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# Multifunctional Silver Nanoparticles From Plant Extracts: Green Synthesis and Applications In Antibacterial Activity and Plant Growth

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**Silver nanoparticles (AgNPs) possess unique physicochemical properties, making them valuable in various fields, including medicine, agriculture, and environmental remediation. Their strong antibacterial activity has significant potential for combating infectious diseases. This study explores the green synthesis of AgNPs using leaf extracts from four plant species, including Cabomba furcata, Limnophila aromatica, Mimosa diplotricha, and Panicum maximum. This eco-friendly approach offers a sustainable and biocompatible alternative to traditional chemical synthesis methods, promoting responsible nanomaterial production. The synthesized AgNPs were characterised using appropriate techniques, and their antibacterial activity against Escherichia coli was evaluated. Additionally, the influence of AgNPs on seed germination was investigated. Molecular docking simulations were performed to explore the potential interactions between AgNPs and Nitrate Reductase A, an essential bacterial enzyme. This in silico approach provides insights into the possible mechanisms underlying the observed antibacterial effects and lays the foundation for developing AgNP-based therapeutics with enhanced efficacy and safety. In summary, this study demonstrates the successful green synthesis of AgNPs using leaf extracts from the four selected plant species and highlights their potential as multifunctional nanomaterials with antibacterial, plant growth-modulating, and molecular docking analysis capabilities.**

**Keywords:** Cabomba furcate; Green Synthesis; Limnophila aromatica; Mimosa diplotricha; Molecular Docking; Panicum maximum.

Silver nanoparticles (AgNPs) exhibit unique physicochemical properties, including optical, electrical, and antibacterial characteristics, leading to widespread applications in diverse fields such as medicine, industry, and consumer products.[1] Conventional AgNP synthesis methods often involve hazardous chemicals and complex procedures.[2] Green synthesis, utilizing plant extracts, offers an eco-friendly and sustainable alternative.[3]

This study investigates the green synthesis of AgNPs using extracts from four plant species: *Cabomba furcata, Limnophila aromatica, Mimosa diplotricha,* and *Panicum maximum*. The synthesized AgNPs were characterized, and their antibacterial activity and effects on seed germination were evaluated. Furthermore, molecular docking studies were performed on *E. coli* Nitrate Reductase A (NRA) to predict the

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potential binding interactions between the protein and AgNPs derived from each plant extract. This research aims to explore the potential of these plant-mediated AgNPs for various applications while emphasizing an environmentally responsible approach.

## MATERIALS AND METHODS

### Plant Materials and Reagents

Fresh aerial parts of *C. furcata*, *L. aromatica*, *M. diplotricha*, and *P. maximum* were collected from Changanassery, Kottayam district, Kerala state, India in April 2024. Plant identification was confirmed by the Department of Botany, St. Berchmans College. Healthy leaves were collected, washed with distilled water, dried, and ground into a fine powder using a domestic blender. The powder was stored in airtight containers for subsequent use. Analytical-grade silver nitrate ($AgNO_3$) was used for nanoparticle synthesis. Collected leaves were washed thoroughly with distilled water and air-dried at room temperature for 2-3 days. The dried leaves were then cut into small pieces and ground into a fine powder. Plant extracts were prepared by boiling 1 g of plant powder in 100 mL of distilled water for 5 minutes. The boiled extracts were filtered through Whatman No. 1 filter paper, and the supernatants were collected and stored at 4°C for further use.[4] 1 mM $AgNO_3$ solution was prepared by dissolving 0.015 g of $AgNO_3$ in 90 mL of double-distilled water. The solution was stored in dark conditions to prevent photo-oxidation of silver ions.

### Biosynthesis of Silver Nanoparticles

For AgNP synthesis, 10 mL of each plant extract was added to 90 mL of 1 mM AgNO3 solution in 250 mL conical flasks. The reaction mixtures were kept at room temperature under constant mechanical stirring. The progress of the reaction was monitored visually by observing colour changes and spectrophotometrically by periodically measuring UV-Vis absorbance.[5]

### Characterization

### UV-Visible Spectroscopic Analysis

UV-Vis spectral analysis of synthesized AgNPs was performed using a Specord-200 Plus spectrophotometer. Absorbance spectra were recorded in the range of 400-700 nm with a resolution of 1 nm. The characteristic surface plasmon resonance (SPR) peak of AgNPs, typically observed in the range of 420-450 nm, was used to confirm their formation. Diluted leaf extracts were used as blanks.

### Dynamic Light Scattering (DLS) Analysis

DLS analysis was employed to determine the size distribution and hydrodynamic diameter of the synthesized AgNPs. DLS is a non-destructive technique that measures the Brownian motion of particles in solution, providing information on their size distribution.

### Studies on the Application of Silver Nanoparticles Assessment of Antibacterial Activity

The antibacterial activity of the synthesized AgNPs was evaluated against *Escherichia coli* using the standard disk diffusion method. Briefly, bacterial cultures were grown on agar plates. Sterile Whatman No. 1 filter paper disks (6 mm diameter) were impregnated with 30 µL of each plant extract, AgNP solution, or AgNO3 solution as controls. These disks were placed on the inoculated agar plates.[6] After overnight incubation, the zones of inhibition around the disks were measured. The size of the inhibition zone was used to assess the antibacterial activity of each sample.

### Effect on Seed Germination and Plant Growth

Four different concentrations (10%, 25%, 50%, and 75% v/v) of AgNP dispersions were prepared in distilled water. Seed germination tests were conducted using Moong Bean (*Vigna radiata*) seeds. Twenty-one seeds were placed on filter paper in sterile Petri dishes. The respective AgNP dispersions were added to each Petri dish to ensure adequate seed wetting. The Petri dishes were kept undisturbed, and the germination percentage was recorded after 24 hours.[7]

### Molecular Docking Studies

In the preliminary phase of the molecular docking investigation, the silver nanoparticles were created using the software Gauss View and optimised using the Hartree-Fock (HF) method, a quantum mechanics approach. Nitrate Reductase A(NRA) crystal structure was obtained from the RCS Protein Data Bank using the PDB ID 1Q16 and subjected to cleaning, which entailed removing unnecessary water molecules, ligands, and chains. At the active site of the cleaned molecule, a grid/box was constructed, and nanoparticles were docked to the active site where the grid/box was present. Blind docking was employed,

selecting the complete protein molecule as the active site. The interaction and binding energy of the selected nanoparticles were determined using AutoDock software, and a 2D diagram was generated to visualize the interactions. These methodologies offer valuable insights into the potential applications of nanoparticles, particularly in drug delivery and other interdisciplinary fields. [Fig 1]

## RESULTS

### Synthesis of Silver Nanoparticles (AgNPs)

The formation of AgNPs was confirmed by the observation of a colour change from colourless to reddish-brown following incubation. This colour shift signifies the reduction of silver ions (Agz ) to metallic silver (Agp ) nanoparticles, an established phenomenon in green synthesis. UV-Vis spectroscopy further corroborated the formation of AgNPs, with a prominent peak around 420 nm, characteristic of surface plasmon resonance in AgNPs [Fig 2].

### Characterization

### UV-visible spectroscopy

The synthesis of AgNPs was further confirmed using UV–Vis spectroscopy, a vital technique for the characterization of nanoparticles. Generally, the UV–Vis spectra of nanoparticles range from about 390 nm to 470 nm depending on

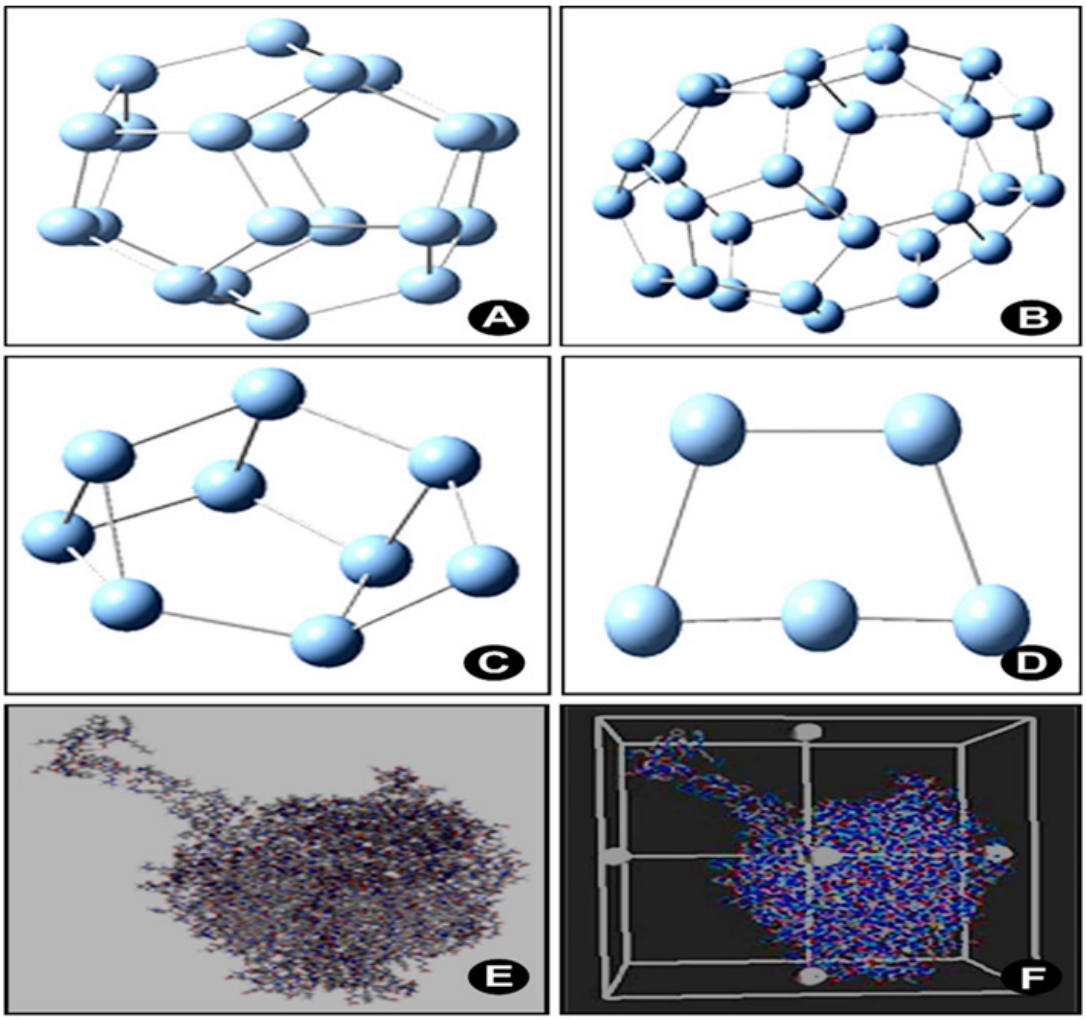


**Fig. 1.** Structure of AgNPs (a) 38nm CF-AgNPs. (b) 48nm LA-AgNPs. (c) 11nm MD-AgNPs. (d) 5nm PM-AgNPs. (e) Crystal structure of NRA and (f) Grid for docking of AgNP., 3.0 mg/L

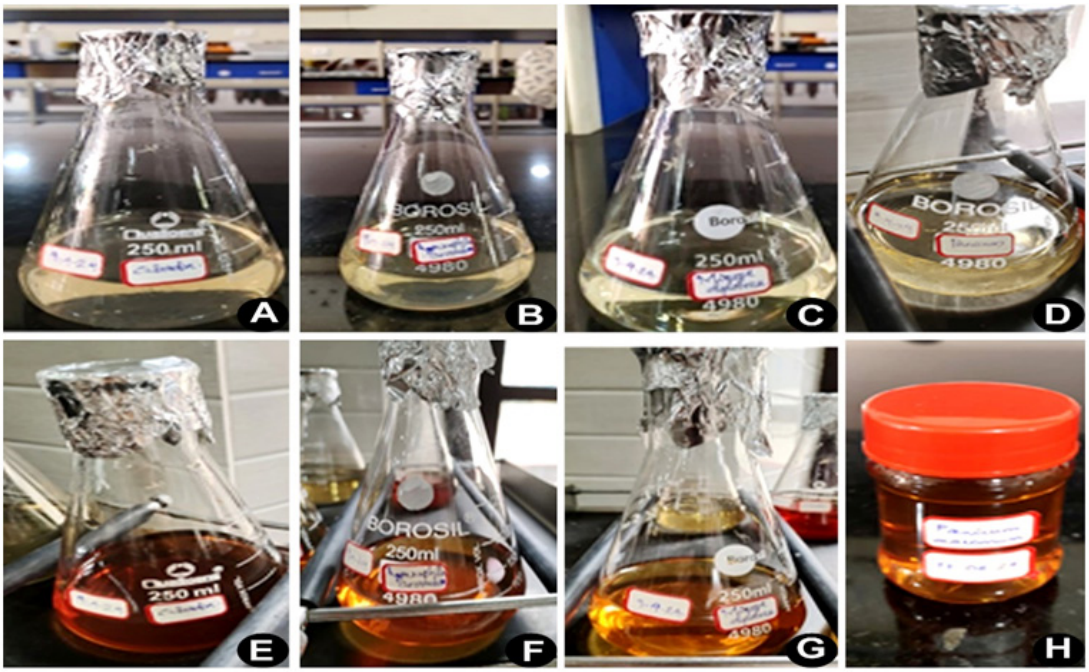


**Fig. 2.** Formation of Silver Nanoparticles from C. furcate (A, E); L. aromatic. (B, F); M. diplotricha (C, G); and P.maximum (D, H).

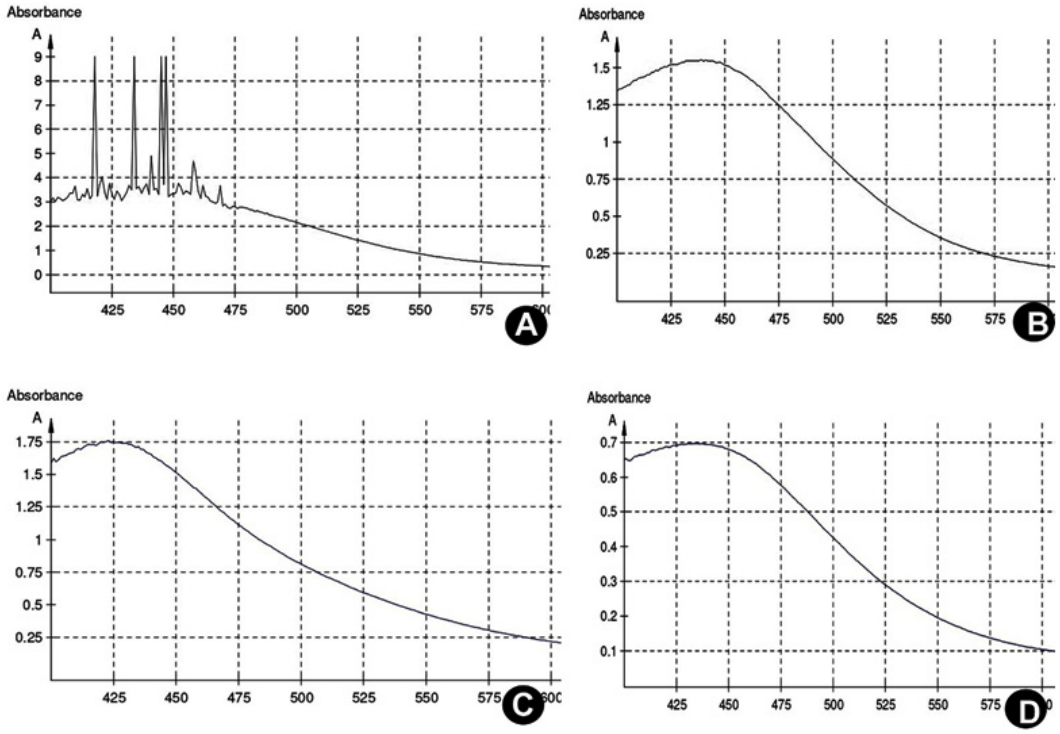


**Fig. 3.** The UV-Vis spectrum of CF-AgNPs (A); LA-AgNPs (B); MD-AgNPs (C) and PM-AgNps (D)

their size, shape, and distribution. UV-Vis spectra of the synthesized AgNPs from the plant extracts revealed major peaks at 420 nm in the 390 to 470 nm range, confirming nanoparticle formation. Peaks at 420 nm were formed by localized surface plasmon resonance. [Fig 3]

**Dynamic Light Scattering (DLS) Analysis**

The scattering angle was set at 173 degrees, with a holder temperature of 25.0 degrees Celsius. The dispersion medium viscosity was 0.894 mPa.s, and the transmission intensity before measurement was 1951 for *C. furcata*-AgNPs (CF-AgNPs), 15347 for *L. aromatica*-AgNPs (LA-AgNPs), 3662 for *M. diplotrica*-AgNPs (MD-AgNPs) and 9443 for *P. maximum*-AgNPs (PM-AgNPs). The distribution form was characterized as narrow, with monodispersity. The representation of the result was in terms of scattering light intensity, with a count rate of 0 kCPS. The mean size of nanoparticles was found to be 38.5nm, 48.2 nm, 11.2nm, and 5.2nm for CF-AgNPs, LA-AgNPs, MD-AgNPs, and PM-AgNPs respectively according to particle size distribution diagrams obtained by the DLS method. [Fig 4]

**Anti-microbial Activity of Silver Nanoparticles**

The green-synthesized AgNPs demonstrated significant antibacterial activity against Escherichia coli, as evidenced by the disk

**Table 1.** The zone of inhibition produced by treated CF-AgNPs, LA-AgNPs, MD-AgNPs, PM-AgNPs and control

| Plant Extracts-AgNPs | Zone of inhibition (mm) - Disk diffusion method | | |
|---|---|---|---|
| | Treated (30µL silver nanoparticles) | Control (30µL) | |
| | | $AgNO_3$ | Extract |
| CF-AgNPs | 12.3 ± 0.29 | 0 | 7.8 ± 0.15 |
| LA-AgNPs | 14.13 ± 0.26 | 0 | 8.03 ± 0.01 |
| MD-AgNPs | 21.96 ± 0.15 | 0 | 7.9 ± 0.1 |
| PM-AgNPs | 23.13 ± 0.26 | 0 | 8.0 ± 0.01 |

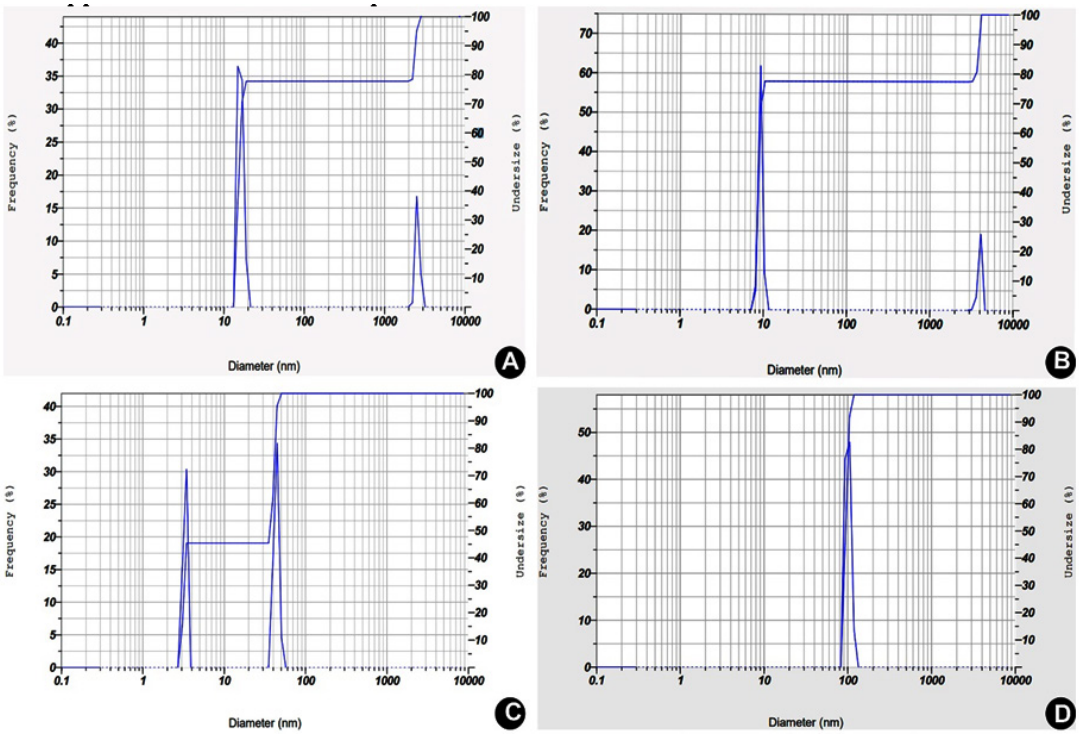


**Fig. 4.** The DLS results of CF-AgNPs (A), LA-AgNPs (B), MD-AgNPs (C) and PM-AgNPs. (D)

diffusion method due to the formation of a zone of inhibition 12.3mm (CF-AgNPs), 14.13mm (LA-AgNPs), 21.96mm (MD-AgNPs) and 23.13mm (PM-AgNPs). [Table 1, Fig 5]

**Effect of silver nanoparticles on Seed Germination and Growth**

The impact of AgNPs on seed germination and growth of Vigna radiata exhibited a concentration-dependent response. Interestingly, the highest germination rate and radicle length were observed at a concentration of 25% CF-AgNPs, and 75% for LA-AgNPs, MD-AgNPs, and PM-AgNPs. [Tables 2 and 3].

**Molecular Docking Studies**

The present study has employed AutoDock software to carry out a docking analysis of the interaction between silver nanoparticles (AgNPs) and Nitrate Reductase A (NRA), a cellular protein ubiquitously expressed in bacteria that employ nitrate as an electron acceptor during anaerobic respiration. The in silico docking analysis revealed a favourable binding affinity of -14 kcal/mol (CF-AgNPs), -17 kcal/mol (LA-NPs), -7kcal/mol (MD-AgNPs), and -5kcal/mol (PM-AgNPs) between these AgNPs produced and the Nitrate Reductase A (NRA) protein of E. coli. [Table 4, Fig 6]

**Table 2.** The radicle length of Vigna radiata seeds measured at different concentrations of CF-AgNPs, LA-AgNPs, MD-AgNPs and PM-AgNPs

| S. No | Concentration of AgNPs | Radicle length(cm) | | | |
|---|---|---|---|---|---|
| | | CF-AgNPs | LA-AgNPs | MD-AgNPs | PM-AgNPs |
| 1 | Control | 1.1±0.10 | 1.0±0.16 | 1.0±0.16 | 1.0±0.16 |
| 2 | 10% | 1.5±0.15 | 1.3±0.25 | 1.1±0.1 | 1.6±0.41 |
| 3 | 25% | 2.5±0.21 | 2.2±0.12 | 1.3 ±0.01 | 1.3±0.41 |
| 4 | 50% | 1.6±0.15 | 1.9±0.16 | 1.7±0.01 | 2.5±0.45 |
| 5 | 75% | 1.5±0.21 | 2.7±0.21 | 2.5±0.01 | 2.3±0.16 |
| 6 | After 2 weeks | 2.5±0.25 | 1.7±0.24 | 2.68±0.02 | 1.7±0.12 |

*Value indicated as mean± SD of 3 samples.

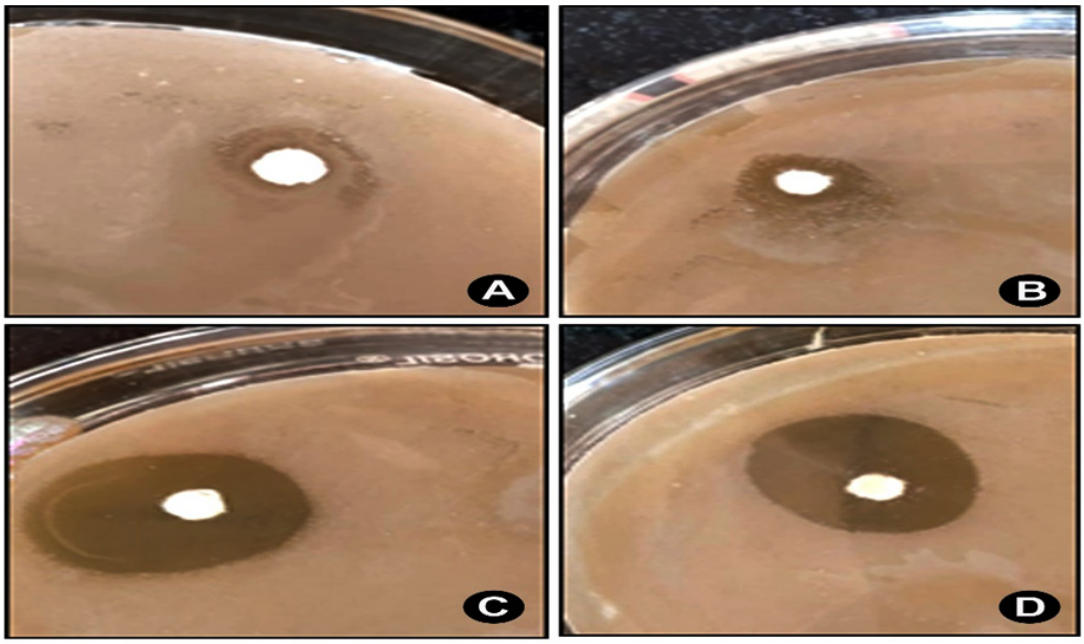


**Fig. 5.** Disk Diffusion method: CF-AgNPs. (A) LA- AgNPs. (B) MD-AgNPs. (C) and PM-AgNPs (D)

## DISCUSSION

The green synthesis of AgNPs from *C. furcata, L. aromatica, M. diplotricha*, *and P. maximum* leaf extracts was confirmed by the observed colour change from colourless to reddish-brown following incubation. This colour shift signifies the reduction of silver ions (Agz ) to metallic silver (Agp ) nanoparticles in green synthesis.[8] UV-Vis spectroscopy further corroborated the formation of AgNPs, with a prominent peak around 420 nm, characteristic of surface plasmon resonance in AgNPs.[9] However, multiple peaks in the DLS data suggest a degree of polydispersity, indicating a variation in nanoparticle sizes. Future studies could explore optimizing reaction conditions for a more uniform size distribution.

**Table 3.** The germination percentage of *Vigna radiata* seeds measured at different concentrations of CF-AgNPs, LA-AgNPs, MD-AgNPs and PM-AgNPs

| S. No | Concentration of AgNPs | Germination Percentage | | | |
|---|---|---|---|---|---|
| | | CF-AgNPs | LA-AgNPs | MD-AgNPs | PM-AgNPs |
| 1 | Control | 80.9% | 80.9% | 80 % | 80.4% |
| 2 | 10% | 76.1% | 90.4% | 80.9% | 90.4% |
| 3 | 25% | 95.2% | 95.2% | 95.2% | 95.2% |
| 4 | 50% | 85.7% | 85.7% | 90.4% | 85.7% |
| 5 | 75% | 76.1% | 100% | 95.2% | 100% |
| 6 | After 2 weeks | 95.2% | 80.9% | 95.2% | 80.9% |

*Value indicated as mean± SD of 3 samples.

**Table 4.** The binding affinities of produced AgNPs and the protein NRA

| S. No. | Plant Extract AgNPs | Binding Affinities (kcal/mol) |
|---|---|---|
| 1 | CF-AgNPs | -14 |
| 2 | LA-AgNPs | -17 |
| 3 | MD-AgNPs | -7 |
| 4 | PM-AgNPs | -5 |

The green-synthesized AgNPs demonstrated significant antibacterial activity against Escherichia coli, as evidenced by the disk diffusion method due to the formation of a zone of inhibition 12.3mm (CF-AgNPs), 14.13mm (LA-AgNPs), 21.96mm (MD-AgNPs) and

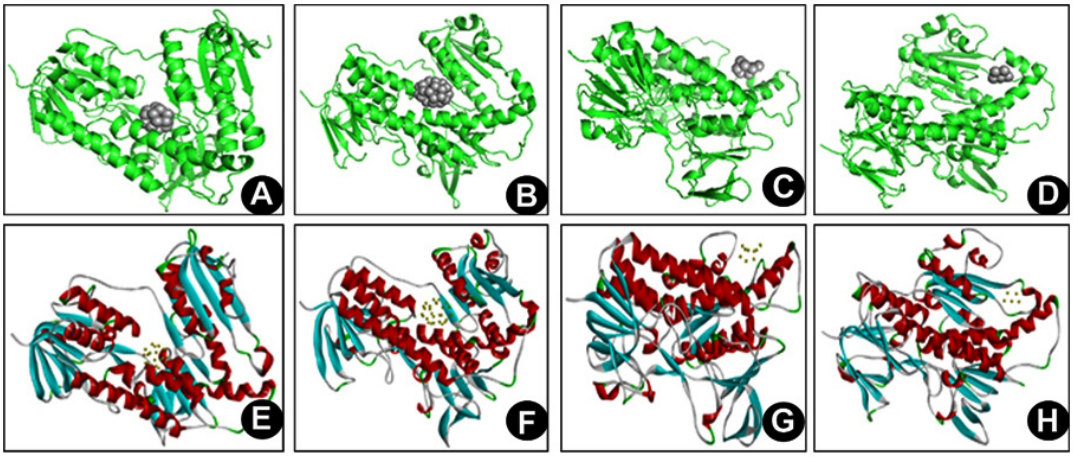


**Fig. 6.** Docked strucutures of CF-AgNPs (A, E), LA-AgNPs. (B, F) MD-AgNPs (C, G), PM-AgNPs (D, H) and Protein NRA

23.13mm (PM-AgNPs). The zone of inhibition formed by AgNPs proved that they have greater antibacterial activity than the extract. This finding aligns with previous reports on the broad-spectrum antibacterial properties of AgNPs.[10] The observed activity likely stems from multiple mechanisms, including cell membrane disruption, interference with essential cellular processes, and generation of reactive oxygen species (ROS) that can damage bacterial components.[11] Notably, the antibacterial activity of AgNPs was superior to that of the extract alone, suggesting that the synergistic effect between plant biomolecules and AgNPs enhances their antimicrobial efficacy. [Table 1, Fig 5]

The impact of AgNPs on seed germination and growth of Vigna radiata exhibited a concentration-dependent response. Interestingly, the highest germination rate and radicle length were observed at a concentration of 25% CF-AgNPs, and at 75% for LA-AgNPs, MD-AgNPs, and PM-AgNPs. This suggests a potential growth-promoting effect of AgNPs at this specific concentration. However, prolonged exposure or higher concentrations resulted in decreased germination rates in LA-AgNPs and PM-AgNPs whereas it increased germination percentage in CF-AgNPs and remained the same for MD-AgNPs, highlighting the importance of optimizing AgNPs concentration for agricultural applications. The underlying mechanisms for these observations remain unclear and warrant further investigation. It's possible that AgNPs, at lower concentrations, might stimulate specific physiological processes in seeds, leading to enhanced germination and growth. Conversely, higher concentrations might induce stress responses or nanoparticle aggregation, hindering seed development. [Tables 2 and 3]

The present study has employed AutoDock software to carry out a docking analysis of the interaction between silver nanoparticles (AgNPs) and Nitrate Reductase A (NRA), a cellular protein ubiquitously expressed in bacteria that employ nitrate as an electron acceptor during anaerobic respiration. The in silico docking analysis revealed a favourable binding affinity of -14 kcal/mol (CF-AgNPs), -17 kcal/mol (LA-NPs), -7kcal/mol (MD-AgNPs), and -5kcal/mol (PM-AgNPs) between these AgNPs produced and the Nitrate Reductase A (NRA) protein of E. coli. This suggests a potential interaction between AgNPs and NRA, which could contribute to their antibacterial activity. NRA plays a vital role in bacterial metabolism by facilitating anaerobic respiration. Disrupting NRA function through AgNP binding could hinder bacterial growth and survival.[12] Silver nanoparticles have received considerable attention due to their broad-spectrum antimicrobial properties, making them promising candidates for combating bacterial infections Given the global concern over infections caused by antibiotic-resistant microorganisms, AgNPs emerge as a promising alternative. They possess unique characteristics that render them suitable for medical and healthcare applications, particularly in treating or preventing infections with utmost efficacy. As novel and effective antibacterial agents are urgently required, the employment of AgNPs in this domain has become increasingly imperative. They can be utilized to prevent infections stemming from these microorganisms, sanitize medical equipment, and even treat ongoing infections.[13] However, in vitro and in vivo studies are crucial to validate the docking results and elucidate the precise mechanisms by which AgNPs interact with NRA and other cellular components to exert their antibacterial effects. [Table 4, Fig 6]

## CONCLUSION

The study has effectively demonstrated a sustainable approach for synthesizing silver nanoparticles (AgNPs) using extracts of *C. furcata, L. aromatica, M. diplotrica and P.maximum.* The synthesis was confirmed through UV-visible spectroscopy, revealing a peak at approximately 420 nm. The Dynamic light scattering (DLS) analysis indicated a mean particle size of 38.5nm, 48.2 nm, 11.2nm, and 5.2nm for CF-AgNPs, LA-AgNPs, MD-AgNPs, and PM-AgNPs respectively. The synthesized AgNPs exhibited notable antibacterial activity against *E. coli*, as evidenced by the formation of a zone of inhibition of 12.3mm (CF-AgNPs), 14.13mm (LA-AgNPs), 21.96mm (MD-AgNPs), and 23.13mm (PM-AgNPs). Furthermore, the impact on seed germination and plant growth was found to be concentration-dependent, with the highest germination rate and radicle length observed at a concentration of 25% for CF-AgNPs, and at 75% for LA-AgNPs, MD-AgNPs and PM-AgNPs. In contrast, higher concentrations resulted in reduced germination rates in LA-AgNPs and

PM-AgNPs. In contrast, it increased germination percentage in CF-AgNPs and remained the same for MD-AgNPs, Molecular docking studies suggested potential interactions between AgNPs and the Nitrate Reductase A protein of *E. coli*, offering insights into the antibacterial mechanisms that warrant further experimental validation.

The green-synthesized AgNPs from the four plant extracts displayed promising antibacterial and seed germination-modulating, suggesting potential applications in both medicine and agriculture. Future research endeavours should concentrate on evaluating the in vivo efficacy and optimizing applications. Additionally, the molecular docking analysis provides a foundation for comprehending the antibacterial mechanism, warranting further exploration of AgNPs interactions with bacterial cellular components. This study underscores the significance of green synthesis methods in producing AgNPs with diverse beneficial properties, ultimately promoting the sustainable development of novel nanomaterials for healthcare and agricultural applications.

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**Conflict of interest**

The authors do not have any conflict of interest.

**Data Availability Statement**

This statement does not apply to this article.

**Ethics Statement**

This research did not involve human participants, animal subjects, or any material that requires ethical approval.

**Informed Consent Statement**

This study did not involve human participants, and therefore, informed consent was not required.

**Clinical Trial Registration**

This research does not involve any clinical trials**.**

**Author Contributions**

Sanisha Attichira Santhosh: Data Collection, Analysis, Writing; Varsha Benny: Data Collection, Analysis; Anjana Kannattu Pushpamgadhan: Data Collection, Analysis; Febamol Shaji: Data Collection, Analysis; Salvy Thomas: Supervision, Project Administration, Writing – Review & Editing.

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