RASĀYAN *J. Chem.*
Vol.19 | *Special Issue* | 58-65 | 2026
ISSN: 0974-1496 | e-ISSN: 0976-0083 | CODEN: RJCABP
http://www.rasayanjournal.com
http://www.rasayanjournal.co.in

PHYTOGENIC SYNTHESIS, OPTICAL, FUNCTIONAL, STRUCTURAL, AND THERMAL PROPERTIES OF *Enicostema axillare*-MEDIATED SILVER NANOPARTICLES

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ABSTRACT

The present study investigates the green synthesis of silver nanoparticles (AgNPs) using an aqueous leaf extract of *Enicostema axillare* as a biological reducing and stabilising agent. Fresh leaves of *E. axillare* were collected and processed into AgNPs using a green chemistry approach. The as-synthesised silver nanoparticles were studied using Ultraviolet-visible spectroscopy (UV), Fourier transform infrared spectroscopy (FTIR), X-Ray Diffraction (XRD), Energy-Dispersive X-ray spectrophotometer (EDX), Thermogravimetric analysis (TGA), and Field Emission Scanning Electron Microscopy (FESEM). XRD analysis shows a clearly defined *hkl* peaks of (111), (200), (211), (220), (221) and (311) of face-centred cubic silver, with crystallite sizes of about 40 - 70 nm estimated using the Scherrer equation. TGA shows the thermal stability of the synthesised AgNPs with 22% total weight loss. With these findings, *E. axillare* leaf extract can be a very effective and environmentally friendly system for synthesising stable AgNPs with nanoscale size and crystalline structure. Due to their stability, phytochemical capping and abundant amount of silver, the AgNPs synthesised have a great potential of being used in diverse areas of biomedical, environmental and nanotechnological practices.

Keywords: *Enicostema axillare,* AgNPs, Green Synthesis, Leaf Extract, Nanoparticles.

RASĀYAN *J. Chem.*, Thematic Issue, 2026

INTRODUCTION

Nanotechnology refers to the scientific study and manipulation of matter at extremely small dimensions, typically within the 1–100 nm range, enabling the development of novel materials, structures, and nanoscale devices with unique properties. Nanoparticles, a key component of nanotechnology, are engineered for specific functions and have found wide-ranging applications across various fields. In medicine, they enhance drug delivery[2] and diagnostics; in electronics, they improve performance and miniaturisation; in energy, they boost efficiency in storage and conversion systems[3], and in environmental science, they aid in pollution control and water purification.[4] The versatility and precision of nanotechnology make it a transformative tool in modern science and industry. Green synthesis, also known as the green chemistry approach, is an emerging field in chemistry and nanotechnology that uses sustainable and environmentally available sources like plant biomass, algae, and fungi to synthesise nanoparticles.[5] Unlike conventional chemical and physical synthesis process completely rely on high-energy sources, heat, toxic chemicals, hazardous solvents, and unsustainable practices.[6] The use of high temperatures and the energy-intensive technologies not only increases energy consumption but also produces harmful byproducts which need expensive and potentially harmful disposal methods. Also, in conventional methods, the use of poisonous chemicals and solvents poses risks to the environment and human health, leading to soil and water pollution. In contrast, green synthesis techniques utilise less

http://doi.org/10.31788/RJC.2026.19510033

harmful reagents, such as extracts of plant biomass, which offer an alternative and more sustainable and eco-friendly way of producing nanoparticles.[7] Green-synthesized nanoparticles have been utilized in medicine, antimicrobial[8], anticancer[9], drug delivery[10], environmental remediation - dye degradation, heavy metal removal[11], agriculture - nano-fertilizers, pesticides[12] and technology - sensors, energy devices. They are biocompatible and stable, offering sustainable solutions in various areas. Among various nanomaterials, silver nanoparticles (AgNPs) have received significant attention for their broad-spectrum antimicrobial properties. Their inhibitory action against microorganisms is attributed to several mechanisms, including membrane damage, liberation of silver ions, alteration of intracellular components, and oxidative stress caused by the formation of reactive oxygen species.[13] These actions cause the bacteria's structure to break down, leading to cell death. Additionally, silver nanoparticles can directly damage bacterial DNA, preventing replication. The antioxidant effect of AgNPs is attributed to the bioactive molecules on their surface, which act as capping agents.[14] The presence of these bioactive constituents enhances antioxidant activity by neutralising various reactive species, such as DPPH, hydrogen peroxide, hydroxyl radicals, and superoxide ions, thereby helping to protect cells from oxidative damage. The trivial size and large surface area of AgNPs enhance their ability to interact with and neutralise reactive species. Anticancer activities of AgNPs exhibit strong anticancer activity by inducing cell death in SKBR3 cell lines through reactive oxygen species (ROS) and triggering autophagy and apoptosis.[15] The mechanism involves mitochondrial dysfunction, oxidative stress, and DNA interaction, as confirmed by proteomic analysis and TEM micrographs. Applications include wound dressing, medical device coating, drug delivery, environmental remediation, catalysis and sensing.[16] *Enicostema axillare* is a perennial plant classified under the family Gentianaceae and is traditionally recognised by names such as Indian Whitehead and Nagajihva. Its natural distribution extends across diverse geographical regions of India, Sri Lanka, Southeast Asia, and selected coastal areas of Africa. The herb is particularly adapted to warm, dry environments and is commonly observed in open lowland regions.[17] The plant is morphologically slender and erect with opposite sessile leaves and small greenish-white flowers in axillary clusters. Historically, it has been very relevant in Ayurveda[18], Siddha and Unani systems[19] of medicine as a result of its extensive medicinal properties. Herbal preparations use the whole plant, particularly the leaves and stems. Traditional knowledge has associated this plant with the management of type 2 diabetes, particularly because of its reported ability to help regulate blood glucose concentrations[20], and in the treatment of liver diseases like jaundice[21], and hepatoprotective activity[22] was observed. The herb has also anti-inflammatory and Painkilling effects; thus can be used to treat pain and swelling and also has anti-microbial properties[23], which have helped prevent bacterial, fungal and parasitic infections. Also, the plant harbours bioactive agents such as swertiamarin, sweroside, and gentiopicroside[24] which make it act as an antioxidant agent and thereby alleviate oxidative stress. Traditional claims have been supported by recent pharmacological studies reporting that *E. axillare* might be a promising source of new therapeutic agents to treat a variety of chronic and infectious diseases.[25] This study explores the utilisation of *E. axillare* leaf extract as a biological resource for the formation of silver nanoparticles (AgNPs), with subsequent evaluation of their characteristic properties.

EXPERIMENTAL

E. axillare Leaf Aqueous Extract Preparation

Fresh *E. axillare* leaves collected from PSG College of Arts and Science, Coimbatore, were thoroughly washed and processed for aqueous extraction. Briefly, 5 g of leaves were homogenised with 100 mL of deionised water and heated at 80 °C for 15 min. The extract was filtered through Whatman No. 1 filter paper and stored at 4 °C until further use for AgNP synthesis and analysis. High-purity chemicals were obtained from Sigma-Aldrich, Mumbai.

Synthesis of AgNPs using *E. axillare* Leaf Extract

An aqueous reaction mixture was prepared by combining 10 mL of *E. axillare* leaf extract with 90 mL of 1 mM $AgNO_3$. The mixture was maintained under dark conditions with continuous stirring for 6 h, allowing the biogenic reduction of silver ions and subsequent formation of AgNPs. Leaf extract-mediated AgNPs are formed by reducing Ag^+ ions, and this was confirmed by observing a change in colour. The separated AgNPs were recovered by centrifugation and subsequently washed with deionised water. The

washed material was dried in a hot-air oven to obtain a dry nanoparticle powder, which was then retained for further characterisation and experimental studies.[26] Graphical abstract of the extraction and synthesis process for *Enicostema axillare* leaf extract-mediated AgNPs was given in the Fig.-1.

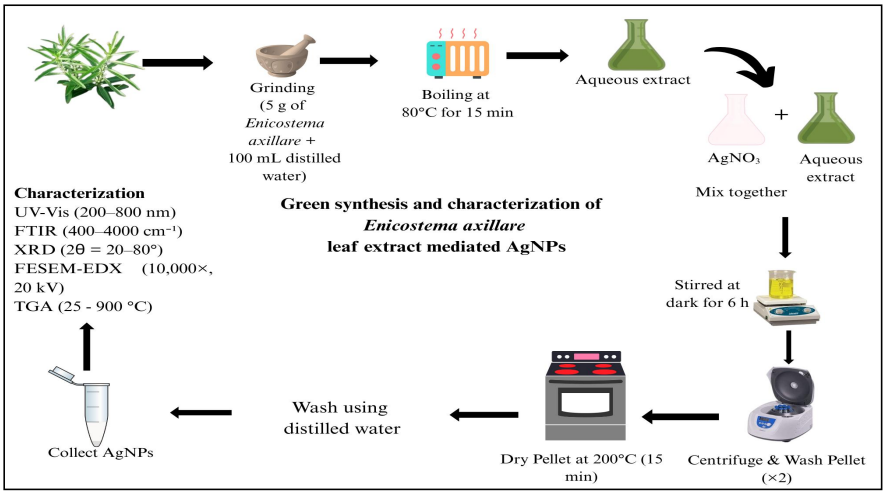

Fig.-1: Graphical Abstract for the Biological Synthesis of *E. axillare* Leaf Extract Mediated AgNPs

Optical and Elemental Characterisation of *E. axillare* Leaf Extract Mediated AgNPs

E. axillare leaf extract-mediated AgNPs were analysed using UV–Vis spectrophotometry, X-ray diffraction analysis, FTIR spectroscopy, FE-SEM imaging, EDX elemental analysis, and thermogravimetric evaluation. The formation of *E. axillare* leaf extract-mediated AgNPs was determined using UV- Vis spectroscopy, which exhibits the specific surface plasmon resonance.[27] XRD spectrum determined the structure of crystalline materials and phase of the *E. axillare* leaf extract-mediated AgNPs.[28] The chemical constituents associated with the leaf extract and AgNP surface were assessed through FTIR spectroscopy. FE-SEM was used to visualize the size, shape, and surface characteristics of the biosynthesised nanoparticles. In addition, EDX analysis was performed to verify the elemental constituents present in the resulting AgNPs.[29] In order to study the thermal stability and thermal decomposition, TG analysis was conducted in the range of 25 – 900 ºC.[30]

RESULTS AND DISCUSSION

Optical Characterisation by UV-Vis Spectroscopy

UV-visible spectroscopy of *E. axillare* leaf extract and *E. axillare* leaf extract-mediated AgNPs (Fig.-2). The leaf extract showed the broad absorption peak, which indicated the occurrence of various phytoconstituents such as flavonoids, phenols, and proteins. Leaf-mediated AgNPs exhibited a characteristic SPR absorption band at 410–420 nm, confirming nanoparticle formation. The intensity of absorption is significantly higher in the synthesised AgNPs than in the *E. axillare* leaf extract that confirms the reduction of Ag^+ ions to AgNPs. Hasanova *et al*.[31] confirmed the formation of *A. lerchiana*-mediated AgNPs by UV–Visible spectroscopy, with an SPR band observed at 480 nm. In comparison, our study observed the SPR peak at 410–420 nm, which could be attributed to different synthesis conditions.

XRD Analysis

Figure-3 presents the XRD pattern obtained for the synthesized AgNPs, showing characteristic diffraction peaks at specific 2θ angles of 32.43, 38.31, 44.49, 55.01, 57.68, and 77.53º corresponds to the (111), (200), (211), (220), (221), and (311) planes that resembles with the JCPDS card no: 04-0783 and application of the Scherrer equation indicated that the AgNPs possessed an estimated crystallite size ranging from 40 to 70 nm. The synthesized silver nanoparticles were crystalline in nature, exhibiting a crystallographic structure. Likewise, Mehta *et al.*,[32] synthesized AgNPs using *Santalum album,* and XRD analysis determined the phase and crystalline nature of AgNPs. The observed XRD peaks at 38.28°, 44.56°, 64.82°, and 77.44° corresponded to the characteristic silver planes and matched JCPDS file No. 04-0783.

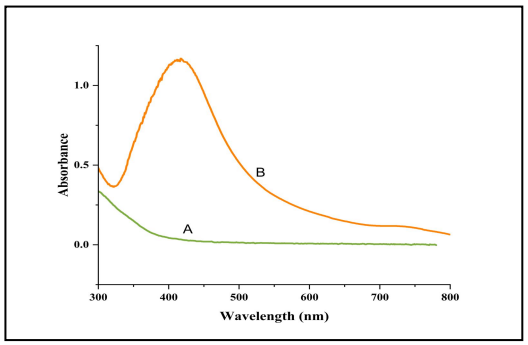

Fig.-2: UV-Vis Spectrum of (a) Leaf Extract of *E. axillare* and (b) AgNPs

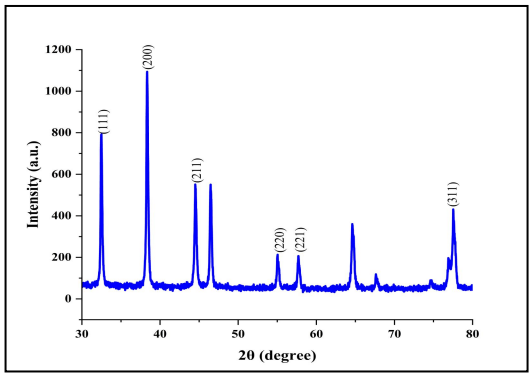

Fig.-3: XRD Spectrum of AgNPs

FTIR Analysis

The functional groups of *E. axillare* leaf extract and *E. axillare* leaf extract-mediated AgNPs was identified using FTIR analysis and displayed in Fig.-4a and 4b. *E. axillare* leaf extract exhibits bands at 3277 cm^{-1}, 1635 cm^{-1}, 455 cm^{-1}, 424 cm^{-1}, and 405 cm^{-1}, which represent OH stretching of hydroxyl groups (alcohols, phenols, carboxylic acids); this indicates the presence of polyphenols and flavonoids, and spectral signals can be attributed to carbonyl groups of amide-containing compounds, aromatic C=C bonds, and vibrations arising from metal–oxygen linkages. The FTIR spectrum of the synthesized AgNPs displayed characteristic bands at 2159, 1023, 433, 446, and 405 cm^{-1}, indicating the involvement of organic functional groups and Ag–O interactions in nanoparticle formation. Similar FTIR-based observations have been reported by Raghupathy *et al.,*[33] for *Psidium guajava*-mediated AgNPs, with a prominent band near 1029 cm^{-1}. Gentle *et al.,*[34] also identified N–H and C=C functional groups in *C. odorata*-derived AgNPs at 3423.76 and 1629.90 cm^{-1}, respectively.

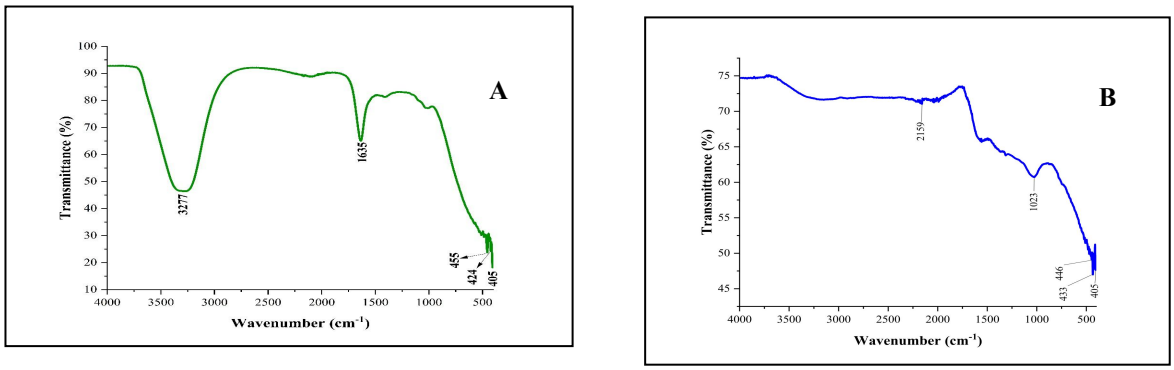

Fig.-4: (a) and (b) FTIR Spectrum of *E. axillare* Leaf Extract and AgNPs

FESEM Analysis

Figure-5 depicts the FESEM analysis conducted to study the structural morphology and dispersion of the *E. axillare* leaf extract-mediated AgNPs. The AgNPs exhibit spherical to quasi-spherical morphology

with aggregation and polydispersity. Similarly, Khan *et al*.,[35] synthesised AgNPs using *Curcumin* and performed FE-SEM to study their morphology, and it showed spherical C-AgNPs.

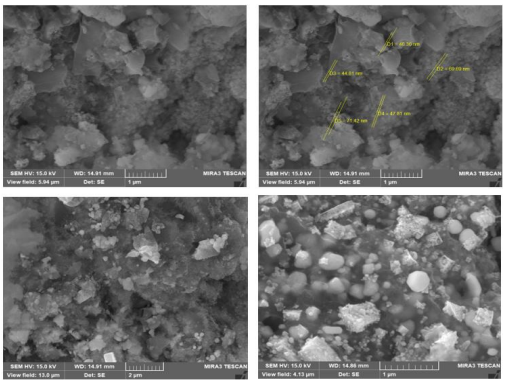

Fig.-5: FESEM Micrographs of AgNPs

EDX Analysis

The elemental composition of the synthesized AgNPs was determined by EDX analysis (Fig.-6). Silver was the predominant element (52.05%), accompanied by C (24.45%), Cl (13.41%), and O (11.08%). The prominent Ag signal verified nanoparticle formation, whereas the C and Cl signals were likely associated with residual phytochemicals from the leaf extract. Comparable Ag signals have been observed in plant-mediated AgNPs synthesized from *E. officinalis*, *E. hybrida*, and *T. catappa* extracts.

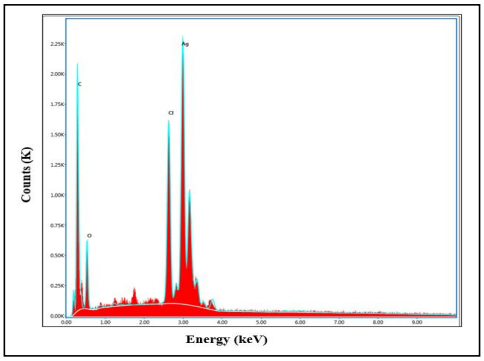

Fig.-6: EDX Spectrum of AgNPs

Thermogravimetric Analysis (TGA)

Thermal stability and decomposition level of the *E. axillare* leaf extract-mediated AgNPs was determined using TG analysis, and the result was displayed in the Fig.-7. The TG analysis was carried out in the heating range of 25 – 900 ºC. TGA revealed an initial mass reduction of approximately 2–3% between 25 and 250 °C, primarily due to the removal of surface-bound water and low-molecular-weight volatile constituents associated with the nanoparticles.

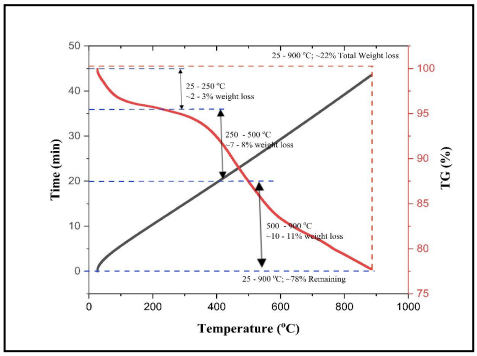

Fig.-7: TG Analysis of AgNPs

A subsequent weight loss of about 7–8% was observed between 250 and 500 °C, which may be attributed to the thermal decomposition of phytochemicals such as polyphenols, flavonoids, and proteins associated with the AgNP surface during nanoparticle synthesis. The final degradation of ~10 – 11% at 500 – 900 °C was because of the decomposition of more stable organic residues and carbonaceous matter associated with the plant extract. Overall, the synthesized AgNPs exhibited a total weight loss of ~22%, indicating that nearly 78% of the mass remained stable up to 900 °C. This high residual mass confirms the excellent thermal stability of the nanoparticles, which is primarily due to the metallic silver core that resists thermal degradation. Rehab *et al.*,[37] reported a total mass loss of 32.2% during TGA of *Psidium guajava*-mediated AgNPs across 160–1600 °C, which was attributed to the removal of bioorganic compounds associated with the nanoparticles.

CONCLUSION

The current research illustrates the successful biological synthesis of *E. axillare* leaf extract-mediated AgNPs. The synthesised material displayed a crystalline structure, with the crystallite size estimated at 40–70 nm. FE-SEM characterisation was performed to examine the surface morphology and particle characteristics of the resulting AgNPs. TGA reveals the thermal stability of the green-synthesised AgNPs, with the total weight loss of 22%, and possesses high stability when exposed to high temperatures. The findings demonstrate that *E. axillare* leaf extract can serve as a cost-effective biological route for producing stable, crystalline AgNPs of nanoscale dimensions. Their observed structural and elemental properties suggest potential applications in biomedical, environmental, and other nanotechnology-based fields.

ACKNOWLEDGEMENTS

The authors have no relevant financial or non-financial interests to disclose.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

The present work is related to *SDG-12: Responsible Consumption and Production*. All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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Cite this article as: S. Narayanan *et al.*, *Rasayan J. Chem.*, Vol.19, Special Issue, 58-65(2026), https://doi.org/10.31788/RJC.2026.19510033

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