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# CHARACTERIZATION AND ANTIBACTERIAL EFFICACY OF *ALBIZIA AMARA* MEDIATED BIOGENIC SILVER NANOPARTICLES

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**Keywords:**

AgNPs, *Albizia amara*, Antibacterial activity, Green synthesis, Nanoparticles

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**ABSTRACT:** Green Nanotechnology techniques have emerged in response to the growing need for sustainable and eco-friendly approaches to synthesize nanoparticles. The present study provides an endurable procedure for the green-mediated synthesis of silver nanoparticles (AgNPs) from *Albizia amara* leaf extract and its antibacterial potential. Various techniques like UV-Vis spectroscopy, FT-IR, XRD and FE-SEM were employed to characterize the properties of the silver nanoparticles. The UV-Vis spectra exhibited a maximum peak for AgNPs at 425nm, confirming their absorption capability. The presence of OH and COOH functional groups was evident from the FT-IR analysis which contributes to the stability of nanoparticles. The XRD patterns displayed distinct diffraction peaks. The morphology of AgNPs was determined by FE-SEM and the elemental composition of synthesized nanoparticles was exhibited in EDS mapping. When the silver nanoparticle was tested against the clinical pathogens, the results demonstrated a considerable inhibition of bacterial growth. The magnitude of the zone of inhibition exhibited by silver nanoparticles showed high antibacterial activity with zones measuring 19mm against *Enterococcus faecalis* strain. The results state that the synthesized nanoparticle may be a potent growth inhibitor against the pathogenic strains.

**INTRODUCTION:** A growing issue in public health is the emergence of drug-resistant disease-causing microorganisms. The upsurge of microbial organisms resistant to several antibiotics has prompted numerous researchers to focus on creating new, potent antibacterial agents that are more efficient causing fewer side effects [1].

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Nanotechnology offers ways to explore and modify biological systems into a green method for synthesizing nanomaterials without causing harm. Throughout human history, medicinal plants have been used to treat illness and heal them [2].

Plants can produce a wide variety of chemical substances that are responsible for different biological functions. Plant-mediated techniques for sustainable production of nanostructures will identify the phytoconstituents that give rise to nanoparticles that are predicated on the biosynthetic mechanism[3]. Nanoparticles have drawn tremendous interest because of their tunable surface characteristics, size and shape.

These special characteristics have encouraged us to research the possible uses of nanoparticles in various therapeutic platforms [4]. Developing dosage forms based on nanotechnology in phyto-formulation research offer numerous benefits for herbal medications.

*Albizia amara*, a member of the Fabaceae family, is a useful medicinal tree with several uses found in India's arid woodlands. Over 100 species of *Albizia* can be found in the tropical and subtropical regions of Asia, Africa and Australia. Most significantly, *Albizia amara*, *Albizia lebbeck* and *Albizia procera* are among the most valuable species in ayurvedic medicine. *Albizia amara* is an ethnomedical and traditional plant used in treating human ailments [5]. Many endemic diseases can be treated with phytoconstituents found in plants, which include anti-inflammatory, anti-cancer, anti-hyperlipidemic, antibacterial, analgesic and antioxidant properties [6].

The rationale behind using biosynthesized nanoparticles is justified by their accessibility over wide range of metabolites. The prominent constituents like stabilizing agents, reducing agents and solvent medium are the factors that determine the biosynthesis of nanoparticles. The richness of bioactive compounds from plant extract can act as both reducing and stabilizing agents. Silver particles, also known as "Rajatha Bhasma" (calcined silver ash), have been utilized extensively in Ayurvedic medicine from ancient times to cure various diseases. The chemical synthesis of AgNPs is undesirable due to the excessive and repetitive use of dangerous chemicals, high temperatures and pressure. In contrast to chemical techniques, the green synthesis of AgNPs has exceptional [7, 8, 9] stability. Plant extract-derived AgNPs are nontoxic and use a variety of metabolites to encourage the bio-reduction of silver into silver ions. Thus, the distinctiveness of this research lies in identifying the structural properties of AgNPs synthesized from *Albizia amara* leaf extract and investigating the antibacterial properties of varying concentrations of silver nanoparticles against strains that are resistant to multiple drugs.

## MATERIALS AND METHODS:

**Collection and Preparation of *A. amara* Leaf Extract:** The fresh and healthy leaves of *Albizia amara* were collected from K. Paramathi, Karur district, Tamil Nadu (Lat 10.957417°Long 77.905468°) and identified by Botanical Survey of India, Southern Regional Campus, Coimbatore, India. 10 g of fresh leaves were taken and washed using distilled water, then finely chopped and ground using a mortar pestle and the ground leaves were added to 100 mL of distilled water and boiled for 15 min and cooled. The filtrate was allowed to centrifuge at 8000 rpm for 10 min and the supernatant was stored [10]. This aqueous extract is further used for the phytochemical screening and synthesis of silver nanoparticles. The extract was tested for the presence of bioactive compounds using standard methods.

**Green Synthesis of Silver Nanoparticles:** 90 mL of an aqueous solution containing 1 mM $AgNO_3$ was mixed with 10 mL of *A. amara* leaf extract in order to reduce Ag into Ag+ ions. The reaction mixture allowed to stand in dark condition for 24 hours of incubation. The silver nitrate solution without leaf extract was maintained as a control. After the incubation period silver nanoparticle solution was centrifuged for 20 min at 10,000 rpm[11]. The pellet was washed with distilled water to wash off impurities. The resulting solution was dried to get powdered silver nanoparticles.

**Characterization of Synthesized Nanoparticles:** The synthesized nanoparticles were investigated for their optical properties using UV-visible spectrophotometer at 300 to 800 nm. The functional molecules present in the nanoparticles is responsible for the reduction and stabilization of any nanostructures [12]. So, FT-IR spectra we rerecorded using KBr discs with a wave number of 4500 $cm^{-1}$ to 400 $cm^{-1}$ to detect the functional groups present in the nanoparticles. The crystalline structure of synthesized silver nanoparticles was determined using X-ray diffraction analysis [13]. The morphology of the nanoparticle was evaluated using the FE-SEM, where the dried nanoparticles were drop casted on the carbon grids. The EDS attached with the FE-SEM shows the elemental composition of the synthesized nanoparticles.

**Antibacterial Activity of Silver Nanoparticles:** The antibacterial activity of the synthesized nanoparticles was tested using the standard agar well diffusion method, which determines the zone of inhibition of selected pathogenic bacterial strains. Both gram-positive (*Enterococcus faecalis, Staphylococcus aureus*) and gram-negative (*Escherichia coli* and *Klebsiella pneumoniae*) multi-drug resistant clinical pathogens were used. The test samples were prepared in different concentrations (10, 20 and 30 μg/mL) against each strain on Muller Hinton Agar plates followed by incubation at 37°C for 24 hours [14]. The respective positive and negative controls for each bacterial strain were used. Antibacterial activity was assessed using a Zone of Inhibition (ZoI) measured after the incubation period of each tested pathogen.

## RESULTS AND DISCUSSION:

**Phytochemical Analysis:** The choice of the plants under investigation was aided by traditional knowledge of inhabitants and literature regarding their ethnomedicinal qualities. The richness of secondary metabolites present in the plant corresponds to its therapeutic efficacy in pharmacological applications. In the present investigation saponins, carbohydrates, phenols and steroids were detected **Table 1**.

In the previous research done with ethanolic extract, phytochemicals like alkaloids, flavonoids and steroids were reported. However, the presence of phenolic compounds was not reported [6]. Disparities between the current and earlier research were noted. The variations may have resulted from differences in the genetic composition of the plant, the climate and geographic location, the extraction process or their phytochemicals. Yet the aqueous extraction worked better in our investigation for extracting a variety of metabolites from this plant. Phenolics are a class of compound that exhibits a wide range of inhibition towards clinical pathogens[15]. The presence of phenolic compound in the present study encouraged to evaluate antibacterial activity which could be a potent therapeutic agent.

**TABLE 1: PHYTOCHEMICAL ANALYSIS OF AQUEOUS EXTRACT OF *ALBIZIA AMARA***

| Secondary metabolite | Qualitative Tests | Result |
|---|---|---|
| Alkaloids | Mayer's test | - |
| Flavonoids | Ferric chloride test | - |
| Terpenoids | Salkowski test | - |
| Carbohydrates | Molisch's test | + |
| Saponins | Foam test | + |
| Glycosides | Fehling's test | - |
| Quinones | Folin-Cioalateu test | - |
| Phenol | Ferric chloride test | + |
| Tannins | Ferric chloride test | - |
| Steroids | Salkowski test | + |

**Green Synthesis of Silver Nanoparticles:** The crude extract was used in the biosynthesis of AgNPs. Color change served as a preliminary confirmation of AgNP synthesis. The color change from pale yellowish green to brown was observed after 12 hours of incubation indicating the reduction reaction [16].

The appearance of a reddish-brown color after 24 hours confirmed the presence of silver nanoparticles **Fig. 1**. The secondary metabolites present in the leaf extract have enhanced the reduction of silver nitrate to silver nanoparticles. Similar to our observation, studies conducted on various species showed the presence of reddish-brown color.

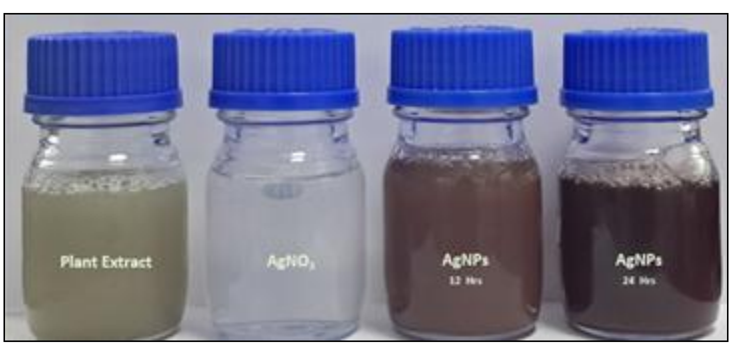


**FIG. 1: GREEN SYNTHESIS OF SILVER NANOPARTICLES USING *ALBIZIA AMARA***

**Characterization of Silver Nanoparticles:** The properties of the silver nanoparticles synthesized using *Albizia amara* were depicted using techniques like UV-Visible spectroscopy, FT-IR, XRD and FE-SEM. The preliminary characterization was carried out in the absorbance region of 300 to 800nm using UV-visible spectrophotometry. The synthesized AgNPs showed highest spectral shift of UV- absorbance peak at 425 nm and found to be stable after 48 hours **Fig. 2**. According to previous reports, AgNPs are known for their UV-visible spectral absorption, which is between 350 and 500 nm[17, 18].

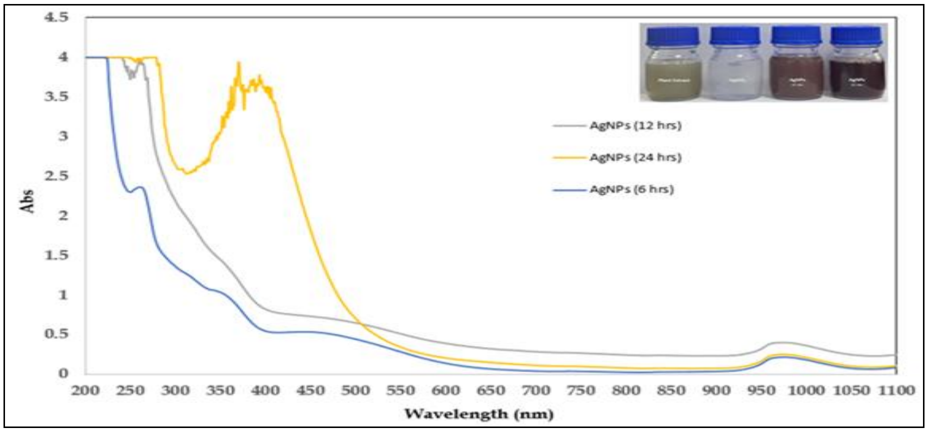


**FIG. 2: UV-VISIBLE SPECTROSCOPIC ANALYSIS OF SILVER NANOPARTICLES**

Fourier Transform Infrared Spectroscopy has become an imperative tool to identify biomolecules present in leaf extract of *A. amara* which are responsible for the reduction, capping, and stabilization of silver nanoparticles. The FT-IR Spectrum showed bands of *A. amara* extract at 678.9 $cm^{-1}$, which correspond to C-Cl stretching vibrations, 1635.6 $cm^{-1}$, corresponding to the amido group (C=O stretching), and 3340.7 $cm^{-1}$, which is related to O-H stretching vibrations which increased due to nanoparticles formation, clearly show their interaction with the silver nanoparticle[19,20]. The FT-IR Spectrum showed bands at 470.6 $cm^{-1}$, 547.8 $cm^{-1}$, 902.7 $cm^{-1}$ and 2916.4 $cm^{-1}$ showing alternations in the peaks as compared to the extract with the formation of Ag nanoparticles **Fig. 3**. By comparing the FT-IR spectra of *A. amara* extract and synthesized nanoparticles, it can be confirmed that the extract plays a dual role as reducing and stabilizing or capping agent.

X-ray Diffraction (XRD) is a widely used technique for characterizing crystalline materials, enabling the measurement of atomic layer spacings and the determination of crystal or grain orientations. The peaks at 2θ = 21.79°, 38.35°, 44.40°, 64.72°, and 77.55° correspond to the crystal planes (53), (111), (200), (220), and (222) respectively **Fig. 4**. These angles confirm the Face Centered Cubic (FCC) lattice structure of the nanoparticles which is consistent with [21] reference. The average crystallite size of the NPs was determined using the Scherrer's equation:

$$D = K\lambda / \beta\cos\theta$$

Where D = crystallite size of the particle,

K = Scherrer constant, which is 0.9, λ = wavelength of light (λ = 1.54 Å), β = FWHM (Full width at Half Maximum) of the diffraction peak and θ = angle of reflection. The average crystallite size was found to be approximately 10.85 nm.

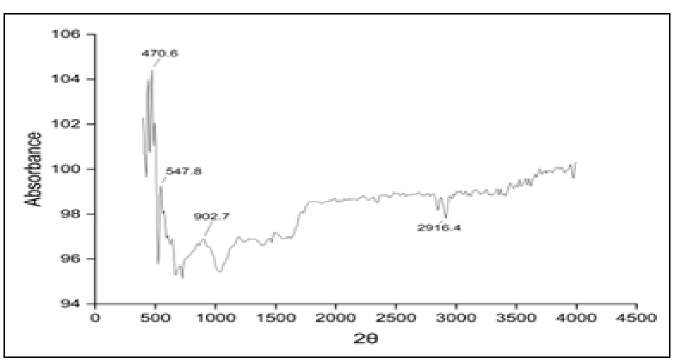


**FIG. 3: FT-IR SPECTRUM OF SILVER NANOPARTICLES**

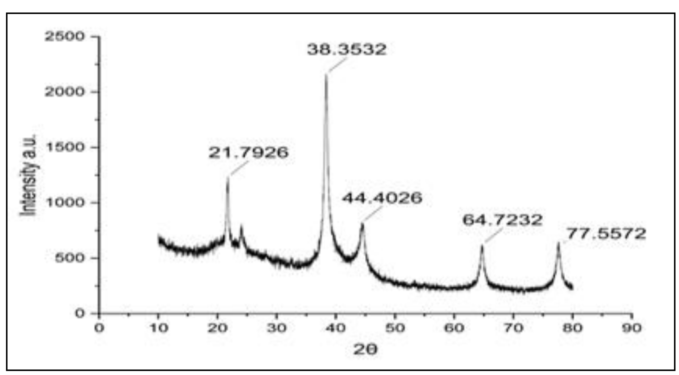


**FIG. 4: XRD PATTERN OF SILVER NANOPARTICLES**

Field Emission Scanning Electron Microscope (FE-SEM) experiments were employed primarily to investigate the surface topography, particle size distribution, crystal composition and texture of AgNPs. An SEI detector was utilized to capture images at 15 Kv[22].

The FE-SEM results revealed that the synthesized AgNPs had an average particle size of 27.75 nm **Fig. 5**.

In XRD analysis, the calculated average crystalline size of 10.85 nm suggests the presence of polycrystallinity, where multiple crystallines aggregate to form larger particles, as evident from the FE-SEM results.

This correlation between FE-SEM and XRD confirms the structural integrity and nanoscale properties of the synthesized AgNPs [23].

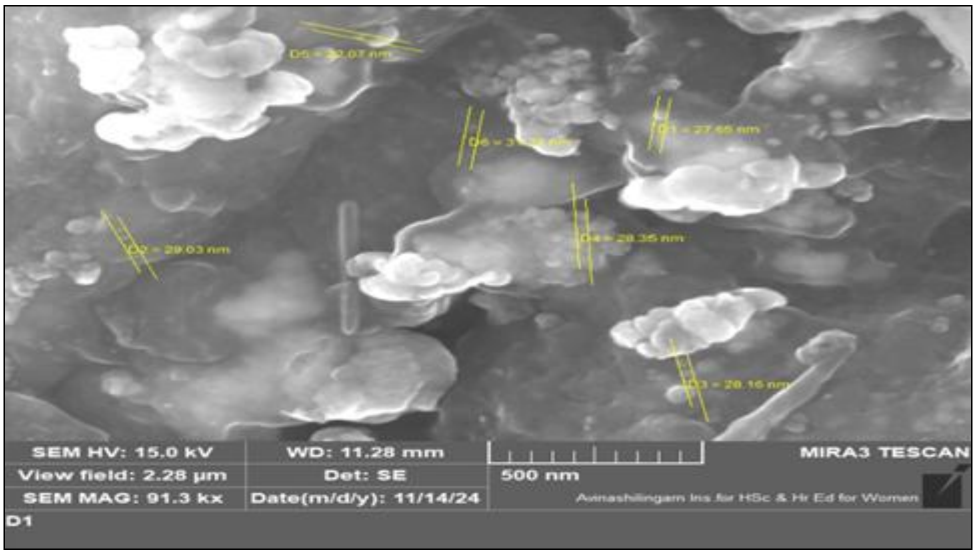


**FIG. 5: FIEL EMISSION SCANNING ELECTRON MICROSCOPIC ANALYSIS OF SILVER NANOPARTICLES**

Using Energy Dispersive X-ray Spectroscopy (EDS), the elemental makeup of the produced AGNPs was examined. The emission of signals from silver (Ag) and oxygen (O) as demonstrated by EDS analysis, clearly illustrated the formation of AgNPs [23]. Additionally, the elemental makeup of the produced silver nanoparticles was ascertained by EDS analysis. Silver (Ag), oxygen (O), and carbon(C) peaks were detected in the resulting EDS spectra **Fig. 6**. The atomic percentage of silver peak in the biosynthesized nanoparticle was 13.15%, while the atomic percentages of carbon (C) and oxygen (O) were 54.21% and 30.71% respectively **Table 2**. Presence of oxygen and Carbon is a common element in most EDS analysis due to the moisture absorption or any residual compounds from plants [23]. The detection of oxygen and silver peaks indicated that AgNPs were present.

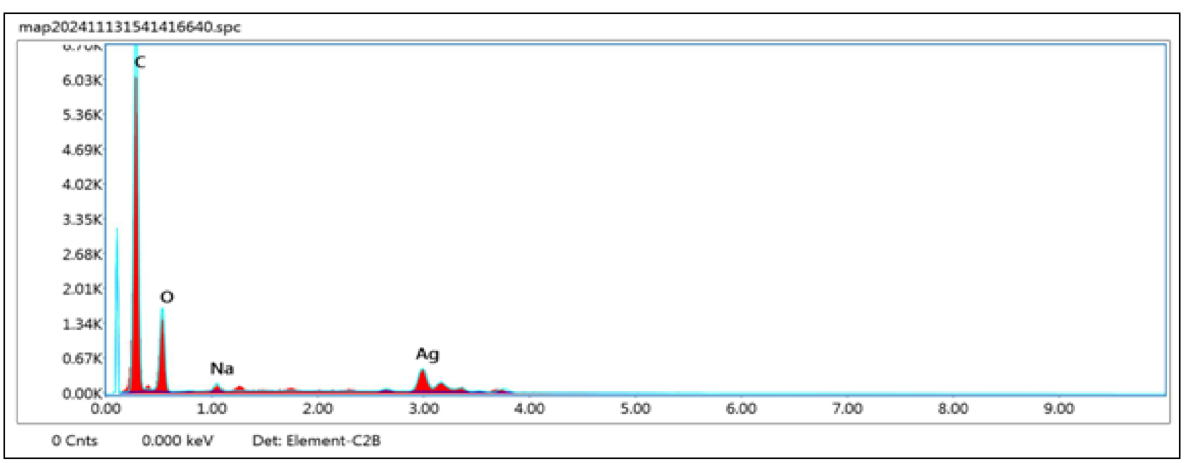


**FIG. 6: EDS ANALYSIS OF SILVER NANOPARTICLES**

**TABLE 2: ELEMENTAL COMPOSITION USING EDS ANALYSIS**

| Element | Weight% | Atomic % |
|---|---|---|
| C | 54.21 | 67.98 |
| O | 30.71 | 28.92 |
| Na | 1.93 | 1.26 |
| Ag | 13.15 | 1.84 |
| Total | 100 | 100 |

AgNPs have been employed as possible therapeutic agents in recent years to effectively control bacterial infections. The antibacterial qualities were assessed against both Gram-positive and Gram-negative microorganisms using agar well diffusion method. The synthesized nanoparticle exhibited enhanced antibacterial properties **Fig. 7**. The highest zone of inhibition was observed in *Enterococcus faecalis* followed by *S. aureus, E. coli* and *K. pneumoniae.* Silver nanoparticle showed potentially the highest antibacterial activity against tested pathogens [24]. This is mainly due to the permeable ability of AgNPs into the cell wall of Gram-positive and Gram-negative pathogens. The synthesized AgNPs demonstrated effective inhibition at a concentration of 10 µg/mL, with zones of clearance measuring 13 mm for *Escherichia coli*, 16 mm *for Enterococcus faecalis*, 8 mm for *Klebsiella pneumoniae* and 14 mm for *Staphylococcus aureus* **Table 3**. These results were compared to those obtained using standard antibiotics, including Erythromycin, Vancomycin, Penicillin and Ciprofloxacin [25]. The magnitude of the zones of inhibition suggests that AgNPs have higher antibacterial activity than the standard antibiotics against the tested strains. The correlation between the presence of phenolic compound as a secondary metabolite from phytochemical evaluation and the penetration ability of silver nanoparticle accounts to the potential inhibition.

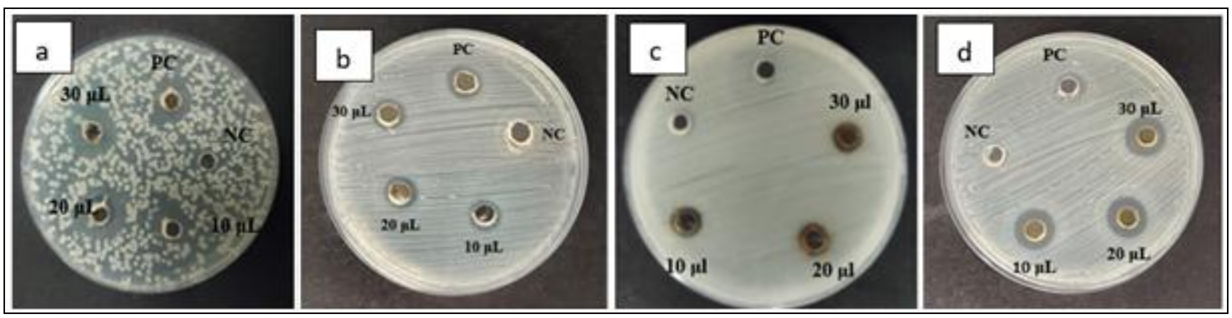


**FIG. 7: ANTIBACTERIAL ACTIVITY OF SILVER NANOPARTICLES.** a. *Enterococcus faecalis* b. *Escherichia coli* c. *Klebsiella pneumonia* d. *Staphylococcus aureus*. Note: PC- Positive control, NC- Negative control

**TABLE 3: ANTIBACTERIAL ACTIVITY OF SILVER NANOPARTICLES**

| Microorganism | Concentration (µg/mL) | Zone of Inhibition (mm) |
|---|---|---|
| *Enterococcus faecalis* | 10 | 17 ± 0.1 |
| | 20 | 16 ± 0.2 |
| | 30 | 19 ± 0.1 |
| *Escherichia coli* | 10 | 13 ± 0.1 |
| | 20 | 13 ± 0.08 |
| | 30 | 14 ± 0.1 |
| *Klebsiella pneumoniae* | 10 | 10 ± 0.1 |
| | 20 | 12 ± 0.1 |
| | 30 | 14 ± 0.1 |
| *Staphylococcus aureus* | 10 | 14 ± 0.1 |
| | 20 | 16 ± 0.2 |
| | 30 | 17 ± 0.1 |

The results support the use of *A. amara*-mediated AgNPs as ecologically benign, bioactive agents and validate the promise of plant-based nanoparticle synthesis for the creation of sustainable and functional materials with therapeutic applications.

**CONCLUSION:** In our investigation, the leaf extract from *A. amara*, which was rich in phytochemicals, contributed to the synthesis and stabilized the AgNPs. The structural stability and consistent dimensions of AgNPs were validated by the characterization study. AgNPs proved to be effective antimicrobial agents by exhibiting potent antibacterial activity against *Staphylococcus aureus* and *Enterococcus faecalis.* The combination of leaf extracts with metal ions in this plant-mediated synthesis produced more stable nanoparticles than the individual plants by imparting synergistic reducing and stabilizing potential. The outcomes suggested that silver nanoparticles produced using aqueous extracts are considered viable options for developing antimicrobial drugs.

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**CONFLICTS OF INTEREST:** The Authors declare no conflicts of interest with regard to this research work.

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