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RESEARCH ARTICLE

The green synthesis and characterization of silver nanoparticles to improve antibacterial activity using *Coscinium fenestratum (Gaertn.) Colebr.:* leaf extracts.

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Abstract

In this study, the silver nanoparticles have been green synthesized using *Coscinium fenestratum (Gaertn.) Colebr.:*. Synthesized silver nanoparticles have been preliminary validated by the color change to rusty brown from greenish yellow and (UV-spectroscopy). Morphological analysis through Field emission scanning microscopy (FE-SEM) showed cubic and spherical shapes of synthesized nanoparticles and the diameter of the synthesized nanoparticles ranged from 30.18 to 184.89nm. The (EDS) energy- dispersive x-ray spectroscopy spectrum has confirmed the synthesis of silver nanoparticles (AgNPs). The peaks around 400 cm^{-1} to 600 cm^{-1} in the IR spectrum indicated the vibration of metals- oxygen (Ag-O), whereas band at 1365.60 cm^{-1} and 1357.89 cm^{-1} shows the hydroxyl group bending which confirms the synthesis of nanoparticles. The synthesised silver nanoparticles exhibited antibacterial activity against *Escherichia coli* with 20±0.3 mm (Zone of inhibition).

Keywords

Silver nanoparticles; green synthesis; antibacterial assay; *Coscinium fenestratum (Gaertn.) Colebr.:* extracts.

Introduction

A significant area of present-day research is nanotechnology, a field concerned with the development, management, and manipulation of particle structures between 1 and 100 nm in size. In recent years, nanoparticles synthesis has received attention because of its various properties like optical, electrical, chemical and magnetic. Chemical analysis, integrated circuits, cell electrode, and antibacterial deodorant fibre are a few applications for silver nanoparticles (1). A range of nanomaterials have applications as antimicrobial agents, which include metal-based nanoparticles, carbon-based nanomaterials, polymers, nanocomposite, nanoemulsions, liposomes, and smart nanomaterials (2).

A crucial area of contemporary nanotechnology research is the creation of dependable green processes for the manufacture of silver nanoparticles. Various methods using plants, fungi and bacteria have been used to synthesize nanoparticles (3). The synthesis of metallic silver nanoparticles from their precursor salts and the stability of the silver nanoparticles in a complicated redox-mediated process is facilitated by a number of qualitative components found in plants. The three main steps for the formation of the silver nanoparticles are Reduction, Agglomeration and Stabilization (4). The chemical reduction of an aqueous solution of silver nitrate is one of the most widely used processes for the synthesis of silver nanoparticles.

The reaction vessels have the appearance of a yellowish-brown color indicating the formation of silver nanoparticles (5). Without any templates, additives, or accelerants, C. *fenestratum* leaf extract was able to produce nanorods at room temperature. The synthesis of silver nanorods may be due to the berberine and other alkaloids found in C. *fenestratum* (1).

Compared to conventional AgNPs, biologically synthesised AgNPs exhibit even better catalytic and electrical properties. The most common application of these catalytic properties is the treatment of wastewater. In order to treat wastewater, the stem extract from *C. fenestratum* was used to create AgNPs (6,7).

AgNPs have been identified as one of the potential metallic nanoparticles for wastewater treatment that can remove heavy metals and dyes. They are frequently used in the treatment of wastewater to get rid of microbes because they have antimicrobial properties that are widely recognized. It has also been widely used as biosensors in the food industry and for the detection of heavy metals (8).

Materials and Methods

The fresh leaf samples were air-dried at room temperature for 72 hr to remove moisture. The dried leaf samples were ground into a fine powder. A total of 3 grams of dried leaf powder was obtained from the initial 100 g of fresh leaf samples. The weight of the dried leaf powder was accurately measured and recorded.

Silver nitrate solution preparation

For the preparation of 1mM, 3mM and 5mM Silver nitrate ($AgNO_3$) 0.018g, 0.05g and 0.09g of $AgNO_3$ were added to 100 ml of distilled water respectively. To prevent the silver from auto-oxidation, the solution was thoroughly mixed and maintained in an amber-colored bottle (1).

Silver nanoparticles green synthesis

Fresh silver nitrate solution was prepared using distilled water at a concentration of about 1 mg/ml in the dark. By combining prepared leaf extracts with silver nitrate solution ($AgNO_3$) at a ratio of 1 to 1 mol (90 ml of aqueous solution containing 1 mM, 3Mm, 5mM silver nitrate was combined with 10 ml of *C. fenestratum* extract), it appeared feasible to convert Ag+ to Ag0. The resulting mixture of plant extracts and $AgNO_3$ has been kept within a range of 27 °C. A visible alteration in colour was used to track the reduction of silver ions in the solution. This shows the first evidence that *C. fenestratum* was created from silver nanoparticles (Cf-Ag nanoparticles). The mixture was then incubated for an entire night at room temperature in the dark (1).

Antibacterial assay

In this antibacterial assay, nanoparticles synthesized using ethanolic leaf extract were tested for their antibacterial activity against two bacterial strains. The antibacterial assay was estimated using the agar disc diffusion method against the bacterial strain *E.coli* and *Bacillus licheniformis* (8).

In summary, the selected bacterial pathogens were evenly distributed using sterile cotton swabs on the surface of solidified (Luria broth) LB media plates. Sterile filter paper discs were then loaded with different concentrations of the synthesized silver nanoparticles of ethanolic leaf extract (25µl and 50µl) and incubated for 24 hours at 37 °C and observed the inhibition zone.

Characterization methods

Using a Shimadzu UV-1800 spectroscopy, the UV-visible spectrum of synthesized Ag NPs was recorded at a wavelength range between 300 and 600 nm. Scanning electron microscopy (FE-SEM-TESCAN MIRA 3) was used to analyze the surface morphology, size, and EDS analysis was used to analyse the elemental makeup of synthesized Ag NPs. Using an IR spectroscopy (FTIR- 00585, PerkinElmer), the chemical composition of *C. fenestratum*, Ag NPs was examined and recorded over 400–4000 cm^{-1} (9).

Results

Formation of Ag nanoparticles

In this study, the synthesis of silver nanoparticles (Ag NPs) from *C. fenestratum* leaf extracts was successfully achieved by combining $AgNO_3$ with both methanolic and ethanolic extracts. One of the striking observations during the synthesis process was the gradual color change from greenish to rusty brown. This visual transformation served as an initial confirmation of Ag NPs synthesis (Fig 1). The color change phenomenon is attributed to the surface plasmon resonance of Ag NPs, which is an indicator of nanoparticle formation. It occurs due to the collective oscillation of free electrons in the metal nanoparticles when exposed to electromagnetic radiation, resulting in the absorption of specific wavelengths and the manifestation of distinct colors [6]. In our study, this change from greenish to rusty brown clearly indicated the reduction of the precursor salt ($AgNO_3$) to Ag NPs.

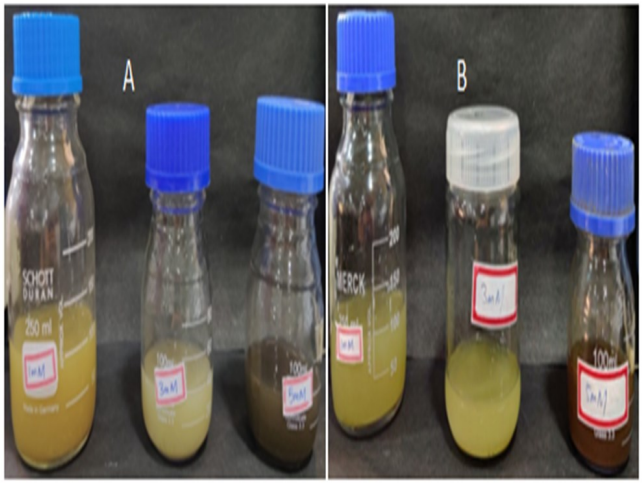

Fig. 1. A: Synthesis of nanoparticles by methanolic extract after 3 days. B: Synthesis of nanoparticles by ethanolic extract after 3 days. In this figure, the process of nanoparticle synthesis is depicted, utilizing an methanolic and ethanolic leaf extract and allowing the reaction to proceed for 3 days. Visual changes or other pertinent observations are presented, confirming the synthesis of nanoparticles with the ethanolic extract.

Notably, the color change was not observed when lower concentrations of leaf extract (1 mM and 3 mM) were employed in the synthesis process. As such, the 5 mM concentration of leaf extract emerged as the optimal choice for the characterization of the synthesized Ag NPs in this study.

UV spectroscopy

In our study, we utilized UV spectroscopy to analyze the methanolic and ethanolic leaf extracts of *C. fenestratum* after the synthesis of silver nanoparticles (Ag NPs). The UV spectra, as depicted in Figure 2, provided crucial insights into the formation and characteristics of Ag NPs within the respective extracts.

Interestingly, in the case of the ethanolic leaf extract, a distinct and blunt peak was observed around 400nm after 72 hours of incubation (Fig 2). This unequivocal peak formation served as strong evidence confirming the successful synthesis of Ag NPs. The sharp peak at approximately 400 nm is a feature associated with the surface plasmon resonance of Ag NPs [3], further validating their presence and stability within the ethanolic extract.

Conversely, the UV spectrum of the methanolic leaf extract (Fig 2) exhibited a different profile. While it indicated the initiation of Ag NPs formation, the peak was not as prominent or distinct as that observed in the ethanolic extract. This observation suggests that in the methanolic extract, the formation of Ag NPs might be in the early stages, and their concentration or size may not be as significant as in the ethanolic extract.

FTIR Analysis

The FTIR spectrum of *C. fenestratum* methanolic (Fig 3A) and ethanolic leaf extract (Fig 3B), Ag NPs were shown. FTIR spectra of methanolic leaf extract (Fig 3A) Ag NPs display C=O stretching at 1728.22cm^{-1},broad and narrow peak of N-H bending at 1589.34cm^{-1},broad peak of O-H bending at 1365.60cm^{-1},sharp peak of S=O at 1033.85 cm^{-1} and weak peak of N-O stretching at 1512.19cm^{-1}.Others peak at 711.53cm^{-1},725.23cm^{-1}and 833.25cm^{-1}contributed for C=C bending. FTIR spectra of ethanolic leaf extract (Fig 3B) Ag NPs displays weak and broad O-H stretching at 2916.37cm^{-1}and 2846.93cm^{-1}.O-H bending at 1357.89cm^{-1}, strong C=O stretch at 1728.22cm^{-1}, C=C stretching at 1597.06cm^{-1}, strong C-OH stretching at 1111.0cm^{-1}, 1072.42 cm^{-1}and 1033.85cm^{-1}and N-O stretching at 1504.48cm^{-1}.

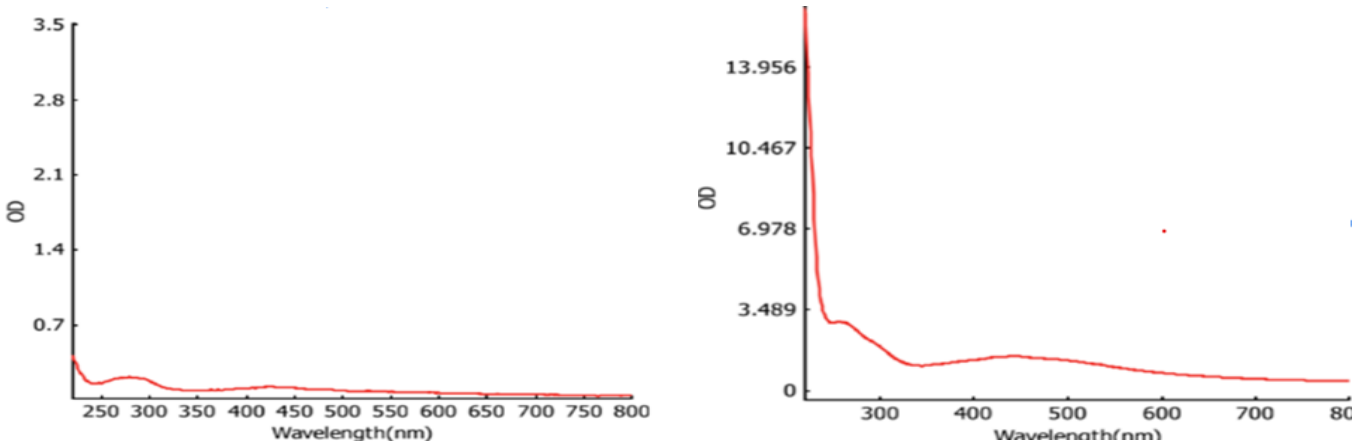

Fig. 2. Absorbance Spectra of Methanolic and Ethanolic Leaf Extract at Various Optical Densities (OD) respectively. Absorbance spectra of the methanolic and ethanolic leaf extract were measured at various optical densities (OD), spanning the range from 0.1 to 3.5 OD units. The graph presents the absorbance profile across the UV-visible spectrum, highlighting the absorbance behaviour of the ethanolic leaf extract at the specified OD values.

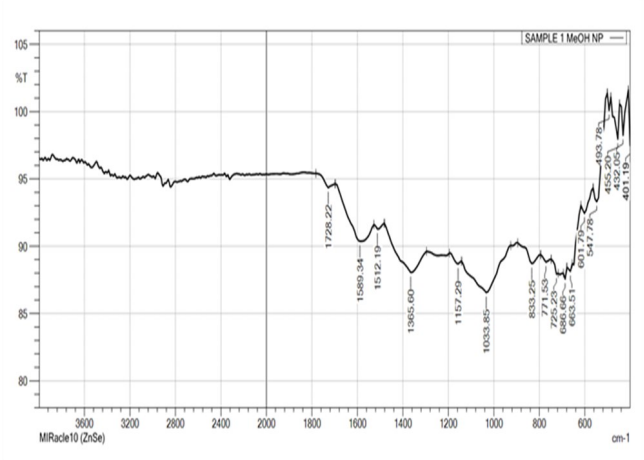

Fig. 3A FTIR Spectrum of Methanolic Leaf Extracts of C. *fenestratum* Containing Silver Nanoparticles (Ag NPs). This FTIR (Fourier-transform infrared) spectrum represents the chemical composition of methanolic leaf extracts from C. *fenestratum* containing synthesized silver nanoparticles (Ag NPs). The spectrum was acquired to elucidate the functional groups and molecular interactions present in the leaf extract following the synthesis process.

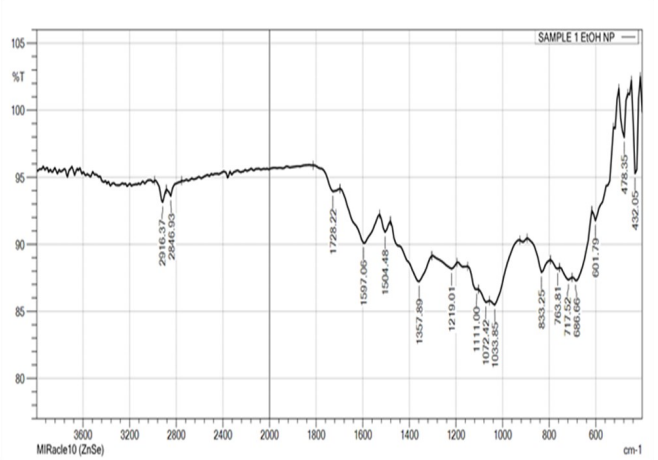

Fig. 3B FTIR Spectrum of Ethanolic Leaf Extracts of C. *fenestratum* Containing Silver Nanoparticles (Ag NPs). This FTIR (Fourier-transform infrared) spectrum represents the chemical composition of ethanolic leaf extracts from C. *fenestratum* containing synthesized silver nanoparticles (Ag NPs). In the spectrum, such as peak positions and intensities, are indicative of the various chemical bonds and functional groups involved in the stabilization and capping of the Ag NPs. The FTIR analysis provides valuable insights into the molecular environment of the Ag NPs within the leaf extract, shedding light on potential bioactive compounds responsible for their synthesis and stability.

These are evidence of the presence of berberine content which is responsible for the formation of nanoparticles. The Ag+ ions may be stabilized by berberine lone electron pairs, and nucleation sites may be created without AgNP clustering. This research found that plant- derived molecules were used to cap AgNPs, which may have decreased the Ag (I) precursors and provided a stabilizing effect.

EDS Analysis

The synthesis of silver nanoparticles (AgNPs) in both the methanolic and ethanolic leaf extracts of *C. fenestratum* was further substantiated through energy-dispersive X-ray spectroscopy (EDS) analysis. EDS provided valuable insights into the elemental composition of the synthesized nanoparticles. In the EDS spectrum of the ethanolic leaf extract (Fig 4B), silver (Ag) exhibited the highest and maximum number of peaks, corroborating the successful synthesis of AgNPs. This pronounced presence of Ag peaks underscores the abundance and purity of silver within the nanoparticles formed in the ethanolic extract.

Conversely, in the EDS spectrum of the methanolic leaf extract (Fig 4B), while silver (Ag) was the element with the highest peak, carbon (C) displayed the most prominent peak. This observation suggests the presence of a significant carbonaceous component in the nanoparticles synthesized in the methanolic extract. The coexistence of carbon peaks with silver peaks may indicate the presence of organic capping agents or stabilizers in the synthesized AgNPs.

SEM Analysis

The structural complexity of silver nanoparticles (Ag NPs) is well-documented and can exhibit variations influenced by numerous factors, including the synthesis method, reaction conditions, as well as size and shape control. In this study, Field-Emission Scanning Electron Microscopy (FE-SEM) was employed to provide valuable insights into the morphological characteristics of the synthesized Ag NPs. According to the FE-SEM images presented in Figure 5, the synthesized Ag NPs exhibited distinct structural features. In Figure 5A, a cube-like structure was observed in the methanolic leaf extract, showcasing the cubic morphology of the nanoparticles. SEM analysis revealed a range of nanoparticle diameters, spanning from 30.18 to 83.12 nm.

Conversely, in Figure 5B, the ethanolic leaf extract displayed spherical nanoparticles with a well-defined spherical morphology. SEM analysis of the ethanolic extract revealed a broader range of nanoparticle diameters, ranging from 45.17 to 184.89 nm.

Antibacterial assay

In this antibacterial assay, two bacterial strains were tested for their susceptibility to the antibacterial agent. For E. coli, the inhibition zone diameter was measured as 20 ± 0.3mm when 25 µL of the ethanolic leaf extract was used and 25 ± 0.2mm when 50µL was used (Fig 6). This suggests that the leaf extract had a moderate inhibitory effect on the growth of E. coli, with a stronger effect observed with the higher concentration (50µL). On the other hand, for *B. licheniformis,* no inhibition zone was detected at either 25 µL or 50 µL of the antibacterial agent. This indicates that the ethanolic leaf extract did not show significant antibacterial activity against *B. licheniformis* under the conditions tested.

It's important to note that the absence of an inhibition zone does not necessarily imply complete ineffectiveness against the bacterial strain, as other factors such as the bacterial resistance mechanisms and the specific properties of the leaf extract may influence the results. Further testing and analysis may be required to fully understand the antibacterial activity of the agent against these bacterial strains.

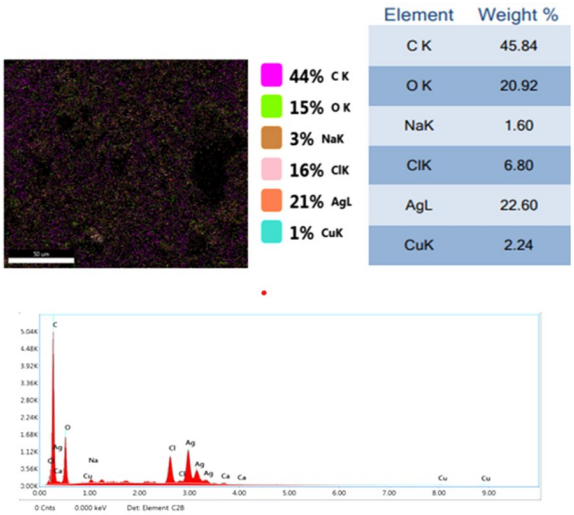

Fig. 4A EDS Spectrum of Methanolic Leaf Extracts of *C. fenestratum* Containing Silver Nanoparticles (Ag NPs). This EDS (Energy-Dispersive X-ray Spectroscopy) spectrum presents the elemental composition of methanolic leaf extracts from *C. fenestratum* containing synthesized silver nanoparticles (Ag NPs). The spectrum was obtained to identify and quantify the elemental constituents within the leaf extract, with a particular focus on the presence of silver (Ag).

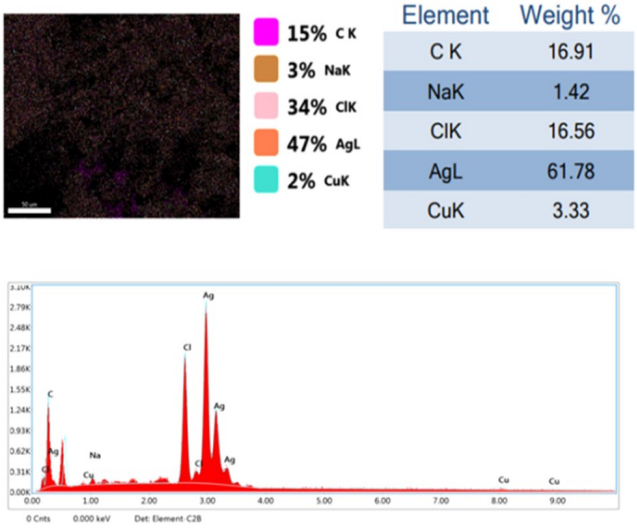

Fig. 4B EDS Spectrum of Methanolic Leaf Extracts of *C. fenestratum* Containing Silver Nanoparticles (Ag NPs). This EDS (Energy-Dispersive X-ray Spectroscopy) spectrum presents the elemental composition of methanolic leaf extracts from *C. fenestratum* containing synthesized silver nanoparticles (Ag NPs). The prominent peaks in the spectrum correspond to the characteristic X-ray emissions of the elements present. The presence of strong silver peaks confirms the existence of Ag NPs in the leaf extract. Additionally, other elemental peaks, if detected, may provide insights into the potential presence of additional elements, which could be attributed to the plant extract's phytochemical composition.

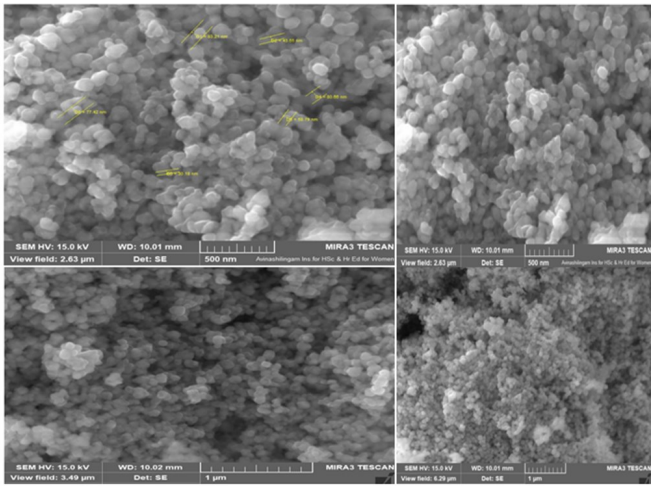

Fig. 5A FE-SEM Images of Silver Nanoparticles (AgNPs) Synthesized from Methanolic Leaf Extract of *C. fenestratum*. These Field-Emission Scanning Electron Microscopy (FE-SEM) images depict the morphological characteristics of silver nanoparticles (AgNPs) synthesized using methanolic leaf extracts of *C. fenestratum*. The FE-SEM analysis was performed to investigate the size, shape, and distribution of the AgNPs. In the images, the silver nanoparticles are visualized as distinct, spherical nanostructures. The size and uniformity of the nanoparticles can be observed, offering insights into the quality of the synthesis process.

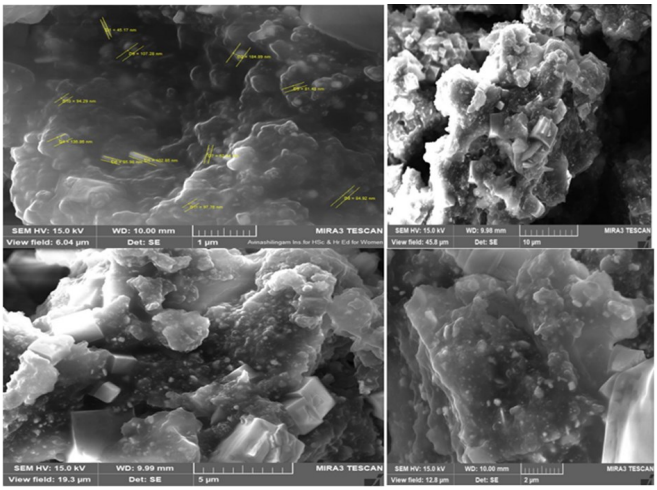

Fig. 5B FE-SEM Images of Silver Nanoparticles (AgNPs) Synthesized from ethanolic Leaf Extract of *C. fenestratum*. These Field-Emission Scanning Electron Microscopy (FE-SEM) images depict the morphological characteristics of silver nanoparticles (AgNPs) synthesized using ethanolic leaf extracts of *C. fenestratum*. The FE-SEM analysis was performed to investigate the size, shape, and distribution of the AgNPs. In the images, the silver nanoparticles are visualized as distinct, cubical nanostructures. The size and uniformity of the nanoparticles can be observed, offering insights into the quality of the synthesis process.

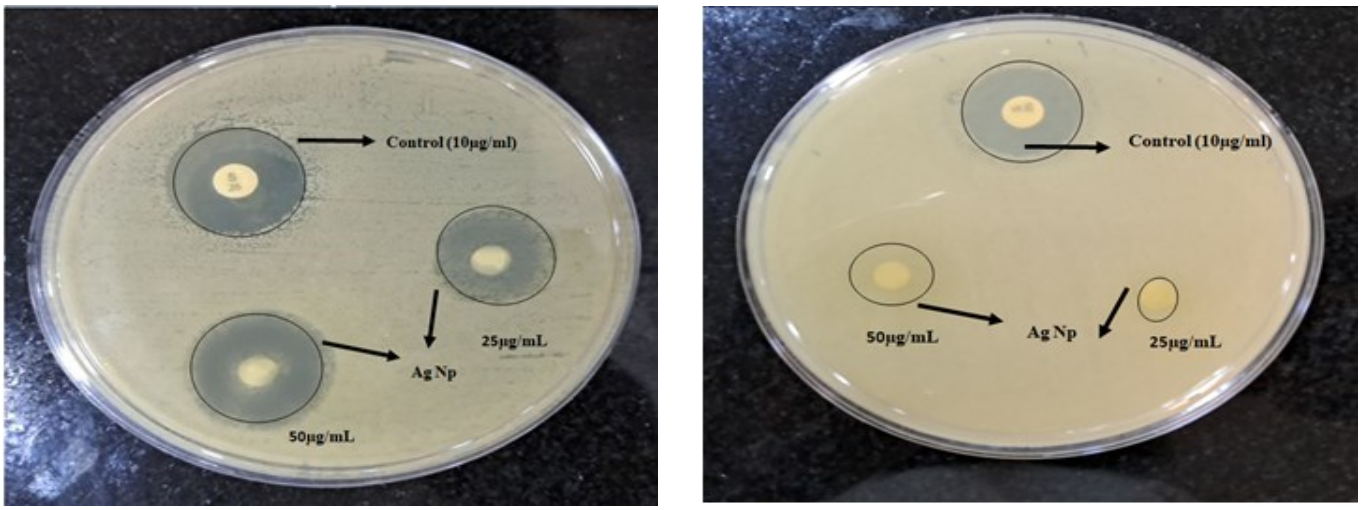

Fig. 6 Antibacterial activity of silver nanoparticles (AgNPs) against A) *Escherichia coli* (*E. coli*) and B) *Bacillus licheniformis* (*B. licheniformis*), with Control: Streptomycin Disc. This figure presents the results of the antibacterial activity assay conducted to assess the effectiveness of silver nanoparticles (AgNPs) against two bacterial strains. For *E. coli* (Panel A), the clear zones of inhibition around the discs indicate the extent of bacterial growth inhibition, This suggests that the AgNPs had a moderate inhibitory effect on the growth of *E. coli*, with a stronger effect observed at the higher concentration. In Panel B, representing *B. licheniformis*, the absence of inhibition zones around both the control Streptomycin disc and the AgNPs treatment discs indicates that the AgNPs did not exhibit significant antibacterial activity against B. licheniformis under the conditions tested.

Conclusion

In this study, we successfully synthesized silver nanoparticles (AgNPs) using the leaf extract of *C. fenestratum* through a green synthesis approach. Our investigation employed a multi-faceted characterization approach, including UV spectroscopy, FTIR analysis, EDS elemental analysis, and SEM imaging, to comprehensively examine the properties and potential applications of these AgNPs. The UV spectroscopy confirmed the synthesis of AgNPs, with distinct spectral changes corresponding to the presence of nanoparticles. FTIR analyses provided insights into the chemical functional groups involved in the reduction and stabilization of AgNPs, notably highlighting the role of O-H stretching. One notable advantage of our green synthesis approach is its scalability to industrial production, offering a sustainable and eco-friendly method for AgNPs production. Moreover, the EDS elemental analyses revealed the elemental composition of the synthesized AgNPs, which included silver alongside other elements, underscoring the complexity of green synthesis processes.

The SEM analysis enabled the visualization of AgNPs size, shape, morphology, and distribution, revealing differences between the methanolic and ethanolic leaf extracts. Notably, the ethanolic extract yielded AgNPs with larger sizes compared to the methanolic extract at the 72-hour incubation period. Furthermore, our results indicated the potential application of AgNPs synthesized from the ethanolic leaf extract as antibacterial agents against specific bacterial strains. These findings hold promise for diverse applications, from nanotechnology to biomedicine.

However, it is essential to acknowledge the limitations of our study. Firstly, our investigation primarily focused on characterizing the synthesized AgNPs and assessing their antibacterial potential against selected strains. Further research is needed to delve into the detailed mechanisms of antibacterial action and potential

cytotoxicity. Moreover, the complexity of natural extracts and the variations in plant material can introduce variability in nanoparticle synthesis, which may require further optimization for consistent results.

In conclusion, this study presents a green synthesis approach for AgNPs using *C. fenestratum* leaf extract, offering valuable insights into their properties and potential applications. While our findings hold promise, additional research is warranted to address the identified limitations and fully unlock the potential of these AgNPs for various practical applications in the future.

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Authors' contributions

All the experiments were conceptualized by MG and SB. The methodology was designed by MG, SB, AS, HS, RP and SK. The original draft of this manuscript was prepared and written by MG and SB. Statistical analysis was carried out by MG and SB. SK performed the investigation, review, editing and supervision.

All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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