Biosynthesis, Characterization and Antimicrobial Activities of Silver Nanoparticles from Leaf Extract of Cissampelos Pareira Leaves

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**Abstract:** New antimicrobial medicines have emerged as a response to the growing resistance of harmful microorganisms to traditional antibiotics. One promising method that has gotten people's attention is the sustainable and environmentally benign route of biosynthesis using plant extracts for silver nanoparticles (AgNPs), with their strong antibacterial properties.This study aims to formulate and assess the productivity of silver nanoparticles synthesized from the leaf extract of Cissampelos pareira, emphasizing their antibacterial properties.The Cissampelos pareira leaf extract was utilized in the formation of AgNPs using the green synthesis technique. TEM, FTIR, and XRD are used to characterize the nanoparticles to confirm size distribution, crystallinity, and purity of the nanoparticles. The antibacterial activity was tested on various microbiological species, such as Staphylococcus aureus, Escherichia coli, and Candida albicans.The AgNPs synthesized displayed excellent antibacterial and anti-biofilm activities. XRD indicated good crystallinity, while FTIR tests showed good purity, and TEM investigation proved the successful biogenesis of the nanoparticles as well as uniform size distribution. In addition to strong inhibition of Klebsiella sp. biofilms, the produced AgNPs demonstrated marked inhibitory activity against C. albicans, E. coli, and S. aureus.The findings highlight the potential of biosynthesized AgNPs from Cissampelos pareira as effective antibacterial agents. This study supports the utilization of plant extracts in nanoparticle synthesis, contributing to sustainable nanotechnology and offering promising applications in combating bacterial infections**.**

**Keywords:** Silver nanoparticles, Cissampelos pareira, biosynthesis, antibacterial activity, anti-biofilm activity, phytochemicals, green nanotechnology, plant-mediated synthesis, sustainable chemistry.

# Introduction

The size of silver nanoparticles (AgNPs), ranging from 1 to 100 nm, influences their physical, chemical, and biological properties. Their antibacterial activity is largely attributed to their high surface area-to-volume ratio [(Ramya et al., 2024)](https://paperpile.com/c/3v6Wqn/1Gm9). Smaller silver nanoparticles (AgNPs) have a higher surface-to-volume ratio, enabling better contact and stronger effects on microbial cells. Their size influences their ability to penetrate cell membranes and disrupt microbial functions. Therefore, controlling particle size and distribution during synthesis is crucial, especially for biomedical applications where stability and uniformity are essential.[(El-Hussein, 2024)](https://paperpile.com/c/3v6Wqn/R5b5o).[(Aparna et al., 2021; Poornima et al., 2021; Verma & Muthuswamy Pandian, 2021)](https://paperpile.com/c/3v6Wqn/oRfp+XDFP+k4Mv) [(Muthuswamy Pandian et al., 2022; Ramakrishnan et al., 2023)](https://paperpile.com/c/3v6Wqn/nT0J+WEz2)Beyond its role in silver nanoparticle (AgNP) synthesis, Cissampelos pareira has long been used in folk medicine for various biological applications [(Muthuswamy Pandian et al., 2022; Ramakrishnan et al., 2023)](https://paperpile.com/c/3v6Wqn/nT0J+WEz2). Its antioxidant properties help combat oxidative stress linked to chronic diseases, while its anti-inflammatory effects make it useful in treating conditions like arthritis and edema.[(Haider et al., 2022a)](https://paperpile.com/c/3v6Wqn/QUK6).Moreover, Cissampelos pareira has antiviral and antifungal effects, making it effective against many microbes[(Wadhwani et al., 2022)](https://paperpile.com/c/3v6Wqn/U4hGf). It contains a variety of bioactive compounds, such as alkaloids and flavonoids, and has proven to trigger apoptosis of malignant cells, suggesting possible anticancer activity[(Muthuswamy Pandian et al., 2022)](https://paperpile.com/c/3v6Wqn/nT0J) . It has also been used throughout history to remedy gastrointestinal disturbances, accelerate healing of ulcers, and control menses, further emphasizing its extensive therapeutic potential [(Laghari et al., 2023; Ramakrishnan et al., 2023)](https://paperpile.com/c/3v6Wqn/WEz2+fUtUb)[(Suresh et al., 2023)](https://paperpile.com/c/3v6Wqn/JMpeU).

Nanotechnology, a multidisciplinary field, has significantly influenced antimicrobial medicine.[(Marya et al., 2022)](https://paperpile.com/c/3v6Wqn/nQtni) . Silver nanoparticles (AgNPs) have gained attention for their remarkable antibacterial properties, making them effective against various microorganisms [(Chokkattu et al., 2023)](https://paperpile.com/c/3v6Wqn/3Tqs8). Traditionally, AgNPs are synthesized using physical and chemical methods, but these often involve toxic reagents, high energy consumption, and hazardous byproducts [(Solanki et al., 2023)](https://paperpile.com/c/3v6Wqn/TQHK). This raises concerns about their safety and sustainability, particularly in biomedical applications where biocompatibility and environmental friendliness are crucial [(Subramanian & Harikrishnan, 2023)](https://paperpile.com/c/3v6Wqn/Frlq5) [(Krishnappan et al., 2024)](https://paperpile.com/c/3v6Wqn/pn9cI). Green synthesis of silver nanoparticles (AgNPs) offers a sustainable, non-toxic alternative using plant extracts as reducing agents. Phytochemicals like alkaloids and flavonoids aid in AgNP formation, enhancing antibacterial properties while minimizing harmful chemicals [(Adel et al., 2023)](https://paperpile.com/c/3v6Wqn/6Lia)[(Farooqi et al., 2024)](https://paperpile.com/c/3v6Wqn/T3m5G))

Research highlights the potential of plant-based nanoparticles, especially for their antiviral, antifungal, and antibacterial properties [(Chokkattu et al., 2022; Ramamurthy et al., 2022)](https://paperpile.com/c/3v6Wqn/XHAl+vCCu). Plant-mediated synthesis offers a sustainable alternative to chemical methods. Cissampelos pareira (velvetleaf), known for its anti-inflammatory and antioxidant effects, is widely used in traditional medicine. Its bioactive compounds, including flavonoids, tannins, and alkaloids, help stabilize metal nanoparticles [(Chokkattu et al., 2022; Ramamurthy et al., 2022)](https://paperpile.com/c/3v6Wqn/XHAl+vCCu)[(Peng et al., 2024)](https://paperpile.com/c/3v6Wqn/HDxab). Cissampelos pareira is an ideal choice for the biosynthesis of silver nanoparticles because of these phytochemicals, which not only improve the stability and natural adequacy of AgNPs but also serve to induce their union [(Chokkattu et al., 2022; Ramamurthy et al., 2022)](https://paperpile.com/c/3v6Wqn/XHAl+vCCu).Using Cissampelos pareira leaf extract for AgNP biosynthesis provides an eco-friendly alternative to chemical methods while enhancing antibacterial properties [(Merchant et al., 2022; Pandiyan et al., 2022)](https://paperpile.com/c/3v6Wqn/sH9ti+JT4C4). AgNPs combat microbes by disrupting cell membranes, generating reactive oxygen species (ROS), and interfering with DNA replication. Their broad-spectrum action is further boosted by plant bioactive compounds, making them effective against various pathogens. This synergy is crucial amid rising antimicrobial resistance. [(Haider et al., 2022b)](https://paperpile.com/c/3v6Wqn/aOaW).

This study examines the antibacterial potential of green-synthesized AgNPs using Cissampelos pareira leaf extract. It aims to provide a cost-effective, eco-friendly alternative to conventional methods while highlighting plant-based nanoparticles as effective antibacterial agents[(Aparna et al., 2021; Poornima et al., 2021; Verma & Muthuswamy Pandian, 2021)](https://paperpile.com/c/3v6Wqn/oRfp+XDFP+k4Mv). The findings contribute to green nanotechnology’s role in combating bacterial infections and biofilms.This study examines the antibacterial potential of green-synthesized AgNPs using Cissampelos pareira leaf extract. It aims to provide a cost-effective, eco-friendly alternative to conventional methods while highlighting plant-based nanoparticles as effective antibacterial agents. The findings contribute to green nanotechnology’s role in combating bacterial infections and biofilms.[(Asif et al., 2021; S. Kumari et al., 2021)](https://paperpile.com/c/3v6Wqn/CYkgh+qwmo4).The aim of this study is to investigate the antibacterial potential of silver nanoparticles (AgNPs) synthesized using Cissampelos pareira leaf extract through green chemistry methods. This research seeks to provide a cost-effective, sustainable alternative to conventional AgNP synthesis techniques while exploring the enhanced antimicrobial properties of plant-mediated AgNPs. Specifically, the study aims to evaluate the efficacy of these nanoparticles against harmful pathogens, contributing to the growing body of knowledge on plant-based nanotechnology and its potential applications in combating bacterial infections and biofilms This study aims to evaluate the antibacterial potential of silver nanoparticles (AgNPs) synthesized using Cissampelos pareira leaf extract through green chemistry. It explores a cost-effective, sustainable alternative to conventional methods while assessing the enhanced antimicrobial properties of plant-mediated AgNPs against harmful pathogens.

# MATERIALS AND METHODS

## Silver Nanoparticles

​Distilled water, Silver Nitrate, Ammonia solution and Sodium Borohydrate Solution from Fisher Scientific, India were used for the synthesis of AgNPs in this study.

## Collection and Preparation of Plant Sample

Cissampelos pareira leaves were collected and washed with distilled water to remove impurities. The leaves were shade-dried to preserve their structure and then ground into a coarse powder using a mortar and pestle. Ten grams of the powdered leaves were added to 200 ml of distilled water in a sterile conical flask, heated, and stirred continuously for 24 hours. The extract was then purified by filtration using Whatman No. 1 filter paper.[(Sasidharan et al., 2010)](https://paperpile.com/c/3v6Wqn/5qtC).

## Synthesis of silver nanoparticles

A 0.4 M Europium nitrate solution was prepared in distilled water, and plant extract filtrate was added. The mixture was boiled and stirred using a magnetic agitator. Gradual addition of 1M sodium borohydride and ammonia solutions led to a blackish-brown color, indicating AgNP formation. The solution was then centrifuged at 5000 rpm for 15 minutes, and the supernatant was transferred to a petri dish for water evaporation.The nanoparticles were further dried in a hot air oven at 100 °C, following the procedure by Sumitha et al. [(Vidhya & Prasad, 2016)](https://paperpile.com/c/3v6Wqn/YDyP). Extensive studies have been carried out to determine the characteristics of Ag nanoparticles obtained using the extract of Cissampelos pareira.

## Characterisation of Ag NPs

Fourier-transform infrared (FTIR) analysis was performed using a Bruker Alpha-II ATR instrument within a 4000–400 cm⁻¹ wavelength range, confirming the involvement of C. bonduc-mediated AgNPs in synthesis. XRD analysis, conducted with a Bruker D8 Advance diffractometer (AgKα radiation, 30 kV, 15 mA), examined the crystal structure with a scanning rate of 4°/min and a step size of 0.05°, covering 2θ angles from 5° to 80°. TEM analysis using a G2 20 S-Twin TEM instrument revealed polydispersed AgNPs.

## Antibacterial activity and minimum inhibitory concentration (MIC)

Antibacterial activity of C. bonduc extract was determined against bacterial samples obtained from the Microbiology Laboratory. The activity against Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, and Bacillus sp. was checked according to the approved protocols of the Clinical and Laboratory Standards Institute, CLSI described by Padil et al. [(Thekkae Padil & Černík, 2013)](https://paperpile.com/c/3v6Wqn/gr46). Muller-Hinton agar plates were prepared, and bacterial cultures (90 CFU/ml) were evenly spread using aseptic cotton swabs. Sterile well borers created 8 mm wells, into which AgNPs (25, 50, and 100 µg/ml) were added. Streptomycin (10 mg/ml) served as the positive control. Plates were incubated at 37 ± 2°C for 24 hours, and inhibition zones were measured to assess antibacterial activity. The MIC assay was conducted using the broth dilution method to determine the minimum AgNP concentration required to inhibit bacterial growth.

# Antibiofilm activity

## Biofilm inhibition assay using Microtiter plate method

The synthesized Ag NPs used to inhibit the biofilm formation at sub – MIC’ s using microtiter plate method by employing the Crystal Violet as a staining agent [(Qais et al., 2021)](https://paperpile.com/c/3v6Wqn/Z500). To assess biofilm inhibition, add 100 µl of fresh Luria-Bertani broth (with or without AgNPs at sub-MIC) to each well of a 96-well microtiter plate. Introduce 1% of the overnight bacterial culture and incubate at 37°C overnight. After incubation, remove free-floating cells and wash wells three times with sterile PBS. Let the plate rest at room temperature for 30 minutes, then stain biofilms with crystal violet for 10–15 minutes. Wash with PBS and add 200 µl of 95% ethanol to dissolve the dye. Measure absorbance at 620 nm using a microplate reader. Biofilm inhibition (%) is calculated as:

Percentage of inhibition = (Control OD620nm − Test OD620nm) / Control OD620nm × 100

## Biofilm inhibition by Ag NPs using Confocal Laser Scanning Microscopy (CLSM)

Biofilm inhibition was further analyzed using CLSM with a representative bacterial strain. In a 12-well microtiter plate with glass coverslips, 100 µl of bacteria was added to each well. AgNPs were applied at two concentrations (50 µl – low, 100 µl – high), while the control contained only live bacteria. After 24 hours of incubation at 37°C, coverslips were washed with PBS and stained. Acridine Orange and Propidium Iodide were used to identify dead cells (red), while ConA-FITC stained the glycocalyx matrix (green). Fluorescence emissions were detected at 620 nm (PI) and 525 nm (ConA-FITC). Bacteria were then visualized, and images were captured at randomly selected sites.

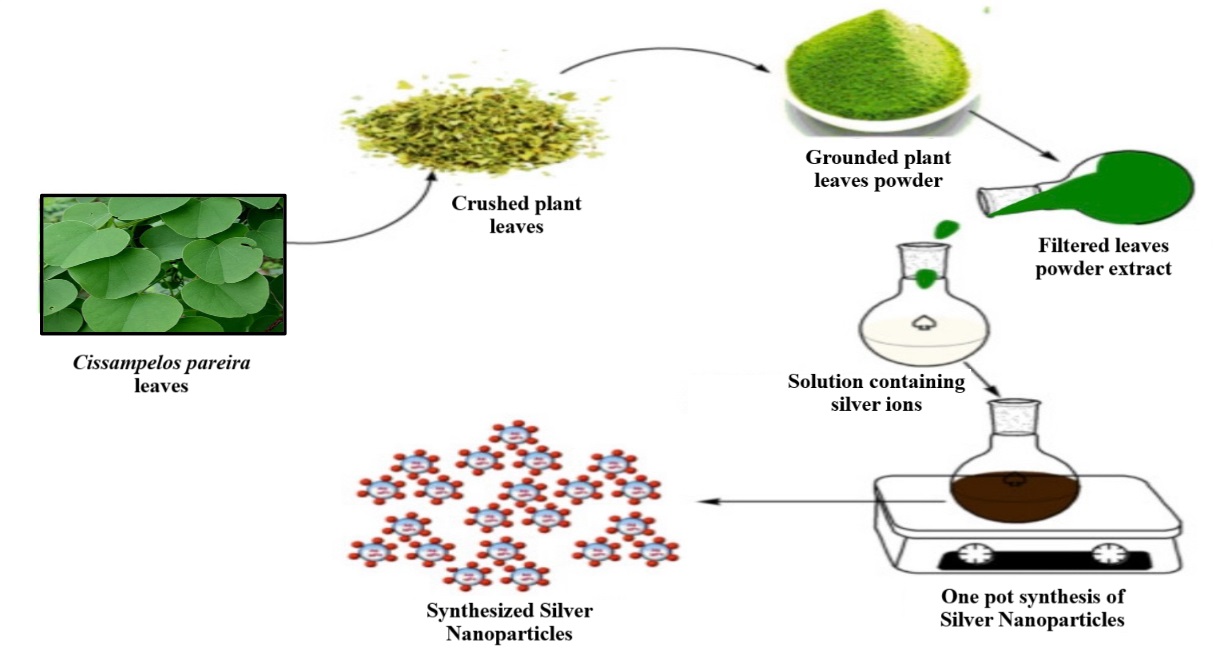


Figure 1: Flowchart

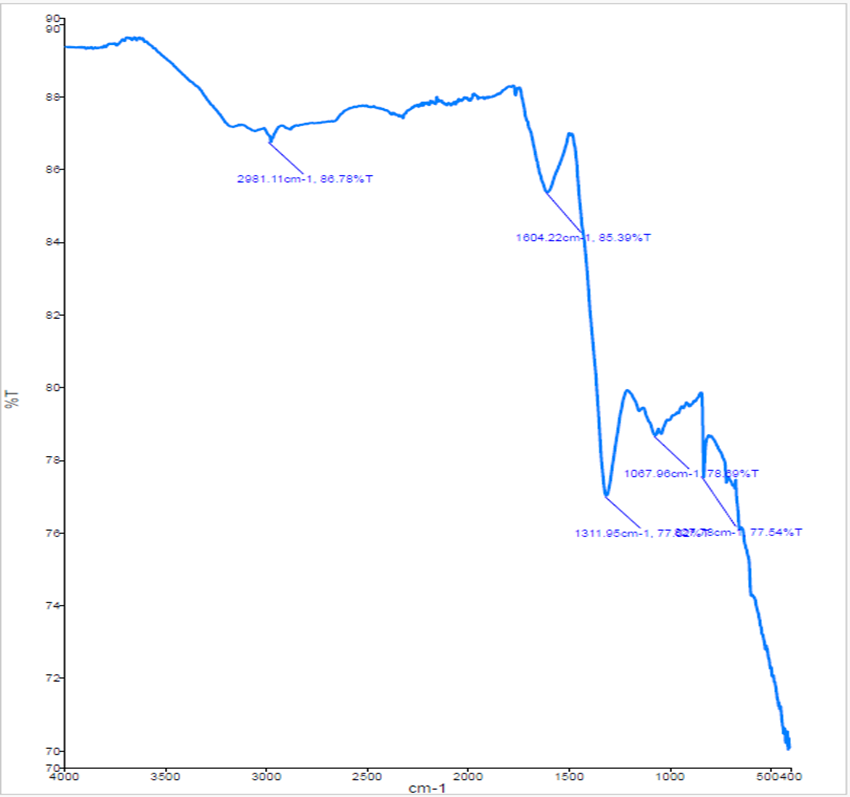
# Statistical analysis

​The experiments were carried out successive times to ensure dependability, and the accuracy of the data was presented as the mean values together with their corresponding standard deviations. The IC50 value, representing the concentration at which 50% inhibition occurred, was accurately measured and confirmed using rigorous statistical analysis, including ANOVA and linear regression. The IC50 values play a crucial role in evaluating the biological effectiveness and possible uses of the nanoparticles being studied.

# Results

## Fourier-transform infrared (FTIR) spectroscopy

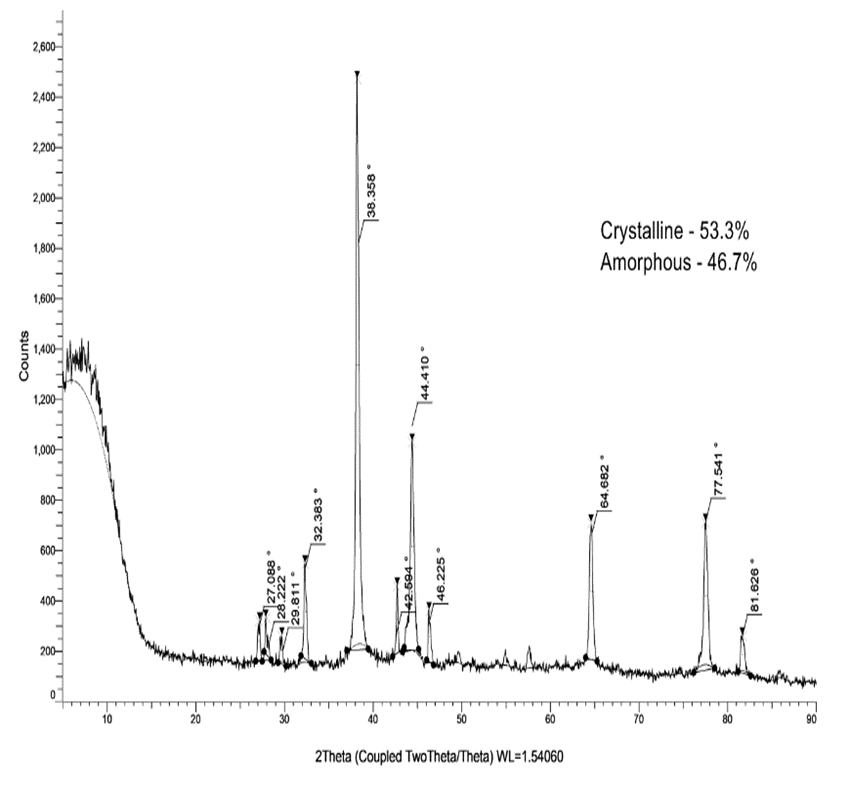
Fourier-transform infrared spectroscopy (FTIR) was used to characterize silver oxide nanoparticles synthesized from Cissampelos pareira leaf extract. The spectrum revealed key peaks at 2981.11 cm⁻¹ (C-H stretching of aliphatic hydrocarbons), 1604.22 cm⁻¹ (C=C stretching of alkenes), and 1311.95 cm⁻¹ (C-H bending). Additionally, the peak at 1067.96 cm⁻¹ corresponds to C-O stretching, indicating the presence of alcohols, ethers, or carboxylic acids, confirming the role of organic compounds in nanoparticle stabilization. [(Gnanam et al., 2024)](https://paperpile.com/c/3v6Wqn/3ZKj).



**Fig. 2** FTIR analysis if Silver nanoparticles from Cissampelos pareira

## X – Ray Diffraction

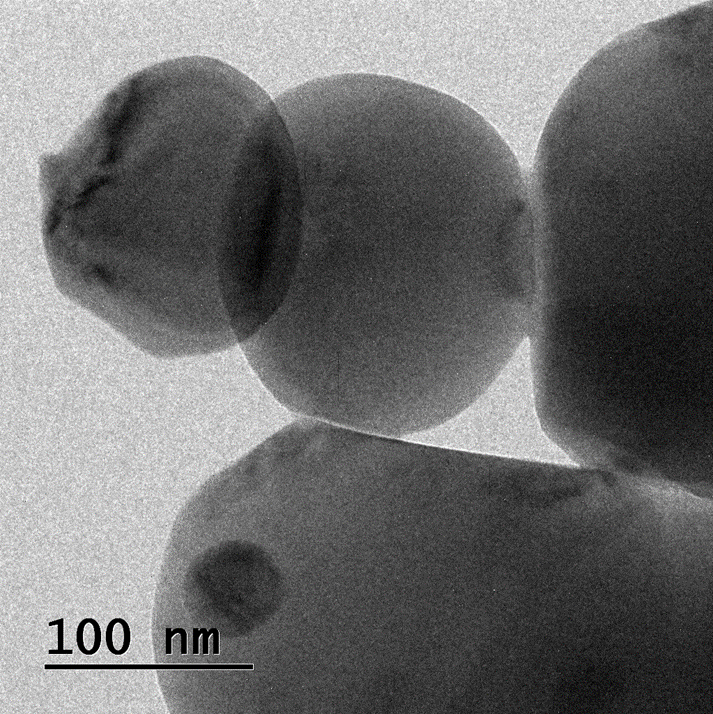
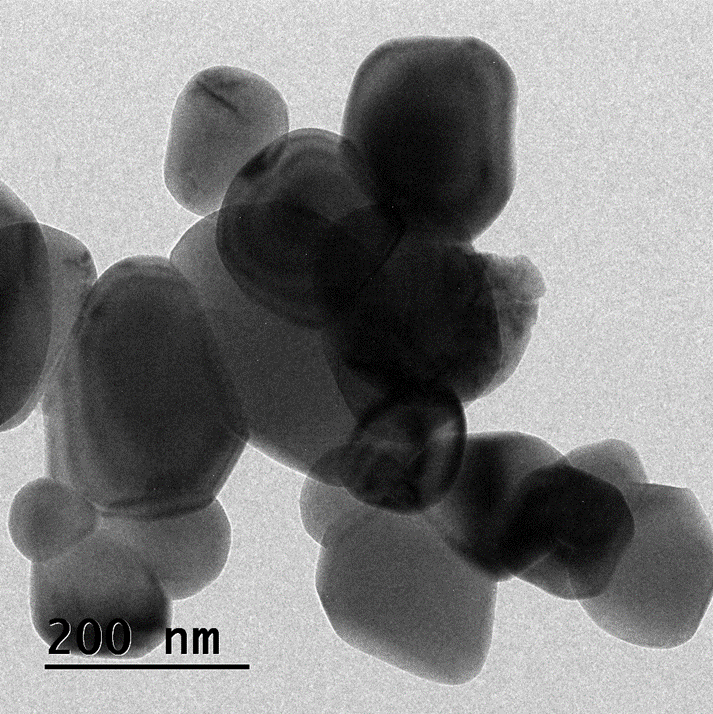
XRD analysis of AgNPs synthesized from Cissampelos pareira shows distinct peaks at 27.08°, 28.22°, 32.38°, 38.35°, 44.41°, 46.22°, 64.68°, 77.54°, and 81.62°, confirming their crystalline nature(Chehelgerdi et al., 2023). These peaks correspond to FCC silver planes, including (111) and (200). The results align with previous studies, highlighting this green synthesis as an eco-friendly alternative that utilizes Cissampelos pareira bioactive compounds for nanoparticle reduction and stabilization.[(Kumar et al., 2017)](https://paperpile.com/c/3v6Wqn/R5tB).Compared to AgNPs synthesized from other plant extracts, subtle differences in XRD peaks are observed. Azadirachta indica (Neem) AgNPs exhibit peaks at 38.18°, 44.36°, 64.46°, and 77.46°, while Moringa oleifera AgNPs show peaks at 38.10°, 44.26°, and 64.56°. These variations may be due to differences in phytochemical composition influencing nanoparticle formation and stabilization. [(S. A. Kumari et al., 2022)](https://paperpile.com/c/3v6Wqn/vpNo). Slight variations in XRD peak positions arise from differences in capping agents, particle sizes, and crystallinity due to the unique phytochemicals in each plant. Despite these minor shifts, the FCC silver pattern remains consistent, confirming the universal crystalline nature of plant-mediated silver nanoparticles.



**Fig. 3** XRD analysis if Silver nanoparticles synthesized from Cissampelos pareira

## Transmission Electron Microscopy (TEM)

The TEM micrographs at different magnifications reveal irregularly shaped, somewhat agglomerated nanoparticles with crystalline structures, indicated by lattice fringes. The size distribution appears broad, averaging around 87 nm. The left image, with a 200 nm scale bar, provides a broader view of nanoparticle distribution, while the right image, scaled at 100 nm, offers a detailed look at individual nanoparticle shape and size.



1. (B)

**Fig. 4 (**A-B) TEM analysis of silver nanoparticles synthesized from Cissampelos pareira

# ANTI - MICROBIAL ACTIVITY OF SILVER NANOPARTICLES

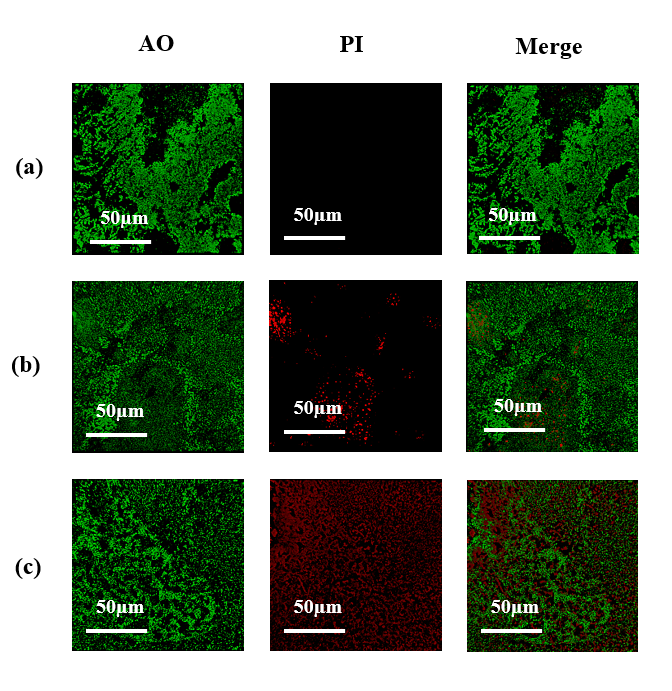
Fig 4 shows the zone of inhibition produced by silver oxide nanoparticles against both Gram-positive and Gram-negative bacterial strains. At very low concentration (10 ug/ml), all the considered pathogens did not show any zone of inhibition, whereas at concentration (100 ug/ml) silver nanoparticles exhibited maximum 9.8 $ 0.90 mm zone of inhibition against Bacillus subtilis due to diffusion of nanoparticles on nutrient agar plates.

Table 1: Treatment

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Treatment (µg/ml) | Zone of Inhibition (mm) | | | |
| Name of Microorganisms | *E. coli* | *Bacillus* sp. | *Klebsiella pneumoniae* | *Lactobacillus* sp. |
| 10 | - | - | - | - |
| 100 | 6.8 ± 0.65 | 9.8 ± 0.90 | 7.6 ± 0.17 | 8.5 ± 0.43 |
| 1000 | 12.4 ± 0.01 | 14.6 ± 0.32 | 13.5 ± 0.87 | 11.7 ± 0.21 |
| Ciproflacin  (12 µg/ml) | 17 ± 0.67 | 19 ± 0.34 | 20 ± 0.54 | 18 ± 0.08 |

# ANTI BIOFLIM ACTIVY

**Figure 5:** Confocal Laser Scanning Microscopy (CLSM) was used to assess the anti-biofilm activity of AgNPs against Klebsiella sp. Staining with AO and PI showed live cells in green and dead cells in red (Saadh et al., 2024). At MIC, the biofilm was significantly disrupted with more dead cells, while at 1/2MIC, biofilm thickness decreased with some dead cells. The control group had a dense biofilm with mostly live cells. These findings confirm AgNPs’ effectiveness in reducing biofilm formation and bacterial viability, suggesting their potential in preventing biofilm initiation and maintenance.[(Sathish et al., 2024)](https://paperpile.com/c/3v6Wqn/MjK5).



**Fig. 5** Anti – Biofilm analysis of silver nanoparticles synthesized from Cissampelos pareira

# Discussion

The eco-friendly biosynthesis of AgNPs using Cissampelos pareira leaf extract aligns with green chemistry principles. Phytochemicals like flavonoids, terpenoids, and alkaloids aid in reducing and stabilizing silver ions under mild conditions.[(Tian et al., 2024)](https://paperpile.com/c/3v6Wqn/8h84). TEM, FTIR, and XRD analyses confirmed the successful synthesis of crystalline, polydisperse AgNPs. FTIR identified functional groups from the leaf extract, highlighting their role in nanoparticle stabilization. This green synthesis method eliminates toxic chemicals, enhancing biocompatibility and potential applications.[(Meng et al., 2024)](https://paperpile.com/c/3v6Wqn/tlR5)

The synthesized AgNPs showed strong antibacterial activity against Gram-positive and Gram-negative bacteria, including Staphylococcus aureus, Escherichia coli, and Bacillus sp. Larger inhibition zones at higher concentrations indicated their effectiveness, attributed to membrane disruption, ROS generation, and DNA interference. Their broad-spectrum action makes them promising alternatives to antibiotics, offering a natural and sustainable solution to combat bacterial infections amid rising antibiotic resistance.[(Azimzadeh et al., 2024)](https://paperpile.com/c/3v6Wqn/gFu4)

The formation of biofilms by bacteria is a significant challenge in clinical settings, usually causing prolonged infections and developing resistance to conventional therapy. This study reports the potent antibiofilm activity of AgNPs against Klebsiella sp. through microtiter plate assays and Confocal Laser Scanning Microscopy. [(Iqtedar et al., 2019)](https://paperpile.com/c/3v6Wqn/XIHL)A reduction in biofilm thickness and an increase in dead bacterial cells with the rise in AgNPs concentration clearly bring out the fact that nanoparticles disrupt biofilm formation. Acridine orange and propidium iodine staining in CLSM clearly outlined living and dead cells, respectively. From these results, it could be shown that AgNPs are inhibitors of biofilm formation; therefore, AgNPs could be an important tool to prevent infections via biofilms, especially those related to devices or implants applied to the human body.[(Khedr et al., 2024)](https://paperpile.com/c/3v6Wqn/JoLT)

The green synthesis of AgNPs using plant extracts highlights sustainable nanotechnology by eliminating hazardous chemicals and utilizing renewable resources. This method aligns with green chemistry principles, enabling eco-friendly biosynthesis with enhanced antimicrobial and antibiofilm properties. The study’s promising results suggest applications in wound healing, infection control, and medical coatings. Further research on optimizing synthesis, scaling production, and evaluating safety is needed. Combining AgNPs with natural compounds or antibiotics could enhance therapeutic potential, making green nanotechnology a sustainable approach to developing advanced antimicrobials.

# Conclusion

The green synthesis of silver nanoparticles (AgNPs) using Cissampelos pareira leaf extract as a reducing and stabilizing agent proved to be an eco-friendly and efficient method, leveraging the plant's rich phytochemical content. The AgNPs demonstrated strong antibacterial and anti-biofilm activities, effective against a broad spectrum of microbial strains. Characterization techniques confirmed their crystalline structure, size, and stability, while their antimicrobial efficacy was established through disk diffusion, MIC, and MBC tests. This sustainable approach aligns with the principles of green chemistry and has potential applications in antimicrobial agents, medical coatings, and water treatment systems. Further optimization of synthesis parameters and exploration of synergistic effects with antimicrobial drugs could enhance their clinical efficiency and broaden their applications.

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