

GREEN SYNTHESIS OF SILVER NANOPARTICLES USING *TINOSPORA CORDIFOLIA* EXTRACT: SYNTHESIS AND COMPREHENSIVE CHARACTERIZATION

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Abstract

The use of plant extracts for the synthesis of silver nanoparticles (AgNPs) represents a sustainable approach that can overcome some of the environmental concerns associated with conventional nanoparticle production methods. In the present study, AgNPs were synthesized using *Tinospora cordifolia* extract as a natural source of reducing and capping biomolecules. The formation of AgNPs was initially indicated by a visible colour change in the reaction mixture and further supported by UV–Visible spectroscopy, which exhibited a characteristic surface plasmon resonance band at 576 nm. The synthesized nanoparticles were comprehensively characterized using FTIR, XRD, DLS, zeta potential, HRTEM, SAED, FESEM, EDS and elemental mapping techniques. FTIR analysis indicated the involvement of plant-derived functional groups in nanoparticle formation and surface stabilization, while XRD and SAED supported the crystalline nature of the synthesized material. HRTEM revealed predominantly spherical to quasi-spherical nanoparticles with localized aggregation, whereas FESEM showed irregularly shaped nanostructures with visible particle aggregation. EDS confirmed silver as the major detected element, and elemental mapping demonstrated its distribution within the analyzed region. DLS revealed a heterogeneous hydrodynamic size distribution with a predominant aggregated population, while the negative zeta potential indicated a negatively charged nanoparticle surface with limited-to-moderate colloidal stabilization. Overall, the findings demonstrate the potential of *T. cordifolia* as a biological resource for the eco-friendly synthesis of AgNPs with physicochemical characteristics that may support their potential applications in textile dye degradation, antimicrobial materials, environmental remediation, biosensing and other nanotechnology-based applications.

Keywords: Silver nanoparticles, *Tinospora cordifolia*, Surface plasmon resonance, Physicochemical characteristics.

1. Introduction

The term “nano” has its origin in the Greek word *nanos*, meaning dwarf, and is used to denote an extremely small scale. Thus, nanotechnology can be simply defined as small-scale technology. But the word “nano” also has another meaning; in scientific terminology, it refers to a “nanometer,” which is one billionth of a meter.¹⁻³ Nanoparticles, typically having a size of less than 100 nm, can be formed from organic materials, metal, carbon, or metal oxides.⁴ Researchers can interact with complex biological processes at the cellular and molecular levels in a variety of ways due to nanotechnology. Nanoparticles (NPs) are used as possible drug delivery agents to treat diabetes, cancer, autoimmune illnesses,⁵ and cardiovascular disease⁶ because of their specific chemical and physical characteristics.

There are numerous applications for nanoparticles in medicine. Eco-friendly or biogenesis of NPs uses plant extracts as reducing agents. Compared to other biological techniques, green synthesis is more stable, economical, and non-toxic. NPs produced by green synthesis exhibit a range of biological and catalytic

properties.⁷ The noble metals⁸ are highly beneficial in nanoparticle synthesis due to their exceptional physicochemical properties, which are strongly influenced by size, shape, and surface features.

Due to their many industrial and biological uses,⁹ silver nanoparticles (AgNPs) have attracted considerable interest among metal nanoparticles. Since ancient times, silver has been used medicinally to cure wounds¹⁰ and diseases. Silver-based formulations are widely used in healthcare and sanitation because they have shown strong antibacterial and antioxidant action over time against a variety of harmful microorganisms.¹¹

Silver nanoparticles (AgNPs) are one of the most researched and promising nanostructures among the several zero-dimensional nanomaterials because of their distinct physicochemical and biological characteristics. Pharmaceutical and biomedical sciences,¹² cosmetic formulations,¹³⁻¹⁴ water treatments, antimicrobial textiles,¹⁵ wound-healing materials,¹⁶⁻¹⁷ anti-infective surface coatings,¹⁸⁻¹⁹ and food packaging systems²⁰ are just a few of their many uses. AgNPs' exceptional antibacterial activity, large surface area, and adaptability have drawn a lot of interest for the creation of cutting-edge, high-performing products in a variety of industries.

Silver-based compounds have been used primarily as antimicrobial agents simply because they can penetrate through biological membranes and have local or systemic effects. As a result, they are utilized for a variety of therapies,²¹ such as the healing of burns,²² wounds,²³ and digestive and dental disorders.²⁴ Nanoparticles are produced using a variety of techniques, including chemical, biological, and physical procedures.²⁵ However, chemical toxicity, high operating costs, and environmental concerns caused by conventional synthesis methods have led researchers to explore more environmentally friendly methods. Plant-mediated synthesis has become a highly desirable approach due to its ease of use, eco-friendliness, and capacity to generate stable nanoparticles.²⁶

Tinospora cordifolia, also known as giloy, is a shrub that belongs to the Menispermaceae family.²⁷ It is a diversified therapeutic plant known for its antioxidant, antimicrobial, anti-inflammatory, antidiabetic, and immunomodulatory properties, as well as novel uses in wastewater treatment, environmental remediation, heavy metal adsorption, and green nanotechnology. Alkaloids,²⁸ flavonoids, terpenoids,²⁹ glycosides, anphenolic compounds³⁰ are among the many bioactive phytochemicals that make it an important biological resource for the environmentally friendly synthesis of metal nanoparticles. *Tinospora cordifolia* has been extensively used in Ayurvedic medicine for the management of various diseases. It is traditionally recognized as a *Rasayana* herb that enhances immunity, improves resistance to infections, and possesses therapeutic properties throughout the entire plant.³¹⁻³²



Figure1: a) Fresh *T.cordifolia* leaves, b) Dried powder of *T.cordifolia*

2. Materials and Methods

2.1. Collection of *T. cordifolia* and Preparation of Extracts

Fresh *Tinospora cordifolia* stems were collected from Udaipur, Rajasthan, India. To get rid of sticking dust, soil particles, and other contaminants, the collected stem material was thoroughly cleaned using tap water and then distilled water. After being cleaned, the stems were cut into small pieces and let to dry in the shade at room temperature until all moisture was evaporated. A mechanical grinder was then used to grind the dried stem material into a fine powder. To prepare the extract, 25 g of dried stem powder was precisely

weighed in a clean Petri dish and added to a 500 mL beaker containing 225ml of distilled water. The mixture was then heated to 60-70°C for 50–55 minutes while continuously stirred to aid the extraction of bioactive phytochemicals. After extraction, it was cooled to room temperature and filtered through Whatman No. 1 filter paper to remove insoluble plant residues. The resulting aqueous extract was collected and stored in a refrigerator at 4 °C for later use in the environmentally friendly production of silver nanoparticles.

2.2. Phytochemical and Morphological Features of *T. cordifolia*

Among Indian species, *Tinospora cordifolia* is widely recognized as an important plant in the Indian System of Medicines (ISM) because of its many medicinal qualities and bioactive components. The pharmacological actions of the plant are caused by chemical components found in the root, stem, and the whole plant.³³⁻³⁴ It has attracted a lot of scientific attention because of its many medicinal qualities in treating different illnesses and its use in environmental fields. It is a large, broadly dispersed, evergreen climbing shrub with a variety of morphologically distinct twisted branches. The plant's bark is white to gray, and its stem is filiform, fleshy, and climbing. The stem powder has a distinct smell and bitter odor, and it can be either pale or dark brown. The starch known as "Guduchi-satva" is extracted from the stem. It has a lot of nutrients.³⁵ To identify the phytochemical constituents, such as flavonoids, glycosides, tannins, alkaloids, saponins, terpenoids, sugar, and proteins, freshly made plant extracts were analysed for typical phytochemical analysis by using standard methods.³⁶

2.3 Green Synthesis of AgNPs from *T. cordifolia*

The aqueous extract of *Tinospora cordifolia* was used as a natural reducing and stabilizing agent in the green synthesis of silver nanoparticles (AgNPs). To produce an adequate amount of nanoparticles required for in-depth characterisation and further applications, the synthesis was carried out in several batches. A 5 mM AgNO₃ solution was made in each batch by dissolving 0.191 g of silver nitrate (AgNO₃) in 225 mL of distilled water while swirling constantly to ensure total dissolution. The silver nitrate solution was then continuously magnetically stirred at 400–600 rpm while 40–50 mL of the plant extract was added dropwise. Ag⁺ ions were reduced into elemental silver nanoparticles by keeping the reaction mixture at 50–60 °C for 1–1.5 h.

The surface plasmon resonance phenomena linked to nanoscale silver production are responsible for the initial gradual color shift from pale yellow to dark brown, signaling AgNP formation. To ensure effective nanoparticle formation and repeatability of results, the reaction conditions—precursor concentration, temperature, reaction time, and extract volume—were chosen based on previously published green synthesis techniques. The produced nanoparticles were collected by centrifugation at 3500 rpm for 30 minutes (4 cycles) to remove any remaining plant biomolecules and unreacted ions. They were then repeatedly washed with distilled water and ethanol.

Once the purified nanoparticles were dried for 10 to 12 hours at 55 to 60 degrees Celsius in a hot air oven, the dried powders from each batch were combined and kept in airtight containers for additional analysis. The general approach used in this work is in line with previously published green synthesis techniques for *T. cordifolia*-mediated silver nanoparticles, with the appropriate adjustments made to optimize reaction conditions and nanoparticle production.³⁷⁻³⁸



Figure 2: Schematic illustration of the green synthesis of Ag nanoparticles using *T. cordifolia* extract, showing the preparation of plant extract, synthesis process.

3. Characterization of AgNPs

The optical characteristics of the synthesized Ag nanoparticles were investigated using a JASCO UV–Vis spectrophotometer (V-750, Japan) within the wavelength range of 190–850 nm. X-ray diffraction (XRD) analysis was carried out using an Empyrean X-ray diffractometer (Malvern Panalytical, Netherlands) equipped with Cu K α radiation ($\lambda = 1.5406 \text{ \AA}$) to determine the crystalline structure, phase composition, and crystallinity of the synthesized Ag nanoparticles. The functional groups present on the nanoparticle surface were examined using a Nicolet Summit X Fourier Transform Infrared (FTIR) spectrometer (Thermo Fisher Scientific, USA) over the spectral range of 4000–400 cm^{-1} .

The morphology and distribution of the synthesized Ag nanoparticles were investigated using a Hitachi SU8010 Field Emission Scanning Electron Microscope (FESEM) (Hitachi High-Tech Corporation, Japan). Further morphological and structural information, including particle size and lattice characteristics, was obtained using a JEM-2100 Plus High-Resolution Transmission Electron Microscope (HRTEM) (JEOL Ltd., Japan), while the crystalline nature of the nanoparticles was further assessed by Selected Area Electron Diffraction (SAED). The hydrodynamic particle size distribution and polydispersity index (PDI) of the Ag nanoparticle suspension prepared in deionized water were determined by Dynamic Light Scattering (DLS) using a Litesizer 500 (Anton Paar GmbH, Austria). The zeta potential of the suspension was measured using the same instrument to evaluate the surface charge and colloidal stability of the synthesized nanoparticles.

4. Results and Discussion

Understanding the physicochemical characteristics of nanoparticles is essential because these properties influence their behaviour, movement within biological systems, safety, and overall effectiveness in various applications.³⁹

4.1 UV–Visible Analysis

Ultraviolet–Visible (UV–Vis) spectroscopy is one of the most widely employed analytical techniques for monitoring the synthesis and stability of metallic nanoparticles.⁴⁰ In the present study, the biosynthesized silver nanoparticles (AgNPs) were characterized using a JASCO V-750 UV–Visible spectrophotometer over the wavelength range of 190–850 nm. The synthesized AgNPs exhibited a characteristic surface plasmon resonance (SPR) absorption band with a maximum absorption wavelength (λ_{max}) at 576 nm and a maximum absorbance of approximately 0.980, confirming the successful reduction of silver ions (Ag^+) into metallic silver nanoparticles (Ag^0). The broad and red-shifted SPR band indicates the formation of nanoparticles with a relatively wide size distribution and possible aggregation in the colloidal suspension. Such a shift in the SPR peak may be attributed to the influence of phytochemicals present in the *T. cordifolia*

extract, which act as both reducing and capping agents during nanoparticle synthesis. These findings demonstrate the successful green synthesis of AgNPs and provide preliminary evidence for their formation.

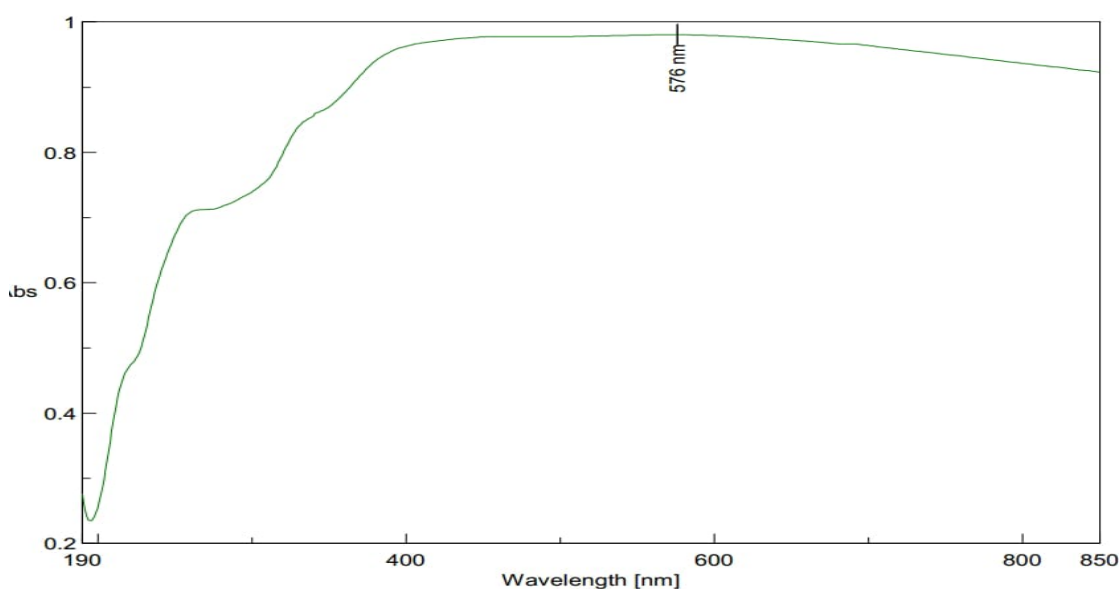


Figure 3: uv-visible spectrum of *T. cordifolia*-mediated Agnps showing an absorption maximum at 576 nm.

4.2 XRD Analysis

X-ray diffraction (XRD) analysis was performed to investigate the crystalline nature and phase composition of the biosynthesized silver nanoparticles. XRD is a reliable technique for determining the crystal structure and crystallinity of nanomaterials by measuring the diffraction of X-rays from atomic planes within the crystal lattice.⁴¹⁻⁴² The biosynthesized silver nanoparticles (AgNPs) exhibited a prominent diffraction peak at $2\theta = 38.04^\circ$ in the X-ray diffraction (XRD) pattern, along with additional reflections at approximately 46.13° , 64.38° , and 77.19° . The prominent reflection at 38.04° is consistent with the (111) plane of face-centered cubic (FCC) metallic silver. Additional diffraction features were observed at approximately 27.72° and 32.14° , which may be associated with additional crystalline phases or components present in the biosynthesized material. These additional reflections may originate from residual plant-derived constituents or other phases formed during the green synthesis process. Overall, the observed diffraction pattern indicates the crystalline nature of the synthesized material, while the characteristic Ag (111) reflection supports the formation of metallic silver nanoparticles.

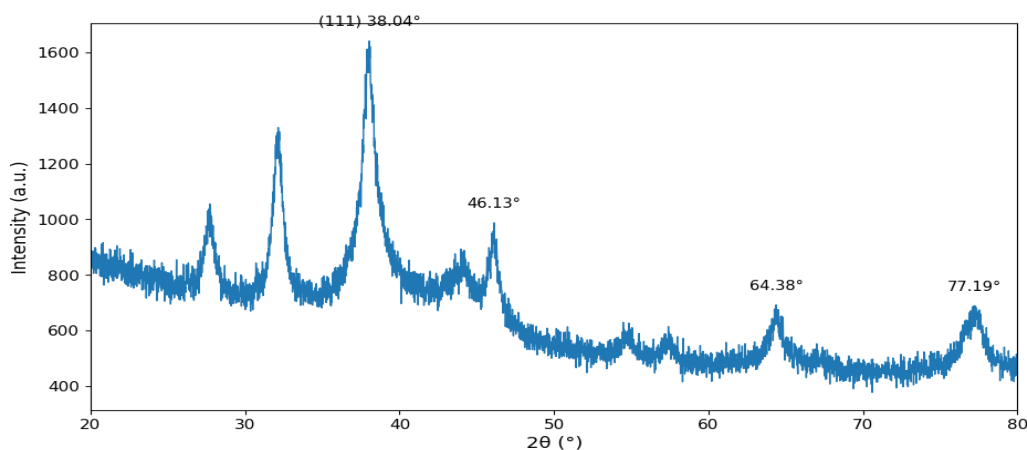


Figure 4: XRD pattern of *Tinospora Cordifolia*-mediated Agnps.

4.3 FTIR Analysis

Fourier Transform Infrared (FTIR) spectroscopy is used to identify the surface functional groups of synthesized metal nanoparticles and to determine the role of biomolecules as reducing and capping agents during nanoparticle synthesis.⁴³ The FTIR spectrum of the green synthesized silver nanoparticles (AgNPs) exhibited several characteristic absorption bands corresponding to the phytochemicals present in the *Tinospora cordifolia* extract. A broad absorption band at 3266 cm^{-1} was attributed to O–H stretching

vibrations of phenolic compounds and alcohol groups. The band observed at 2923 cm^{-1} corresponds to C–H stretching vibrations of aliphatic compounds. The absorption peak at 1627 cm^{-1} was assigned to C=O stretching (amide I) and/or aromatic C=C vibrations, indicating the presence of proteins and polyphenolic compounds. The peak at 1515 cm^{-1} represents amide II vibrations and aromatic ring stretching. The absorption bands at 1374 cm^{-1} and 1228 cm^{-1} were attributed to C–N and C–O stretching vibrations, respectively, whereas the peak at 1022 cm^{-1} corresponds to C–O–C stretching of carbohydrates and polysaccharides. These functional groups indicate that phytochemicals present in the *Tinospora cordifolia* extract acted as reducing as well as stabilizing (capping) agents during the biosynthesis of silver nanoparticles.

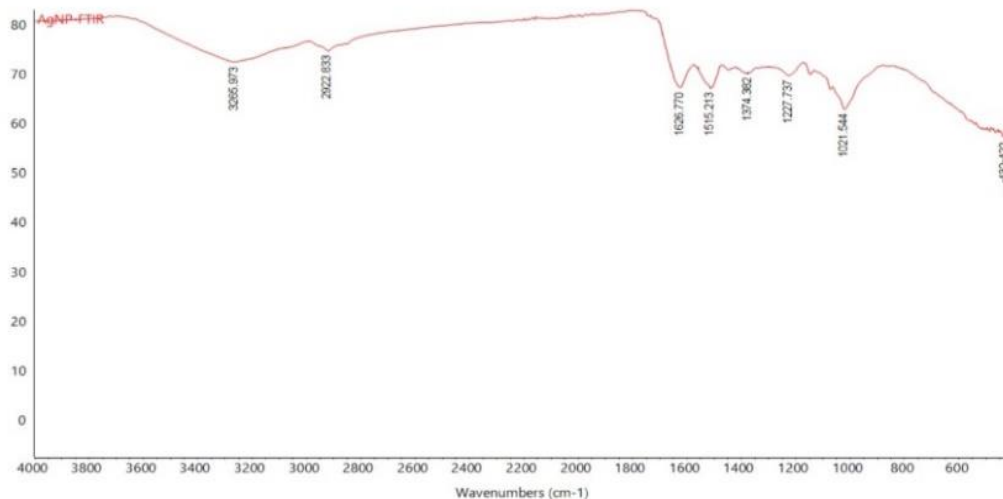


Figure 5: FTIR spectrum of *Tinospora Cordifolia*-mediated AgNPs showing characteristic absorption bands of phytochemical functional groups.

4.4 FE-SEM, EDS and Mapping

FESEM, EDS and elemental mapping analyses were performed to investigate the surface morphology, elemental composition and spatial distribution of the synthesized AgNPs. The FESEM micrograph showed irregularly shaped nanostructures with visible particle aggregation within the analyzed region. EDS analysis showed distinct characteristic signals of Ag, confirming the presence of silver in the synthesized nanomaterial. The quantitative EDS analysis showed Ag as the major detected element with a normalized content of 56.81 wt%, while O, Cl and Mo were also detected at 33.06, 6.36 and 3.77 wt%, respectively. The elemental mapping further demonstrated the distribution of Ag throughout the analyzed region, providing additional evidence for the presence and distribution of silver in the synthesized nanomaterial.

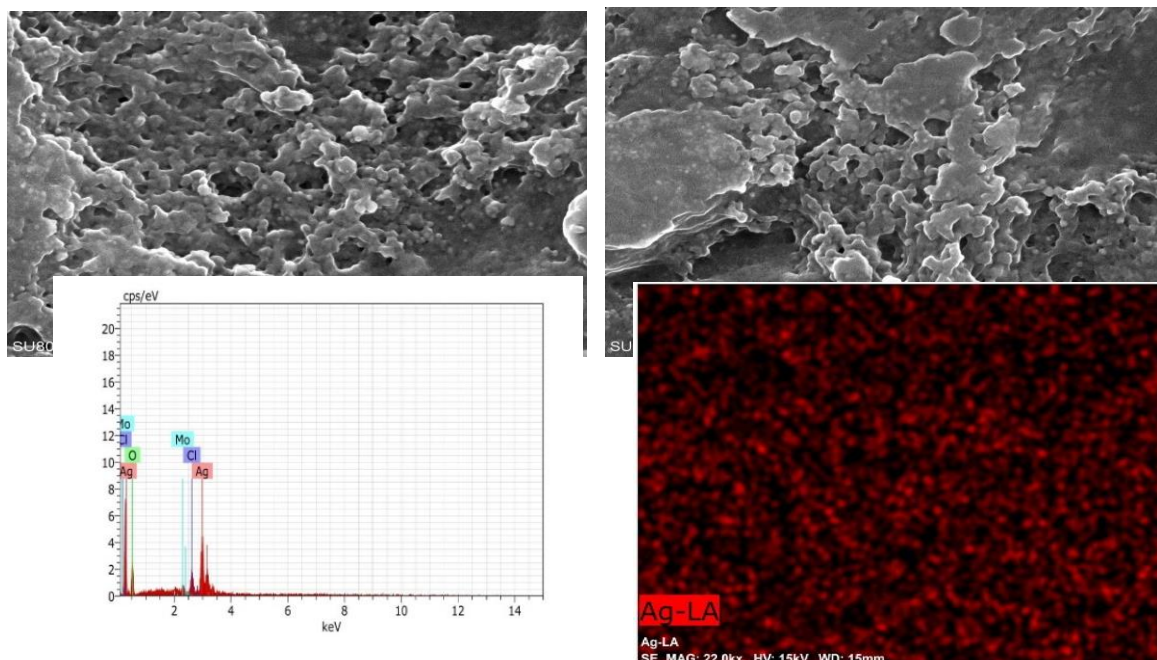


Figure 6: FE-SEM image, EDS spectrum and elemental mapping of *Tinospora Cordifolia*-mediated agnps.

4.5 HR-TEM and SAED

HRTEM analysis confirmed the successful green synthesis of silver nanoparticles (AgNPs) using *T. cordifolia* extract. The HRTEM images revealed that the synthesized AgNPs were predominantly spherical to quasi-spherical in morphology with a generally dispersed distribution and localized aggregation. The observed agglomeration may be attributed to the adsorption of phytochemicals from the plant extract, which acted as natural reducing and capping agents during nanoparticle synthesis. The nanoparticles exhibited well-defined boundaries, indicating controlled nucleation and growth during the biosynthesis process. Furthermore, the selected area electron diffraction (SAED) pattern showed diffraction features supporting the crystalline nature of the synthesized silver nanoparticles. These observations, together with the HRTEM results, support the formation of crystalline nanoscale silver nanoparticles through the green synthesis approach.

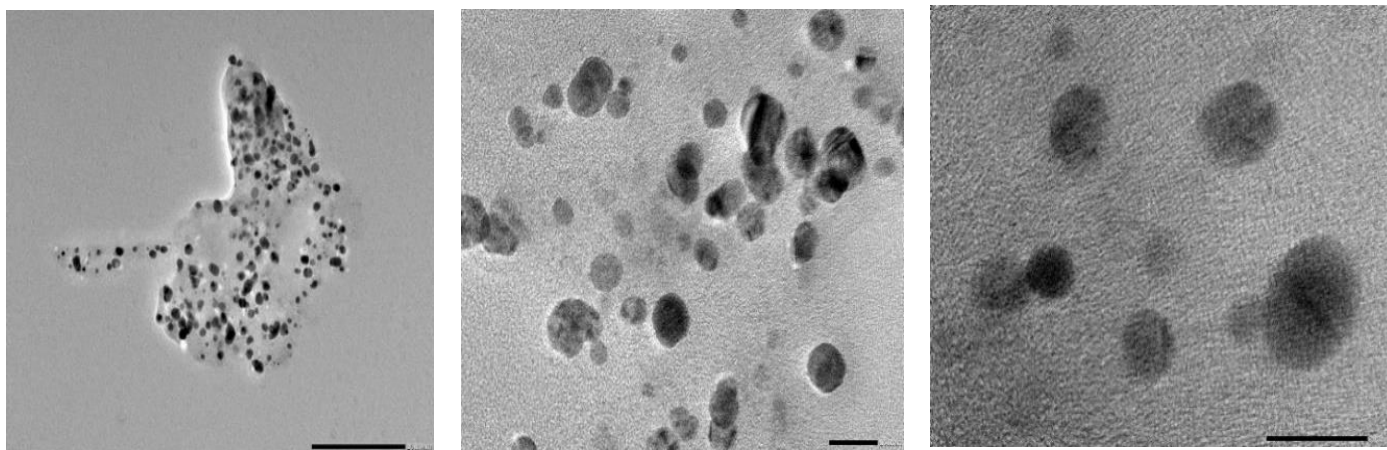


Figure 7: HRTEM images of *Tinospora Cordifolia*-mediated Agnps showing their spherical to quasi-spherical morphology.



Figure 8: SAED pattern of *T. Cordifolia*-mediated Agnps indicating their crystalline structure.

4.6 DLS

Dynamic light scattering (DLS) provides an effective means of evaluating the hydrodynamic size and size distribution of nanoparticles dispersed in a liquid medium.⁴⁴ The DLS analysis revealed a hydrodynamic diameter of 1772.68 nm with a polydispersity index (PDI) of 0.254, indicating a moderately polydisperse colloidal system. The intensity-weighted particle size distribution displayed two distinct populations, with a dominant peak at 1169.79 nm accounting for 96.74% of the scattering intensity and a minor peak at 37.36 nm contributing 3.26%.

The comparatively large hydrodynamic diameter may be attributed to particle aggregation and the presence of phytochemical capping layers on the nanoparticle surface, which can increase the effective hydrodynamic size in aqueous dispersion. The smaller population at approximately 37 nm indicates the presence of a nanoscale fraction within the dispersion. Overall, the DLS results demonstrate a

heterogeneous particle-size distribution with a predominant aggregated population, while a smaller nanoscale population is also present.

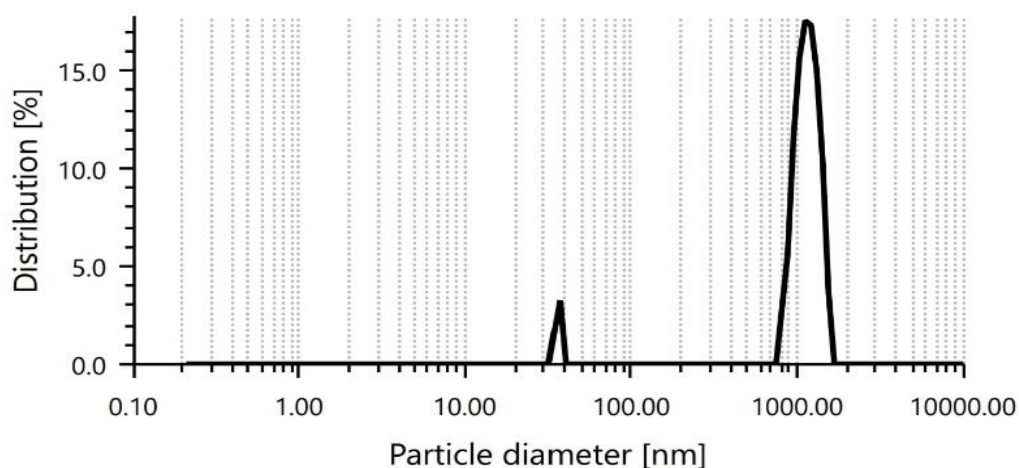


Figure 9: Intensity-weighted DLS particle size distribution of *T. cordifolia*-mediated AgNps.

4.7 Zeta Potential

Zeta potential analysis of the biosynthesized silver nanoparticles (AgNPs) revealed a mean zeta potential of -16.2 ± 0.6 mV, with a distribution peak at -13.9 mV, indicating that the nanoparticles possessed a net negative surface charge. The negative surface charge is most likely attributed to the adsorption of phytochemicals from *Tinospora cordifolia*, including phenolic compounds, flavonoids, proteins, and other biomolecules, which acted as natural reducing and capping agents during the green synthesis process.

These surface-bound biomolecules contribute to electrostatic repulsion between nanoparticles and may also provide steric stabilization, thereby enhancing the stability of the colloidal suspension and reducing particle aggregation. Although the measured zeta potential did not exceed ± 30 mV, the obtained value indicates moderate colloidal stability, which is commonly observed for plant-mediated silver nanoparticles. The narrow standard deviation (± 0.6 mV) further suggests a relatively uniform surface charge distribution, indicating successful and reproducible biosynthesis of the AgNPs.

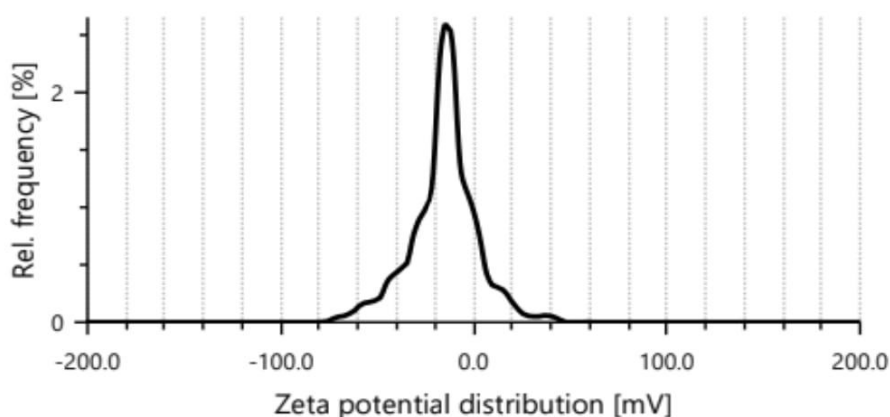


Figure 10: zeta potential distribution of *T. Cordifolia*-mediated AgNps showing a negative surface charge.

5. Conclusions

The present investigation demonstrates that *Tinospora cordifolia* extract can be effectively employed as a biological medium for the preparation of silver nanoparticles through a green synthesis route. The appearance of the UV–Visible absorption band at 576 nm provided initial evidence for AgNP formation. The FTIR findings indicated the involvement of plant-derived functional groups in the nanoparticle formation

and surface capping process, while the XRD and SAED observations supported the crystalline nature of the synthesized material. HRTEM observations showed predominantly spherical to quasi-spherical nanoscale particles with localized aggregation. FESEM further revealed an irregular surface morphology, and EDS confirmed silver as the major detected element (56.81 wt%) together with O, Cl and Mo. Elemental mapping demonstrated the distribution of silver within the examined region. DLS revealed a hydrodynamic diameter of 1772.7 nm, with the major intensity contribution (96.74%) occurring around 1169.8 nm, suggesting substantial aggregation in aqueous dispersion. The negative zeta potential (-16.2 ± 0.6 mV) indicated a negatively charged nanoparticle surface with limited-to-moderate colloidal stabilization. Overall, the findings establish *T. cordifolia* as a promising biological resource for AgNP synthesis through an eco-friendly route. Based on their physicochemical characteristics, the synthesized AgNPs may have potential applications in textile dye degradation, antimicrobial and antibacterial materials, environmental remediation, biosensing, and other nanotechnology-related fields.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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