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BIOSYNTHESIS AND CHARACTERIZATION OF SILVER NANOPARTICLES USING *CLEOME VISCOSA* LEAVES EXTRACT AND EVOLUTION OF ANTIBACTERIAL ACTIVITY

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Abstract

Green synthesis of silver nanoparticles was successfully done using *Cleome viscosa* leaf extract by simple, rapid, eco-friendly and a cheaper method. In this study, silver nanoparticles were synthesized using leaf extract from *C. viscosa*, which facilitated the reduction of silver nitrate into silver ions. The obtained AgNPs were characterized by UV, FTIR, XRD and SEM-EDX analysis. The presence of biosynthesized AgNPs was confirmed by UV-visible spectroscopy and also crystal nature of AgNPs confirmed through XRD analysis; FT-IR spectrum was used to confirm the presence of different functional groups in the biomolecules which act as a reducing and capping agent for the nanoparticles. The morphology of the synthesized silver nanoparticles (AgNPs) was examined using SEM, and their silver content was verified through elemental analysis. The green-synthesized AgNPs displayed effective antibacterial activity against both Gram-negative and Gram-positive bacteria.

Keywords: *Cleome viscosa*; FTIR; XRD; SEM-EDX; Antibacterial

1. Introduction

Nanoscience has emerged as a leading field, offering vast opportunities for both fundamental and applied research across various branches of cognitive sciences. The rise of nanoscience and nanotechnology is catalyzing a global technological revolution (Bayda *et al*., 2019). Nanotechnology is typically utilized for materials within the size range of 1 to 100 nm. Nanomaterials exhibit a variety of properties distinct from their bulk counterparts, including differences in size, mechanical strength, chemical reactivity, electrical conductivity, magnetic behaviour, and optical characteristics (Binns, 2021). Various physical, chemical, and biological methods have been developed to synthesize stable nanomaterials with precise control over their size and shape (Singh *et al*., 2020).

For synthesizing nanomaterials Physical methods often face challenges such as poor reproducibility due to sensitivity to process parameters, limited control over particle size and composition, difficulties in scaling up for commercial production, and the generation of hazardous waste that necessitates proper disposal (Fiedler *et al*., 2022). Chemical synthesis involves the use of costly synthetic chemicals and produces hazardous chemicals and by-products as a result of the synthesis process (Pollok & Waldvogel, 2020). Biological synthesis offers several advantages over chemical and physical methods, including lower cost, environmental sustainability, and ease of scalability for large-scale production (Tilwari & Saxena, 2022). The biological approach utilizes beneficial microorganisms, including bacteria, fungi, and plants making it an environmentally friendly, cost-effective, and safe method (Huq *et al*., 2022). The synthesis of nanoparticles using plants is preferred over other biological sources due to its simplicity. It is a straightforward one-step process that eliminates the need for maintaining cell cultures and a sterile environment, which can be time-consuming (Khan *et al*., 2022). Plants are valuable for manufacturing nanoparticles because they are abundant, easily available, easy to handle, rich in several metabolites, and contain pharmacologically active constituents that act as both reducing and capping agents in nanoparticle synthesis (Sumathi & Thomas, 2017). Metal nanoparticles can be synthesized using various parts of plants including leaves, roots, seeds, stems, latex, flowers, and buds (Rajoriya *et al*., 2021). Different plants and their parts, such as the leaf extract of *Curcuma longa* L. (Maghimaaa & Alharbi, 2020), peel of *Citrus reticulata* (Ituen *et al*., 2020), the fruit extract of *Phyllanthus emblica* L. (Dhar *et al*., 2021) and the leaves of *Fenugreek* (Rizwana *et al*., 2021), have been reported for nanoparticle formation.

The green synthesis of nanoparticles is influenced by various factors such as temperature, pH, reaction time, the volume of metal salt, and the volume of plant extract. The interaction of these factors is crucial in determining the shape and size of the synthesized nanoparticles (Ibrahim & Ali, 2018). The temperature is a key factor in the synthesis of silver nanoparticles. While the reaction typically occurs at room temperature, which is a slower process, raising the temperature of the reaction mixture can significantly speed it up. The reaction temperature is generally set between 30 and 100°C. Additionally, silver nanoparticles produced at higher temperatures are generally smaller in size (Bélteky *et al*., 2021). Increasing the temperature reduces the rate of Ag^+ ion reduction, promoting the homogeneous nucleation of silver nuclei and leading to the production of smaller-sized silver nanoparticles (Long *et al*., 2022). Research has indicated that higher temperatures in the reaction mixture result in a slower rate of nanoparticle synthesis but enhance stability.

The reaction time is crucial in nanoparticle synthesis because it facilitates proper interaction between the metal salt and the reducing complex components in the plant extract (Barzinjy & Azeez, 2020). The reaction time starts when the reactant is introduced into the beaker and continues until the reaction takes place. Plants with higher concentrations of secondary metabolites or phytochemicals reduce salts more efficiently. In contrast, plants with fewer reducing compounds require more time to reduce salts. However, even plants with lower secondary metabolites can still produce nanoparticles quickly. The reaction time is affected by various factors, including the pH of the reaction mixture, temperature, the reducing capability of the plant extract, light intensity, enzymatic activity, and the presence of secondary metabolites in the extract (Lade & Shanware, 2020).

The pH level significantly affects biomolecules by altering their electrical charges, which can potentially interfere with their ability to cap and stabilize nanoparticles. This, in turn, can influence the growth and size of the nanoparticles (Godoy-Gallardo *et al*., 2021). During nanoparticle synthesis, the pH level plays a critical role in determining their properties and reaction kinetics. Different plant extracts contain various bioactive components such as polyphenols, flavonoids, and proteins that can act as reducing agents, but their reactivity varies with pH. Optimizing the pH conditions is essential to enhance reduction efficiency and control the size and shape of the nanoparticles. The pH level also affects the stability and aggregation behaviour of the synthesized nanoparticles. The surface charge of nanoparticles is strongly dependent on pH, influencing their colloidal stability and tendency to aggregate. By adjusting the electrostatic repulsion at specific pH values, it's possible to prevent or minimize aggregation, which is crucial for achieving uniform and stable dispersions of silver nanoparticles (Bélteky *et al*., 2021; Din *et al*., 2022).

Despite the numerous advantages and the expanding research in this field, there remains a need for critical analysis of the green synthesis process. Factors such as the choice of plant extract, reaction conditions, and characterization methods can significantly impact the properties and quality of the resulting silver nanoparticles (Ag NPs) (Philip, 2010; Reddy *et al*., 2013; Mallikarjuna *et al*., 2014; Siddiqi *et al*., 2018; Singh *et al*., 2018).

This study focused on the green synthesis of silver nanoparticles (AgNPs) utilizing plant extracts. *C. viscosa* Linn. (family Cleomaceae, formerly known as Capparidaceae) is an annual herb that thrives during the rainy season. It is self-compatible, sexually reproducing, and characterized by its sticky texture. Commonly referred to as "wild mustard" or "dog mustard," This plant is widely distributed as a weed across the plains of India and throughout tropical regions worldwide (Ahmed *et al*., 2011). It is an annual weed commonly found in wastelands, characterized by yellow flowers and a strong, penetrating odor. In Indian traditional medicine, the plant is widespread across most parts of India and is often seen in woodlands, fallow lands, roadsides, refuse heaps, waste grounds, and agricultural areas (Asolkar *et al*., 1965-1981). The leaves of the plant exhibit rubefacient, vesicant, and sudorific properties. The seeds are small, dark brown or black, and granular, and they are known for their rubefacient, vesicant, and anthelmintic properties. Occasionally, the seeds are used as a condiment in curries (Devi *et al*., 2003). Fresh leaves of *C. viscosa* are traditionally used in Indian folklore medicine for the treatment of jaundice (Gupta & Dixit, 2009).

C. viscosa is well documented in Indian and Chinese traditional medicine systems, including Ayurveda and Unani, as a folklore remedy for various human ailments. The plant is believed to contain numerous bioactive metabolites with potential therapeutic applications, including antimicrobial, anti-inflammatory, hepatoprotective, antifibrotic, anticonvulsant, antioxidant, antinociceptive, antibacterial, and antitumor activities. Additionally, it is used as a carminative, cardiac stimulant, and anti-rheumatic agent, as well as for treating bronchitis, diarrhea, hypertension, malaria, neurasthenia, wound healing, and skin disorders (Kumar *et al*., 2009; Mali, 2010; Mishra *et al*., 2010; Chaudhary *et al*., 2010).

This research aims to provide a comprehensive and critical perspective on the green synthesis of crystalline Ag NPs using aqueous leaf extracts of *C. viscosa*. This study aims to enhance sustainable nanotechnology and its applications through an analysis of current knowledge, identification of key challenges, and exploration of potential future directions.

2. Methodology

2.1. Materials

For the synthesis of silver nanaoparticles, Analytical grade Silver Nitrate ($AgNO_3$) was obtained from Department of Zoology, Annamalai University, Chidhambaram, Tamil Nadu, India. Mueller-Hilton agar medium and Standard antibacterial drug, gentamycin was purchased from the local market. *C. viscosa* was collcted from Cuddalore, Tamil Nadu, India. The experiments were done using double-distilled water. The extract was selected as a reducing and capping agent based on its cost-effectiveness, easy availability, and medicinal properties.

2.2. Methods

2.2.1. Preparation of *Cleome viscosa* leaf extract

The *C. viscosa* leaves are thoroughly washed several times with running tap water to remove dust and then rinsed with distilled water. The leaves are allowed to dry at room temperature and they are grinded into a fine powder. 10 g leaf powder was mixed with 100 ml distilled water in the 250ml Erlenmeyer flask. These leaves are then boiled at 100°C for 30 min and then allowed to cool down. The prepared extract was then filtered through Whatman No.1 filter paper. The filtered extract was used as the reducing and stabilizing agent in the Synthesis of Nanoparticles. The filtrate is stored at - 20°C to prevent contamination of the aqueous leaf extract.

2.2.2. Green synthesis of AgNPs

The green synthesis of AgNPs is performed using aqueous extraction from *C. viscosa* leaves through a simple reduction method with $AgNO_3$. 1 mM solution of silver nitrate was prepared. 10ml of plant extract was mixed with 90ml of 1mM silver nitrate solution, with stirring. The solution is then stored at room temperature for 24 hours to ensure the complete settlement of nanoparticles. Reduction of Silver ions (Ag^+) to Silver Nanoparticles (Ag^0) was confirmed by the color change of solution from yellow to reddish brown. The rapid formation of a reddish brown solution indicates the successful formation of AgNPs through the reduction of silver ions in the solution. The reaction mixture was centrifuged for 15 minutes at 6000 rpm and the supernatant was removed. The centrifugation process was repeated two times to obtain clear nanoparticles. The collected pellet was dried in a hot air oven at 35-40°C. The dried nanoparticles were collected and utilized for further characterization and application studies.

3. Characterization of AgNPs

3.1. UV–vis spectrophotometer

The bioreduction of Ag+ ions in solutions was monitored by measuring the UV-VIS spectrum of the reaction medium. The UV-VIS spectral analysis of the sample was done by using U-3200 Hitachi spectrophotometer at room temperature operated at a resolution of 1 nm between 200 and 800 nm ranges.

3.2. Fourier Transform Infrared Spectroscopy (FTIR)

Shimadzu IR-IR Affinity1 model in the diffuse reflectance mode operating in the range 4000 - 400 cm^{-1} at a resolution of 4 cm^{-1} was used. The sample was mixed with KBr pellets. A thin sample disc was prepared using a disc press machine and analyzed with Fourier Transform Infrared (FTIR) spectroscopy to study the nanoparticles.

3.3. X-Ray Diffractometer (XRD)

The X-ray diffraction (XRD) analysis of drop-coated films containing silver nanoparticles was conducted to confirm their formation. This was carried out using an X'Pert Pro X-ray diffractometer operating at 40 kV and 30 mA with Cu Kα radiation.

3.4. SEM with EDS

The morphology, structure, and chemical composition of the silver nanoparticles were examined using SEM-EDX analysis, performed with a JEOL JSM-6610-LV microscope equipped with EDS.

4. Antibacterial activity of the synthesized CvAgNPs

4.1. Bacterial strains used in this study

The antibacterial efficacy of CvAgNPs was evaluated using two bacterial strains: a Gram-negative strain (*Escherichia coli*) and a Gram-positive strain (*Staphylococcus aureus*).

4.2. Inoculums preparation

The bacterial strains were subcultured at 35°C overnight in Mueller-Hilton agar slants. The bacterial growth was then collected using 5 mL of sterile water.

4.3. Antibacterial activity of synthesized CvAgNPs

The disk diffusion technique was employed to assess the antibacterial activity of the synthesized CvAgNPs. Agar medium (Mueller-Hilton, 10 mL) was poured into sterilized Petri dishes, followed by the addition of 15 mL of the previously prepared bacterial suspension. Filter paper discs loaded with 10 mg/mL of CvAgNPs were placed on top of the agar plates, along with a positive control (Gentamycin, 5 µg) on a separate disc. The Petri plates were refrigerated at 4°C for 120 minutes to allow for the diffusion and dispersion of CvAgNPs, then incubated at 35°C for 24 hours. The inhibition zones were indicated as antibacterial activity.

5. Results and discussion

5.1. UV–vis spectrophotometer

The UV-visible spectroscopy of the green-synthesized silver nanoparticles shows two characteristic absorption peaks at wavelengths 391.3 nm and 486.9 nm with absorbance values of 1.153 AU and 1.164 AU, respectively. Peak at 391.3 nm explains this absorption is indicative of the surface plasmon resonance (SPR) of silver nanoparticles. The peak at 391.3 nm suggests the formation of small, spherical nanoparticles. The SPR band was generated as a result of the excitation of free electrons in the silver nanoparticles upon absorbing visible light (Baia *et al*., 2007). The observation of a single peak suggests the formation of spherical nanoparticles (He *et al*., 2002). The broadened peak indicates the presence of poly-dispersed silver nanoparticles (Ashraf *et al*., 2019). Similar results were reported for Aloe Vera leaf extract (Ashraf *et al*., 2016), confirming the formation of AgNPs. The UV-visible spectrum demonstrates the successful synthesis of silver nanoparticles, with characteristic SPR peaks confirming their formation in Fig.1.

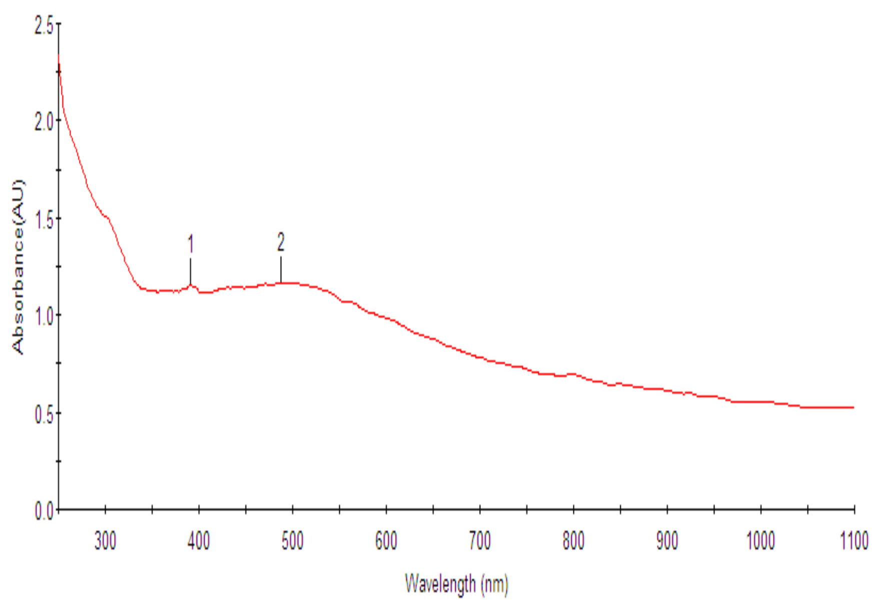


Fig.1. UV–vis absorption spectrum of the synthesized CvAgNPs

5.2. Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR spectrum of *C. viscosa* involved in the green synthesis of silver nanoparticles provides insight into the functional groups responsible for nanoparticle formation and stabilization.

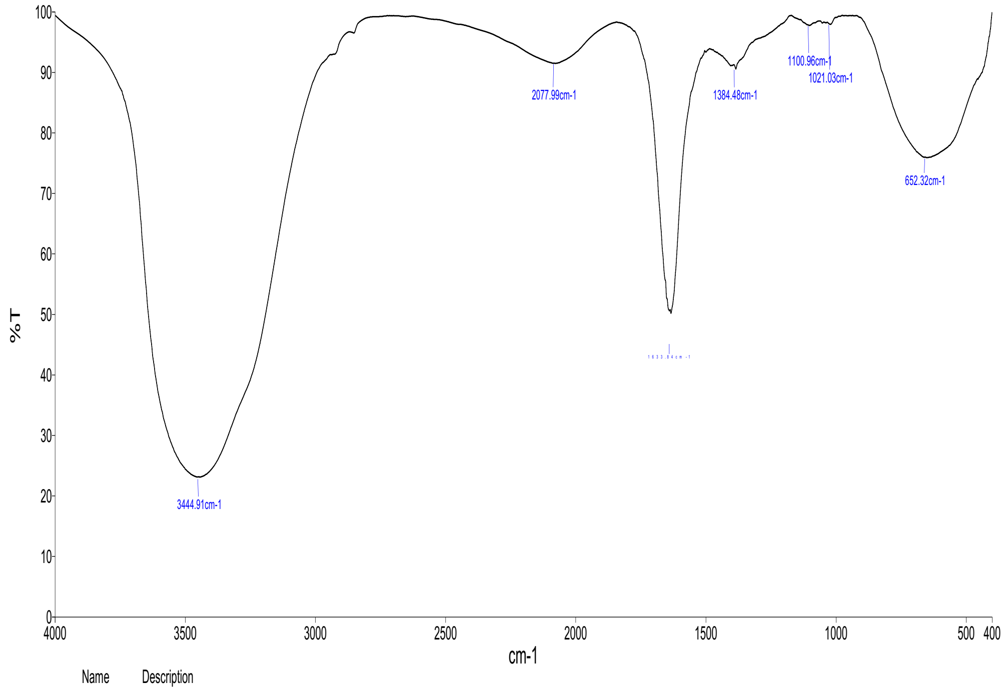


Fig.2. FT-IR spectra of the synthesized CvAgNPs

The FTIR analysis of silver nanoparticles synthesized using *C. viscosa* revealed significant peaks that highlight the involvement of various functional groups from the plant extract in the synthesis and stabilization of the nanoparticles. In Fig.2 the silver nanoparticles exhibit an O–H stretching vibration in carboxylic acids, indicated by a broad peak at 3444.91 cm^{-1}. The rapid reduction and stabilization of silver ions into nanoparticles in this analysis are likely facilitated by flavonoids and proteins. Flavonoids in the leaf extract act as potent reducing agents, suggesting their role in the conversion of silver nitrate to silver nanoparticles. These compounds in the aqueous extract of *C. viscosa* are likely key contributors to the reduction of Ag^{+} to Ag^{0} (Zuas *et al*., 2014).

The peak at 2077.99 cm^{-1} is associated with C≡C or C≡N stretching vibrations, suggesting the involvement of alkynes or nitriles in the stabilization process. The sharp peak at 1633.84 cm^{-1} is characteristic of C=C stretching vibrations, typically found in alkenes or aromatic compounds, indicating the possible role of metabolites like flavonoids or polyphenols in nanoparticle synthesis confirming the presence of aromatic groups (Reddy *et al*., 2014).

The peak at 1384.48 cm^{-1} corresponds to C-H bending vibrations of alkanes or $-NO_2$ symmetric stretching, suggesting the presence of organic compounds that act as reducing or capping agents. Peaks at 1100.96 cm^{-1} and 1021.03 cm^{-1} are attributed to C-O stretching vibrations, indicative of alcohols, esters, or ethers contributing to the capping process. Finally, the peak at 652.32 cm^{-1} is linked to C-H bending in aromatic compounds or metal-ligand interactions, confirming the binding of silver nanoparticles to functional groups in the plant extract. Overall, the FTIR analysis confirms the successful green synthesis of silver nanoparticles, where bioactive compounds from *C. viscosa* act as natural reducing and stabilizing agents.

The FTIR analysis reveals that biomolecules in *C. viscosa* act as reducing and stabilizing agents in the green synthesis of silver nanoparticles. Functional groups like -OH, C=C, C-H, and C-O are actively involved in the synthesis process, demonstrating the plant's role in the eco-friendly production of nanoparticles (Oluwaniyi *et al*., 2015).

5.3. X-Ray Diffractometer (XRD)

The XRD analysis was performed to determine the structural characteristics of silver nanoparticles synthesized using the *C. viscosa* extract. The analysis confirms the crystalline nature of the synthesized nanoparticles and provides insight into their phase, crystallite size, and the role of the plant extract in the synthesis process.

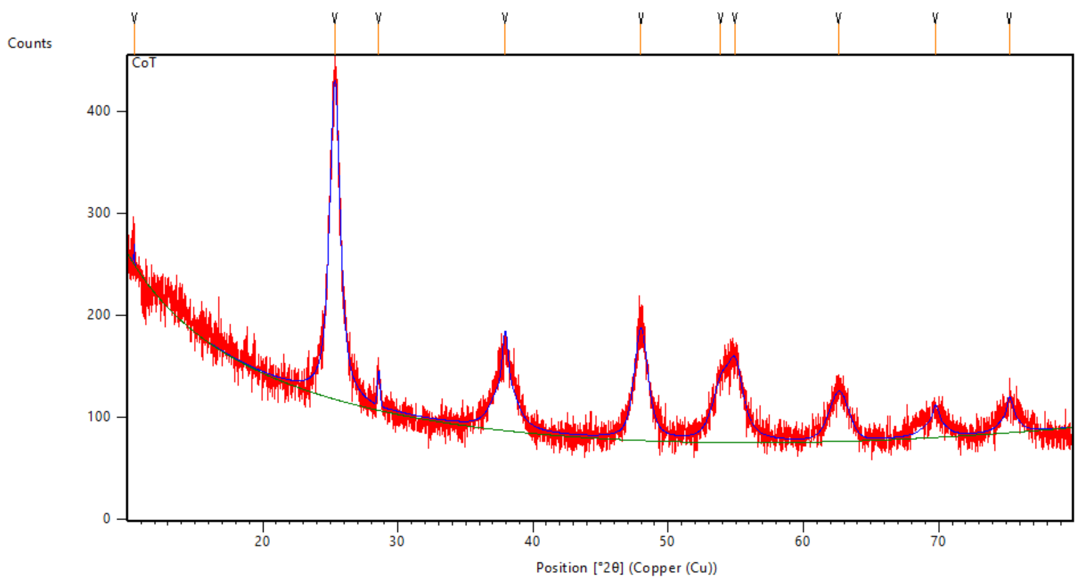


Fig.3. XRD spectra of CvAgNPs

The XRD pattern exhibits sharp and distinct peaks, confirming the crystalline structure of the synthesized nanoparticles. The positions of the diffraction peaks correspond to specific planes of face-centered cubic (FCC) silver, which aligns with standard JCPDS (Joint Committee on Powder Diffraction Standards) card data for silver nanoparticles. On examining the XRD pattern (Fig. 3) of silver nanoparticles synthesized using *C. viscosa*, the prominent peaks at $2\theta = 25.31°$, 37.90°, 47.96° and 69.77° represent the (111), (200), (220), and (311) Bragg's reflections of the face-centered cubic (FCC) structure of silver, respectively. The size of the Ag nanoparticles was determined from X-ray line broadening using the Debye–Scherrer formula (Ajitha et al 2014).

The average particle size is estimated to be 88.4 nm based on the width of the (111) Bragg's reflection.

Additionally, two unassigned peaks appeared at 37.90° and 47.95°, which were weaker than the silver peaks. These may be attributed to the bioorganic compounds present on the surface of the AgNPs. The unanticipated crystalline structures at 37.90° and 47.95° could also be linked to the organic compounds in the leaf extract (Suvith & Philip, 2014).

Other peaks, such as at 28.5518°, 53.8868°, and 62.5909°, might indicate the presence of other crystalline phases, which could stem from bio-organic residues of *C. viscosa* used in the synthesis.

5.4. SEM with EDS

The scanning electron microscopy (SEM) analysis confirms the successful green synthesis of silver nanoparticles (AgNPs) using *C. viscosa* as a biological reducing and capping agent. The micrograph reveals a layered surface morphology with visible ridges and grooves, which may result from the biomolecules in the plant extract or the substrate used during the synthesis process. Bright spots distributed across the surface are indicative of silver nanoparticles, although their sizes and distribution appear non-uniform. The irregular larger clusters observed could be attributed to residual organic compounds or by-products from the synthesis process (Fig.4). Despite these variations, the analysis demonstrates the formation of silver nanoparticles, with their morphology reflecting the influence of the biological synthesis route.

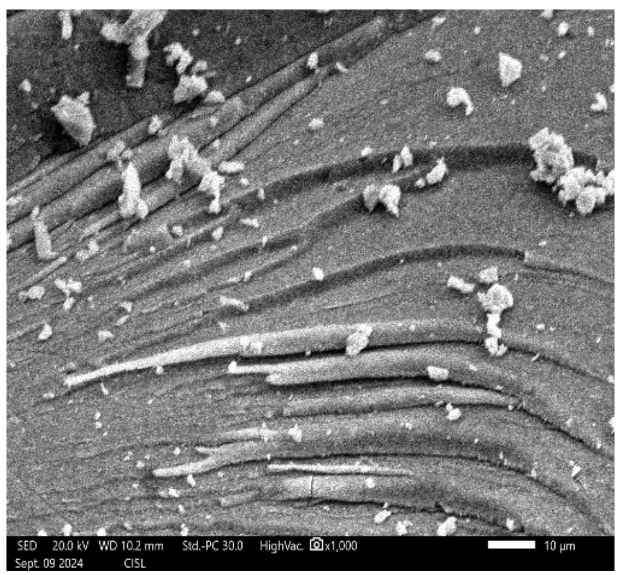


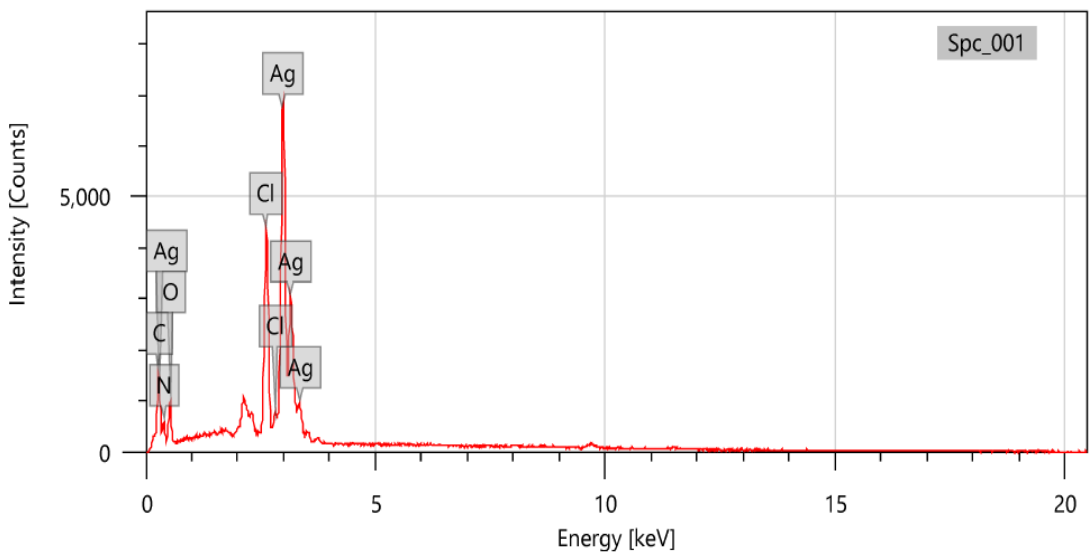
Fig.4. SEM images of synthesized CvAgNPs

5.4.1. Energy Dispersive X-ray Spectroscopy (EDS)

The Energy Dispersive X-ray Spectroscopy (EDS) spectrum of CvAgNPs confirms the presence of silver (Ag) as the predominant element. Metallic AgNPs typically exhibit a distinct and prominent peak around 3 keV, attributed to surface plasmon resonance (Anandalakshmi *et al*., 2016; Ismail *et al*., 2018) reported that the biosynthesized AgNPs exhibit prominent peaks within the 3–4 keV range

The analysis revealed a high silver content, with 55.19% by mass and 15.51% by atom, underscoring its dominance in the sample composition. Additionally, the presence of carbon (16.10% by mass, 40.63% by atom) and oxygen (12.51% by mass, 23.70% by atom) suggests the involvement of organic compounds from the *C. viscosa* extract in reducing and stabilizing the nanoparticles. Nitrogen (4.80% by mass, 10.40% by atom) and chlorine (11.41% by mass, 9.75% by atom) were also detected, likely originating from phytochemicals or precursor compounds present in the extract.

An advantage of synthesizing nanoparticles with plant extracts, as opposed to chemical methods, is evident in this study. In Fig.5 the silver nanoparticles produced in this investigation demonstrated strong absorption at 2.98 keV. Comparable findings were previously observed, with silver nanoparticle formation occurring within the 2–4 keV range using *Artemisia nilagirica* leaf and *Artocarpus heterophyllus* seed extracts, as reported by (Jagtap and Bapat, 2013; Vijaykumar *et al*., 2013).



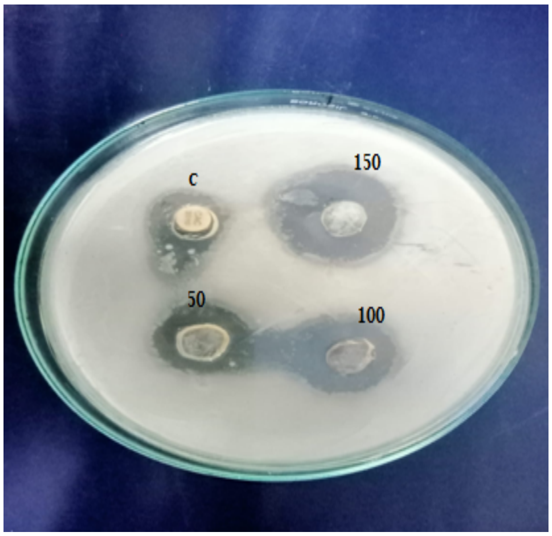
Element	Line	Mass%	Atom%
C	K	16.10±0.10	40.63±0.26
N	K	4.80±0.13	10.40±0.28
O	K	12.51±0.19	23.70±0.36
Cl	K	11.41±0.08	9.75±0.07
Ag	L	55.19±0.24	15.51±0.07
Total		100.00	100.00
Spc_001			Fitting ratio 0.0381

Fig.5. EDS pattern of synthesized CvAgNPs

These findings highlight the dual role of *C. viscosa* extract in the green synthesis process, acting as both a reducing and a capping agent, contributing to the stability and functionalization of the synthesized silver nanoparticles.

5.5. Antibacterial activity of synthesized CvAgNPs

Silver nanoparticles show strong antibacterial activity against a wide range of bacteria isolated from various sources (Rajeshkumar *et al*., 2012; Jelin *et al*., 2015). The antibacterial activity of green-synthesized silver nanoparticles from *C. viscosa* was evaluated against two bacterial strains: Gram-negative *Escherichia coli* (Fig.6) and Gram-positive *Staphylococcus aureus* (Fig.7). The study revealed a concentration-dependent increase in the zone of inhibition for both strains.



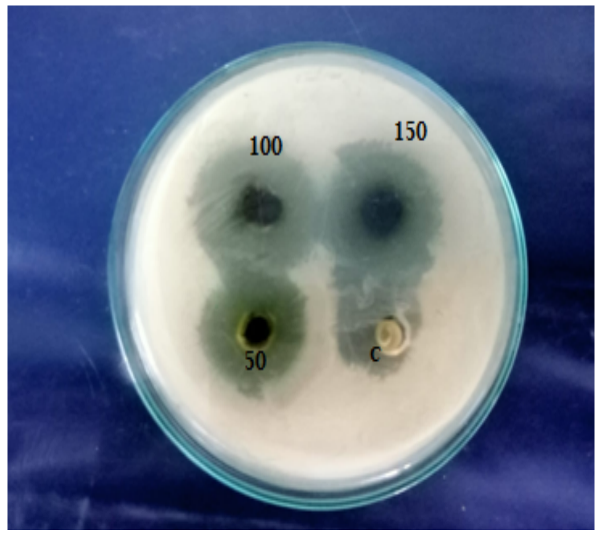


Fig.6. Antibacterial activity of E.coli **Fig.7.** Antibacterial activity of S.aureus

For *E. coli*, the zones of inhibition were 6 mm, 12 mm, and 18 mm for nanoparticle volumes of 50 µL, 100 µL, and 150 µL, respectively, compared to 20 mm for the standard antibiotic gentamicin. Similarly, for *S. aureus*, the zones of inhibition were 5 mm, 10 mm, and 17 mm for the respective concentrations, with a standard control zone of 18 mm. These results suggest that the synthesized nanoparticles exhibit significant antibacterial activity, effectively inhibiting both Gram-negative and Gram-positive bacteria. This indicates the potential application of *C. viscosa*-mediated silver nanoparticles as effective antibacterial agents.

6. CONCLUSION

It is concluded that the extract of *C. viscosa* has demonstrated the ability to extracellularly synthesize stable silver nanoparticles. The rapid synthesis achieved, as confirmed through various analytical methods, highlights the efficiency of this environmentally friendly approach. Utilizing natural resources as an alternative to conventional chemical synthesis offers a cost-effective and sustainable solution. Therefore, these eco-friendly synthesized silver nanoparticles exhibit excellent antibacterial activity against highly pathogenic microorganisms. The findings suggest that silver nanoparticles could serve as effective antibacterial agents, potentially playing a significant role in a variety of commercial products within the pharmaceutical and chemical industries due to their antibacterial properties.

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