silver nanoparticles. Certain volume of the *Mimusops elengi* flower extract (8mL) was added to 40 ml of 1 mM AgNO₃ solution for the reduction of Ag⁺ ions. The reduction process Ag⁺ to Ag⁰ nanoparticles was followed by the color change of the solution from yellow to brownish-yellow to deep brown. The synthesis of silver nanoparticles was carried out at room temperature (25°C±2°C) for 24 h in dark. The silver nanoparticle solution thus obtained was purified by repeated centrifugation at 10,000 rpm for 10 min followed by redispersion of the pellet of silver nanoparticles into acetone. After air drying of the purified silver particles they were stored at 4°C for further analysis.



Fig. 1. Picture of *Mimusops elengi* flower

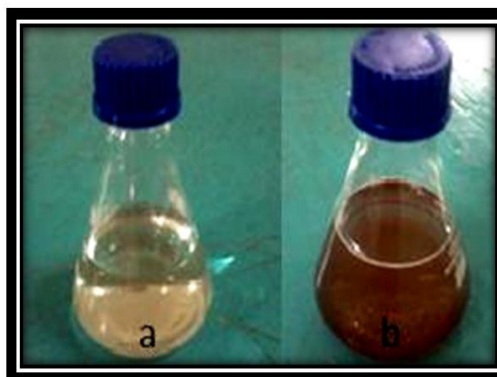


Fig. 2. Digital picture of *Mimusops elengi* flower extract with 1 mM AgNO₃ solution before and after the *Mimusops elengi*-AgNPs

3. CHARACTERIZATIONS

3.1 UV-vis Spectral Analysis

UV-visible spectroscopy is one of the most widely used techniques for structural

characterization of SNPs. The preliminary characterization of silver nanoparticles was monitored by measuring the UV–Vis spectrum of the reaction medium in the wavelength range from 190 to 1100 nm. The reduction of silver nitrate to silver nanoparticles was analyzed by recording the spectra of silver nanoparticles from 340 to 700 nm (Shimadzu UV 150-02, Japan). A spectrum of silver nanoparticles was plotted with wave length on x-axis and absorbance on y-axis. The absorbance peaks can be observed in Fig. 3.

3.2 X-ray Diffraction (XRD)

The crystal structure of the produced AgNPs was determined and confirmed by using X-ray diffractometer (Model PW 1710 control unit Philips Anode material Cu, 40 KV,30 M.A, optics: Automatic divergence slit) with Cu K α radiation $\lambda=1.5405 \text{ \AA}$ over a wide range of Bragg angles ($30^{\circ}\leq 2\theta\leq 80^{\circ}$). An elemental analysis of the sample was examined by energy dispersive analyses of X-rays with JED-2300 instrument. The crystallite domain size was calculated from the width of the XRD peaks, assuring that they are free from non uniform strains. The particle size of the prepared samples were determined by using Scherrer's equation as follows $D\approx 0.94\lambda/\beta\cos\Theta$, where D is the average crystallite domain size perpendicular to the reflecting planes, λ is the X-ray wavelength, β is the full width at half maximum (FWHM) and Θ is the diffraction angle.

3.3 Fourier Transform Infrared (FTIR) Spectroscopy

For the Fourier transform infrared (FTIR) spectroscopy analysis, the vacuum dried AgNPs were mixed with potassium bromide (KBr) and the spectra were recorded with a Perkin Elmer Spectrum Express version 1.03.00. The scanning data were obtained from the average of 47 scans in the range $4000\text{--}400 \text{ cm}^{-1}$ with the resolution of 4 cm^{-1} .

3.4 Scanning Electron Microscopy–Energy Dispersive X-ray Spectrometry (SEM–EDX) Analysis

The microstructure and composite homogeneity of the obtained samples were investigated using a SEM/EDX scanning microscope JEOL-JSM 64000 LV. Energy dispersive X-ray analysis measurements were performed under standard

conditions. The silver nanoparticles were centrifuged at 10,000 rpm for 30 min and the pellet was redispersed in 10 mL ethanol and washed 3 times with sterile distilled water to obtain the pellet. The pellet was dried in an oven and thin films of dried samples (10 mg/mL) were used for compositional analysis.

3.5 Antimicrobial Activity by Well Diffusion Method

The antibacterial activity was done by standard disc diffusion method. Nutrient agar(NA) plates were seeded with 8 h broth culture of different bacteria. In each of these plates, well were cut out using sterile cor borer. Using sterilized dropping pipettes, different concentrations (10, 20, 30, 40 $\mu\text{l/well}$) of sample was carefully added into the wells and allowed to diffuse at room temperature for 2 h. The plates were then incubated at 37°C for 18-24H. Gentamicin (30 μg per disk) was used as the positive control and sterile distilled water as the negative control. The antibacterial activity was evaluated by measuring the diameter of inhibition zone.

4. RESULTS AND DISCUSSION

The detailed study on biosynthesis of silver nanoparticles by natural flower extract such as *Mimusops elengi* was employed and is reported in this work.

4.1 UV–visible Studies

Noble metals are known to exhibit unique optical properties due to the property of surface plasmon resonance (SPR) [17]. The optical properties of the synthesized AgNPs using UV–Vis spectroscopy were studied and the recorded spectra are shown in Fig. 3. The absorption behavior shown in Fig. 3a arises due to surface plasmon resonance (SPR) [18], which is the result of the coherent oscillation of the electrons in the conduction band of the AgNPs induced by the electromagnetic field. It is well known that AgNPs exhibit yellowish brown color in aqueous solution due to the excitation of surface plasmon vibrations in Ag NPs [19]. Fig. 3a shows the UV–Vis spectra of the synthesized AgNPs, which give a sharp absorbance band at around 431.90 nm at room temperature. Broadening of peak indicated that the particles are polydispersed. The UV-Visible spectrum Fig. 3b of the leaf extract shows a band at 234.09 nm arising due to $n\text{--}\pi^*$ transition.

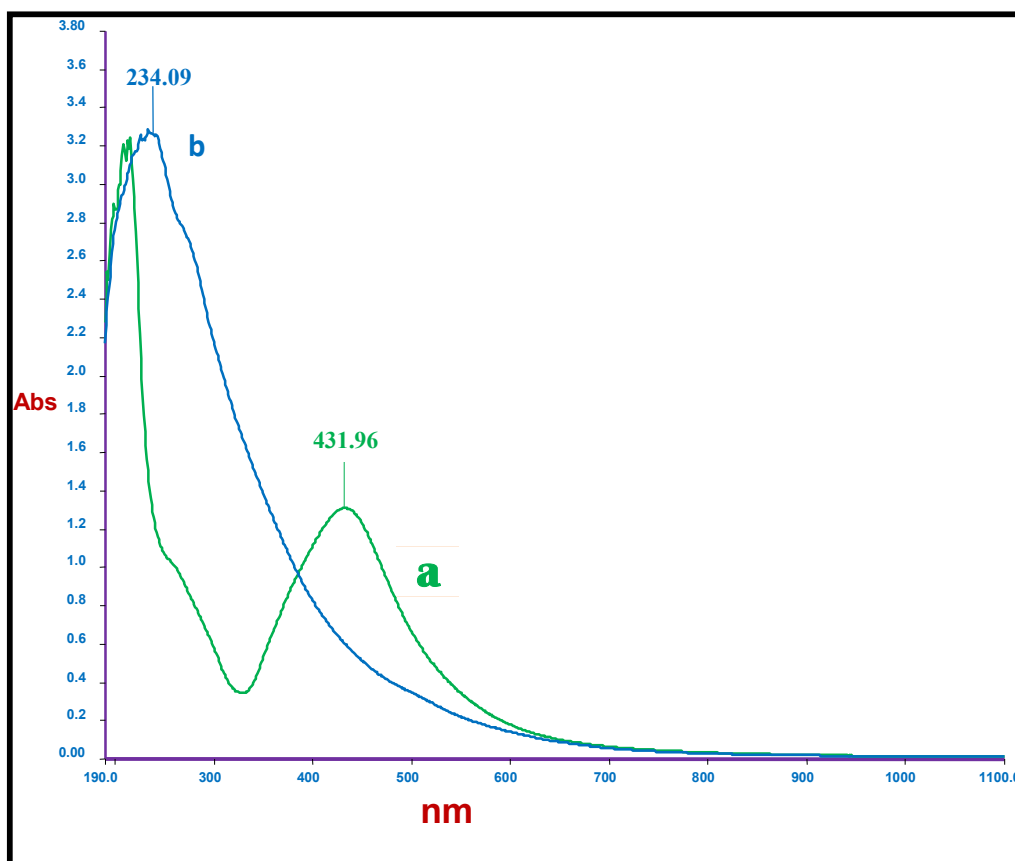


Fig. 3a). UV-visible absorption spectra of synthesized silver nanoparticles, showing the surface plasmon resonance peak at 431.96 nm
b). UV-Visible absorption spectra of *Mimusops elengi* flower extract

4.2 Characterization of SNPs by XRD

The sample of SNPs could be also characterized by X-ray diffraction analysis of dry powders. The diffracted intensities were recorded from 10° to 80° at 2 theta angles (Fig. 4). The results revealed four diffraction peaks [38.05 (111), 46.12 (200), 67.19 (220), 76.3 (311)] are indexed as fcc (JCPDS file no-04-0783). The (200), (220), (311) Bragg reflections are weak and broadened relative to the intense (200) reflection. This feature indicates that the nano crystals are highly anisotropic. The mean size of nanoparticles is calculated using Debye-scherrer's equation by determining the width of the (200) peak [20].

All the peaks in XRD pattern can be readily indexed to a face-centered cubic structure of silver as per available literature. The broadening

of the Bragg peaks indicates the reduction in grain size. The secondary phases are 17(002), 33(004), 55(006), that shows hexagonal, but no sharp peak obtained. The environmental distortion may be involved. The XRD report shows that it may be mixed crystal (cubic, hexagonal). The size of the samples was calculated from the Scherrer's formula:

$$\begin{aligned}
 D &= \frac{0.9 \times 1.5408 \times 10^{-10}}{2.5748 \times 10^{-3} \times 0.9599} \\
 &= \frac{1.38672 \times 10^{-10}}{2.47155052 \times 10^{-3}} \\
 &= 56.10 \times 10^{-9} \text{ nm}
 \end{aligned}$$

The size of silver nanoparticles synthesized by green synthesis was estimated to be 56.10 nm.

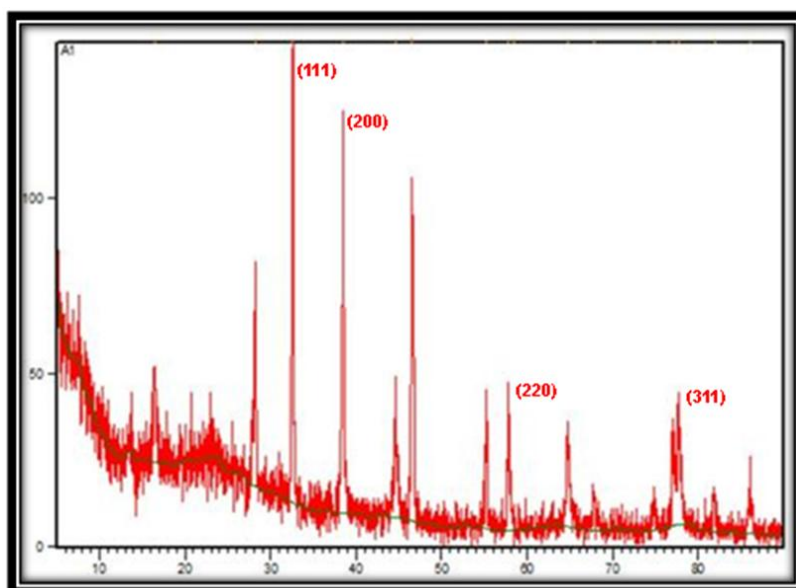


Fig. 4. XR diffractogram of silver nanoparticles

4.3 FT-IR Analysis

FTIR is used to predict the role of bonding (stabilizing) and reducing capability of *Mimusops elengi* flower extract. The nature of the biomolecules involved in the reduction and formation of silver nanoparticle was studied by FTIR.

The FTIR spectroscopy of *Mimusops elengi* flower extract (Fig. 5a) shows prominent peaks at 1050.08 cm^{-1} , 1385.77 cm^{-1} , 1679.89 cm^{-1} and 3501.01 cm^{-1} due to C–N stretching (aliphatic amines), C–H group (aromatic), C=O stretching and O–H stretching respectively. Other minor peaks indicate that flower extract contains proteins, terpenoids and other secondary metabolites.

Fig. 5b which shows comparatively similar absorption bands at 3465.46 cm^{-1} , 3434.08 cm^{-1} , 2074.44 cm^{-1} , 1636.95 cm^{-1} , 674.55 cm^{-1} in the FT-IR spectrum of silver nanoparticles synthesized by flower extract of *Mimusops elengi*.

The IR spectrum of silver nanoparticles shows a band at 3465.46 cm^{-1} which corresponds to O–H and N–H stretching of alcohols and phenols and lower wave number shoulder at 3434.08 cm^{-1} . The peak at 2074.44 cm^{-1} corresponds to C≡N stretching vibrations. The peak corresponding to 1636.95 cm^{-1} indicates carbonyl stretching and

674.55 cm^{-1} corresponds to aromatic stretching vibrations [21,22]. The suppression of C=O stretching in the 1636.95 cm^{-1} region has been related to evidence of silver ion reduction by *Mimusops elengi* as consequence of silver nanoparticles.

These observations indicate the presence and binding of proteins with silver nanoparticles which can lead to their possible stabilization. FTIR results revealed that secondary structure of proteins have not been affected as a consequence of reaction with silver ions or binding with silver nanoparticles. It is important to understand though, that it is not just the size and shape of proteins, but the conformation of protein molecules that plays an important role.

4.4 SEM–EDX Analysis

The compositional analysis using EDAX showed strong characteristic signal of silver, along with weak signals from few other elements like C and O (Fig. 6). These signals could have arisen from proteins and enzymes that had stabilized the nanoparticles. The vertical axis displays the number of X-ray counts while the horizontal axis displays energy in k.eV. Identification lines for the major emission energies for silver (Ag) are displayed and these corresponds with peaks in the spectrum, thus giving confidence that silver has been correctly identified [23].

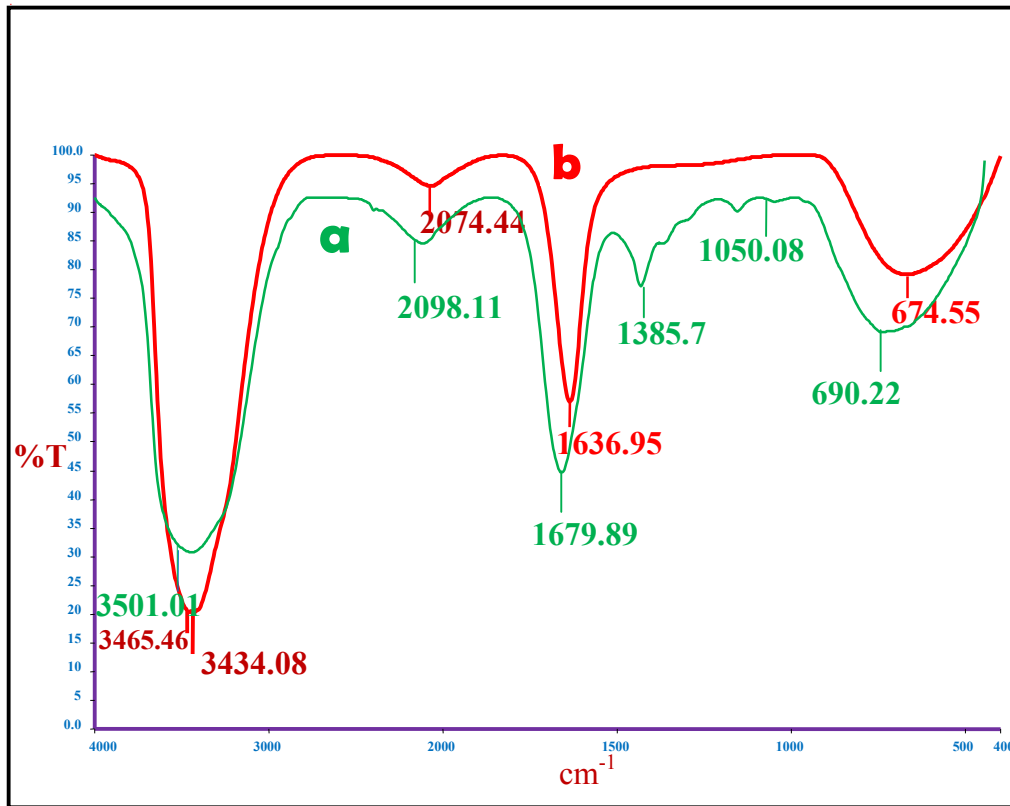


Fig. 5. FTIR spectra of a). *Mimusops elengi* flower extract and b). Silver nanoparticles synthesized using the flower extract of *Mimusops elengi*

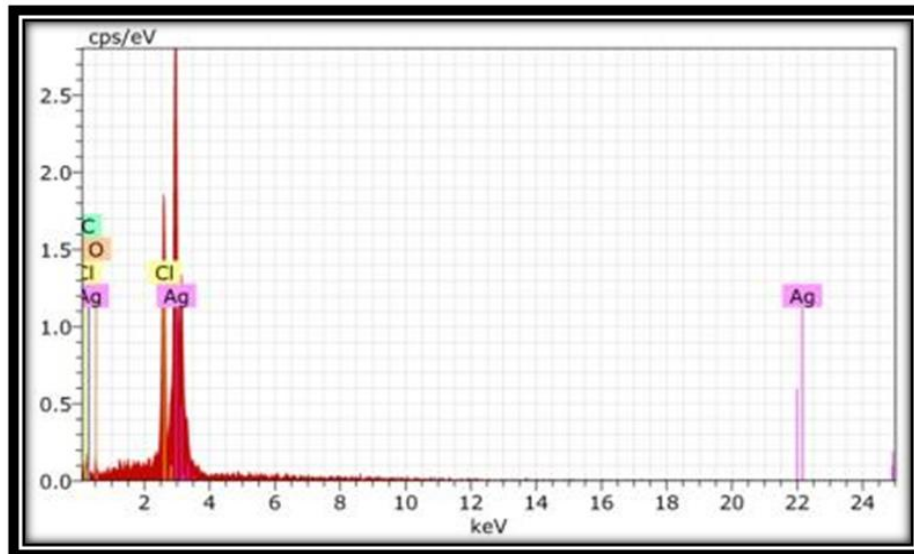


Fig. 6. SEM-EDX profile of the synthesized silver nanoparticles

Metallic silver nano crystals generally show typical optical absorption peak approximately at 3 Kev due to surface plasmon resonance. The presence of chlorine peak arise from the

phytochemical compounds like alkaloids, glycosides, tannins, flavonoids, terpenoids, saponins, fats and oil.

4.5 Antibacterial Activity

Silver ions as well as AgNps were known to have strong antimicrobial Activities [24]. The results showed that the highest enhancing effect was observed for Gentamicin against *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli* and *Klebisella pneumonia*. Therefore, we selected this antibiotic to examine the antibacterial activity in Gram-negative and Gram-positive bacteria, respectively.

The AgNPs solution exhibits good antibacterial activity against the Gram-negative and Gram-positive bacteria. The radial diameter of the inhibiting zones of *Bacillus cereus*, *Escherchia coli*, *Staphylococcus aureus*, *Klebisella pneumonia* is 13, 15, 13 and 16 mm respectively (Table 1).

Fig. 7 represents the clear inhibition zone made by the AgNPs solution. The clear zone indicates bacterial growth restriction by the diffused AgNPs solution. The synthesized AgNPs have great potential due to their antibacterial activity. Thus, the green synthesized nanoparticles have more effective antibacterial activity to the pathogens [25]. Siddhartha Shrivastava et al concluded that the antibacterial effect of silver nanoparticles was more pronounced against gram (-) than gram (+) bacteria. A comparative analysis was done on the basis of this concept [26]. In this investigation, we found that AgNPs synthesized using *Mimusops elengi* flower have potential antibacterial activity against Gram-negative bacteria.

In addition, the exact mechanism of the inhibition of the bacteria is still unknown, but some hypothetical mechanisms show that the inhibition is due to ionic binding of the silver nanoparticles on the surface of the bacteria which creates a great intensity of the proton motive force, and the one hypothesis from the research states that the silver nanoparticles invade the bacterial cell and bind to the vital enzymes containing thiol groups.

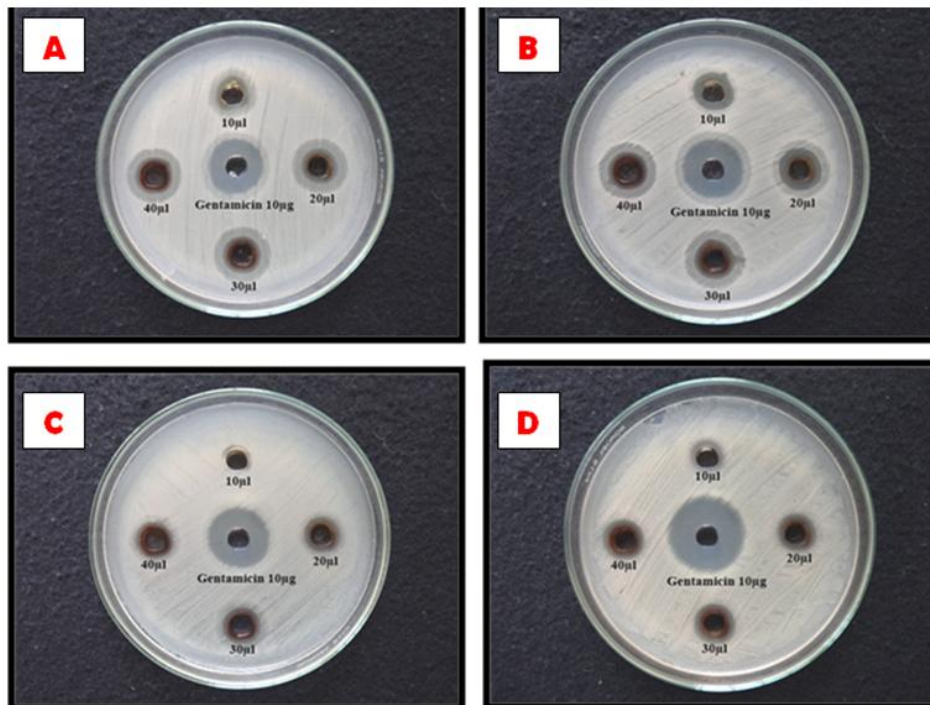


Fig. 7. Antibacterial activity of silver nanoparticles synthesized using the flower extract of *Mimusops elengi* against a). Human pathogenic *Bacillus cereus* b). *Escherchia coli* c). *Klebisella pneumonia* d). *Staphylococcus aureus* by the standard disc diffusion method

Table 1. Effect of silver nanoparticles on human pathogens

Pathogens	Variety of bacteria	Zone of inhibition	
		Plant silver	Gentamicin
<i>Bacillus cereus</i>	Gram-positive	13 mm	23 mm
<i>Escherchia coli</i>	Gram-negative	15 mm	25 mm
<i>Klebisella pneumonia</i>	Gram-negative	16 mm	26 mm
<i>Staphylococcus aureus</i>	Gram-positive	13 mm	27 mm

Also, the findings of Sereemasun et al. suggested the inhibition of oxidation-based biological process by penetration of metallic nanosized particles across the microsomal membrane. The molecular basis for the biosynthesis of these silver crystals speculated that the organic matrix contains silver binding properties that provide amino acid moieties that serve as the nucleation sites [27-33].

5. CONCLUSION

In this investigation the biosynthesized silver nanoparticles were characterized by UV-Visible, FTIR, SEM-Eds and XRD. This green synthesis method is alternative to chemical method, since it is cheap, pollutant free and eco-friendly. The potential antibacterial activity of silver nanoparticles was performed and the antibacterial activity was observed against *Bacillus cereus*, *Escherchia coli*, *Klebisella pneumonia*, and *Staphylococcus aureus* bacteria. Applications of such eco-friendly nanoparticles bactericidal, wound healing and other medical and electronic application, makes this method potentially exciting for the large-scale synthesis of other inorganic nanomaterials. In addition, we showed a clear synergistic effect between silver nanoparticles and tested antibiotics. Thus, our results show that AgNPs synthesized using an aqueous extract of *Mimusops elengi* flower extract have potential antibacterial activity against Gram-negative bacteria. Toxicity studies of silver nanoparticles on human pathogen open a door for a new range of antibacterial agents and anticancer agents. Further studies with other plant-mediated synthesis of silver nanoparticles are in progress.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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