RESEARCH PAPER

Eco-Friendly Synthesis and Characterization of Silver Nanoparticles Using Wrightia tinctoria Leaf Extract: Evaluation of in vitro Cytotoxicity in HaCaT Cells

Neha Shivathaya[1*], Siddalingappa Bhagawati[2], Manjunath Kopparam[3], Pradeep H K[4], Reshma Sawant[5], Rashmi Surve[6], Seema Paragannavar[7]

[1*,5,6,7] Department of Pharmaceutics, Rani Chennamma College of Pharmacy, Belagavi, Karnataka, India
[2,3] Department of Pharmaceutics, Sree Siddaganga College of Pharmacy, Tumkur, Karnataka, India
[4] Department of Pharmaceutics, GM Institute of Pharmaceutical Sciences and Research, Davanagere, Karnataka, India

ABSTRACT

Silver nanoparticles (AgNPs) have gained significant research interest owing to their unique physicochemical characteristics and potential biological applications. The present study focused on the green synthesis of AgNPs using aqueous leaf extract of Wrightia tinctoria, their physicochemical characterization, and preliminary cytotoxicity assessment in HaCaT human keratinocyte cells. Nanoparticles were synthesized through phytochemical-mediated reduction of silver nitrate, followed by calcination at 400°C. Characterization was performed using UV–Visible spectroscopy, Fourier-transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), scanning electron microscopy (SEM), transmission electron microscopy (TEM), dynamic light scattering (DLS), zeta-potential analysis, and energy-dispersive X-ray spectroscopy (EDX). UV–Visible analysis revealed a characteristic absorption maximum at 455 nm, indicating nanoparticle formation. FTIR spectra suggested the involvement of phytochemical functional groups in surface interactions. XRD patterns confirmed diffraction features consistent with face-centered cubic metallic silver, with an estimated crystallite size of 79 nm. TEM revealed predominantly spherical and quasi-spherical particles measuring approximately 15–35 nm, while SEM and DLS indicated larger aggregated structures. EDX confirmed silver alongside oxygen, magnesium, and phosphorus without establishing silver oxide formation. The zeta potential of −39.6 mV suggested considerable negative surface charge and potential electrostatic stability. MTT analysis demonstrated concentration-dependent cytotoxicity against HaCaT cells, with an IC_{50} value of 6.68 µg/mL, highlighting possible keratinocyte safety concerns. These findings demonstrate successful plant-mediated AgNP synthesis and provide preliminary insights into their physicochemical properties and cellular effects. Further investigations are essential to establish nanoparticle stability, biological selectivity, mechanisms of toxicity, and application-specific safety before considering therapeutic applications.

Keywords: Green synthesis; Silver nanoparticles; Wrightia tinctoria; HaCaT cells; MTT assay

How to cite this article: Shivathaya N, Bhagawati S, Kopparam M, Pradeep H K, Sawant R, Surve R, Paragannavar S, Eco-Friendly Synthesis and Characterization of Silver Nanoparticles Using Wrightia tinctoria Leaf Extract: Evaluation of in vitro Cytotoxicity in HaCaT Cells. Int J Drug Deliv Technol. 2026;16(87s): 534-545; DOI: 10.25258/ijddt.16.87s.53

Source of support: Nil

Conflict of interest: None

INTRODUCTION:

Nanotechnology is one of the fastest developing branches of science and technology, in which materials or objects are designed, synthesized, characterized, and applied with dimensions ranging from 1–100 nm1. Due to their nanoscale dimensions, nanoparticles exhibit unique physicochemical properties, including a significantly higher surface area-to-volume (SA/V) ratio2, enhanced reactivity, improved catalytic activity3, and distinct optical, electrical, and biological properties compared with their bulk counterparts4. These remarkable characteristics have led to their extensive applications in pharmaceuticals, medicine, biotechnology, electronics, environmental remediation, and agriculture industries5.

Silver nanoparticles (AgNPs) have attracted considerable attention because of their remarkable biological and physicochemical properties6. Owing to their potent antimicrobial, antioxidant, and anti-inflammatory activities, as well as excellent catalytic and sensing properties, silver nanoparticles (AgNPs) have gained significant attention in pharmaceutical and biomedical applications. Their broad-spectrum antimicrobial efficacy

has led to their use in wound dressings, antimicrobial coatings, drug delivery systems, biosensors, and medical devices. The common approaches for synthesizing silver nanoparticles include chemical reduction, thermal decomposition, hydrothermochemical processing, sol-gel methods, and electrochemical processes5. Although conventional synthesis methods produce nanoparticles with desirable properties, they often involve hazardous chemicals, high energy consumption, expensive equipment, and harsh reaction conditions. These limitations have driven the development of green synthesis, an eco-friendly, cost-effective, and sustainable approach that employs biological resources such as plant extracts, microorganisms, enzymes, and natural biomolecules as reducing and stabilizing agents for nanoparticle production7. Plant-mediated synthesis offers several advantages, including simplicity, rapidity, scalability, and the elimination of toxic chemicals during the synthesis process8. The phytochemicals present in plant extracts, such as flavonoids, phenolics, alkaloids, terpenoids, tannins, and glycosides, play an essential role in reducing metal ions and stabilizing the synthesized nanoparticles.

Among the various green synthesis techniques, the precipitation method is one of the most widely employed because of its simplicity, reproducibility, low cost, and ease of scale-up. This method involves the controlled precipitation of metal ions by reacting them with suitable precipitants in the presence of phytochemicals extracted from plants. The synthesized nanoparticles can subsequently be separated, purified, and characterized to evaluate their biological activity, particle size distribution, crystallinity, and morphology. Furthermore, the precipitation method offers the advantages of uniform particle formation, high yield, and better control over nanoparticle growth and aggregation9.

Several investigations have reported the biosynthesis of silver nanoparticles using medicinal plant extracts, with many studies focusing on physicochemical characterization or antimicrobial and antioxidant properties. However, comparatively limited information is available on *Wrightia tinctoria*-mediated silver nanoparticle preparations evaluated specifically in human keratinocyte cells. The present study was therefore designed as a preliminary investigation of the physicochemical characteristics and in vitro cytotoxic response of *Wrightia tinctoria*-mediated AgNPs in HaCaT cells. Because cytotoxicity toward normal keratinocytes may indicate a potential safety concern, the present study does not by itself establish therapeutic efficacy or suitability for topical use.

Wrightia tinctoria (Roxb.) R. Br., commonly known as the Pala Indigo Plant or Sweet Indrajao, belongs to the Apocynaceae family and is widely distributed throughout India and Southeast Asia. The plant has been extensively used in traditional herbal medicine for the treatment of psoriasis, various skin diseases, diarrhoea, dysentery, inflammation, wound healing, and microbial infections. The leaves are rich in bioactive phytoconstituents such as flavonoids, alkaloids, triterpenoids, phenolic compounds, steroids, and glycosides11. These phytochemicals possess excellent antioxidant and reducing properties, making them suitable for the synthesis and stabilization of silver nanoparticles10.

The utilization of *Wrightia tinctoria* leaf extract for plant-mediated AgNP synthesis is scientifically relevant because of the plant's traditional dermatological use and its reported phytochemical diversity. Phytochemicals present in the leaf extract may participate in Ag^+ reduction and may interact with the nanoparticle surface during synthesis. However, the present study did not identify the individual phytochemicals responsible for reduction or capping, and therefore these roles are considered proposed rather than conclusively demonstrated. The objective of the present investigation was to prepare and characterize *Wrightia tinctoria*-mediated AgNPs and to obtain preliminary information on their effect on HaCaT human keratinocyte cells. The findings are intended to provide a basis for future application-specific investigations rather than to establish therapeutic efficacy or clinical suitability.

MATERIALS:

Fresh leaf of *Wrightia tinctoria* were collected from medicinal nurseries at Kelamangala, Hosur, Tamil Nadu, India and was authenticated by Harsha Hegde, Scientist-E at ICMR- National Institute of Traditional Medicine, Belagavi, Karnataka, India. Silver nitrate, ≥99.9% purity and analytical reagent grade was purchased from Merck Life Science Pvt. Ltd., Mumbai, India. All analytical grade chemicals used in the study were obtained from SD Fine Pvt. Ltd. Mumbai, India. A laboratory reagent-grade water was used for all the experimental works, which was double-distilled.

METHADOLOGY:

Preparation of *Wrightia tinctoria* Aqueous Leaf Extract: The fresh leaves of *Wrightia tinctoria* were picked, washed with running tap water and then distilled water to remove dust and other impurities and shade dried at room temperature. The dried leaves were finely ground by mechanical grinding. About 10g of the powdered leaves was added to 100mL of distilled water and heated at 60-80ºC for 20-30min with continuous stirring to extract bioactive phytoconstituents. The extract thus obtained was cooled to room temperature and filtered first over muslin cloth and then through Whatman No.1 filter paper to get a clear aqueous extract. The filtrate was then kept in 4°C for further use in the synthesis of nanoparticles14.

Green Synthesis of Silver Nanoparticles: A 1 mM aqueous silver nitrate (AgNO3) solution was prepared in 100 mL double-distilled water and cooled for 2 h in a refrigerator. In a separate beaker, 20 g of dried Wrightia tinctoria leaf powder was mixed with 100 mL double-distilled water and heated on a hot-plate magnetic stirrer for 20 min at 70°C, followed by filtration. The silver nitrate solution was added dropwise to the aqueous leaf extract at an extract-to-silver nitrate solution ratio of 1:9. The reduction of Ag+ ions was monitored by the visible colour change. Following overnight incubation at 25°C, the solution changed from light yellow to dark yellow/brown. The reaction mixture was subsequently subjected to UV–Visible spectroscopic analysis. The synthesis is described here as a plant-mediated bioreduction process; the observed precipitate represents the recovered nanoparticulate material rather than a separately established chemical precipitation mechanism.

Isolation of Silver Nanoparticles: The reaction mixture was transferred to centrifuge tubes after the reaction, and these tubes were centrifuged at 8,000 rpm for 15 min to collect the nanoparticles in the form of precipitate. The precipitate was repeatedly washed with distilled water and ethanol to remove the unreacted silver ions, plant metabolites and other soluble impurities. The purified precipitate was dried in a hot air oven at 60ºC until a constant mass was achieved15.

Calcination: The dried nanoparticle powder was placed in a porcelain crucible and calcined in a muffle furnace for 3 h at 400°C. The purpose of this step was to remove residual organic material and obtain a more crystalline powder. Because high-temperature treatment can remove or alter thermolabile plant-derived surface constituents, the calcined product should not be assumed to retain all of the original phytochemical capping layer. The calcined nanoparticles were cooled naturally to room temperature inside the furnace, finely ground using an agate mortar and pestle, and stored in an airtight container for further physicochemical characterization. The possible influence of calcination on surface chemistry and phase composition is considered in the Discussion.

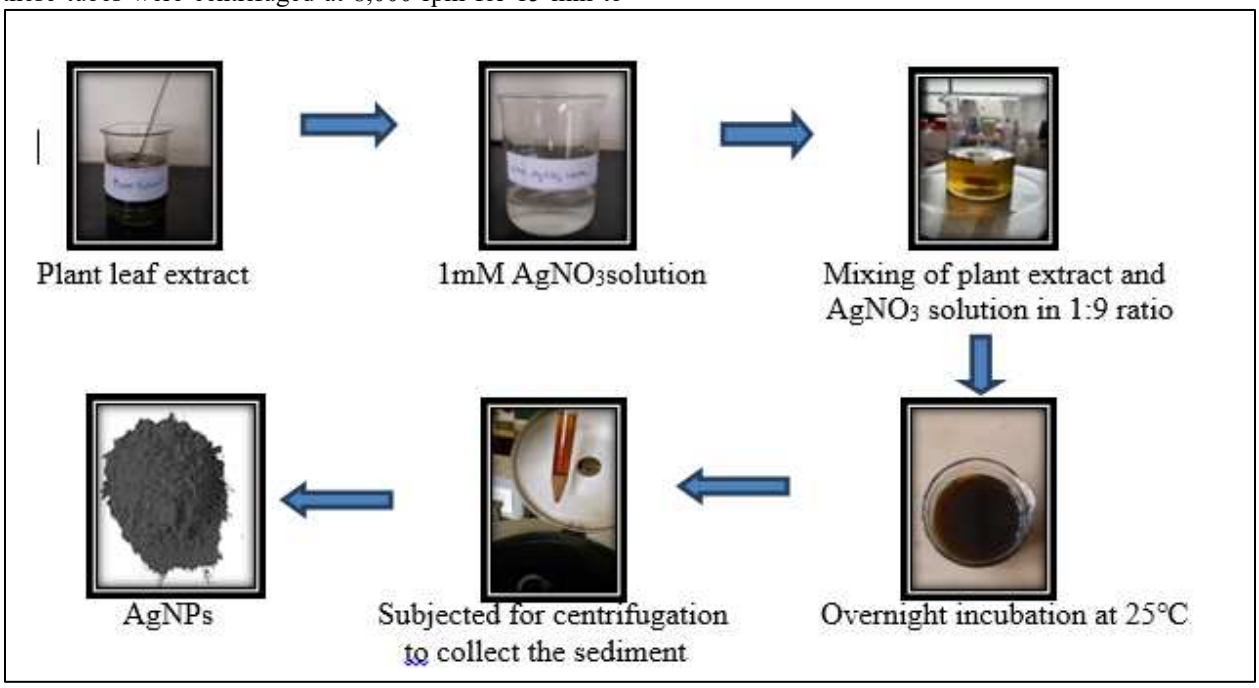


Figure 1: Synthesis of silver nanoparticles from the *Wrightia tinctoria* leaf extract

Characterization15-18

UV–visible spectroscopy: The synthesized AgNPs synthesized were then subjected to the investigation of their optical properties and structural conformation by performing UV-Visible (UV-Vis) spectroscopy under ambient conditions on a UV-Vis spectrophotometer (Shimadzu, UV-1900I). A spectral measurement was carried out in the range of 200-800 nm to look for absorption bands associated with the formation of nanoparticles.

Fourier Transform Infrared Spectroscopy (FTIR) of AgNPs: FTIR spectroscopy was employed to identify functional groups associated with phytoconstituents present in the plant extract and nanoparticle preparation. Dried AgNPs and the corresponding plant extract were mixed with potassium bromide (KBr) in a 1:100 ratio and compressed into thin pellets. The analysis was performed using a Bruker FTIR spectrophotometer at Poornayu Research Lab, Bangalore, Karnataka, India. Characteristic absorption bands were evaluated for functional groups such as –OH, –NH, –C=O, and –C–O that may be

involved in reduction or surface association. FTIR findings were interpreted as supportive evidence of phytochemical functional groups and surface interactions and were not used alone to conclusively assign Ag–O bonding.

X-ray Diffraction: The initiation of the crystallinity of the AgNPs samples was studied to grade the crystalline ordering inside the required size range. The Powder X-ray diffraction (P-XRD) patterns were obtained on a Rigaku MiniFlex 300/600 diffractometer. The AgNPs were first transferred into a dried powder form using the solvent exchange method without damaging the crystal structure dipping in acetone as exchanging solvent to ensure the presence of no water remaining.

Surface morphology (SEM): The surface morphology and elemental composition of developed AgNPs was further analysed by SEM (S-3400, Hitachi, Japan) equipped with energy dispersive X-ray (EDX) detector. The charging effects were minimised by adding the powder of the nanoparticles to a carbon tape attached to an aluminium stub and then coating the stub with a thin layer of gold. Particles were sized by direct measurement of several particles in the SEM micrograph images obtained at an accelerated voltage of 10 kV and a working distance of 6.7 mm using the image analysis software of the SEM.

Transmission Electron Microscopy (TEM): The optimized AgNPs were characterized in terms of their morphological properties by Transmission Electron Microscopy (TEM) analysis. The images were taken by using FEI Tecnai G2 transmission electron microscope (TEM) with a liquid nitrogen cryostage (LiN_2 cryostage) with tilting of up to ±80 degrees, equipped with a Field Emission Gun (FEG) Source at an accelerating voltage of 200kV

Particle size distribution and Zeta Potential: The hydrodynamic diameter and surface charge of the synthesized AgNPs were determined using a Microtrac FLEX Particle Size Analyzer (Version 11.0.0.2). For DLS analysis, a suitable dilution of the AgNP suspension was prepared in double-distilled water to avoid multiple scattering effects. The sample was then placed in a quartz cuvette, and the average particle size, polydispersity index (PDI), and size distribution were measured. Zeta potential was measured to evaluate the surface charge and stability of the nanoparticles. The AgNP suspension was loaded into a folded capillary cell, and the electrophoretic mobility was recorded. Measurements were performed in triplicate, and the mean ± standard deviation was reported, providing insights into nanoparticle colloidal stability and aggregation tendencies.

MTT cytotoxicity assay:19-21 The cytotoxicity of *Wrightia tinctoria*-mediated silver nanoparticles (AgNPs) was evaluated using the MTT assay in HaCaT human keratinocyte cells. Cells were seeded into 96-well plates at a density of 5×10^4 cells/well and incubated for 24 h to allow cell attachment. Subsequently, the cells were treated with various concentrations of AgNPs (10–320 µg/mL) and incubated for an additional 24 h. Following treatment, the medium was removed and 100 µL of MTT solution (0.5 mg/mL in phosphate-buffered saline) was added to each well. After incubation for 4 h at 37°C, the resulting formazan crystals were dissolved in 100 µL dimethyl sulfoxide (DMSO), and the absorbance was measured at 570 nm using a microplate reader. All experiments were performed in triplicate, and the results are expressed as mean ± standard deviation. The cell growth inhibition was determined on the basis of the following equation:

$$\%\ of\ Cell\ Inhibition = \frac{(OD\ Control - OD\ Sample)}{OD\ Control}\ X\ 100$$

RESULTS AND DISCUSSION

UV–visible spectroscopy: Ultraviolet-visible (UV–Vis) spectroscopy was used as a preliminary analytical method to confirm the preparation of silver nanoparticles by using the aqueous leaf extract of Wrightia tinctoria. The optical properties of the synthesized nanoparticles have been measured between 200–800 nm.

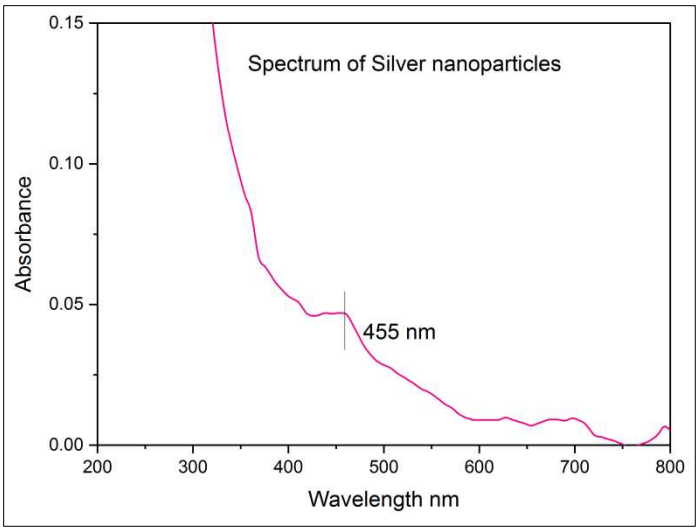


Figure 2: UV- visible spectrum of Silver Oxide nanoparticles

The UV–Visible spectrum (Figure 2) exhibited a distinct absorption peak at 455 nm, confirming the successful formation of silver nanoparticles through surface plasmon resonance (SPR). The slight red shift from the typical AgNP SPR range (400–450 nm) may be attributed to larger particle size, surface oxidation, and phytochemical capping by *Wrightia tinctoria* constituents, including flavonoids, phenolics, alkaloids, and terpenoids, which function as reducing and stabilizing agents. The relatively broad absorption band indicates a moderate particle size distribution, while the absence of additional peaks at higher wavelengths suggests minimal nanoparticle aggregation and good colloidal stability.

Fourier Transform Infrared (FTIR) analysis:

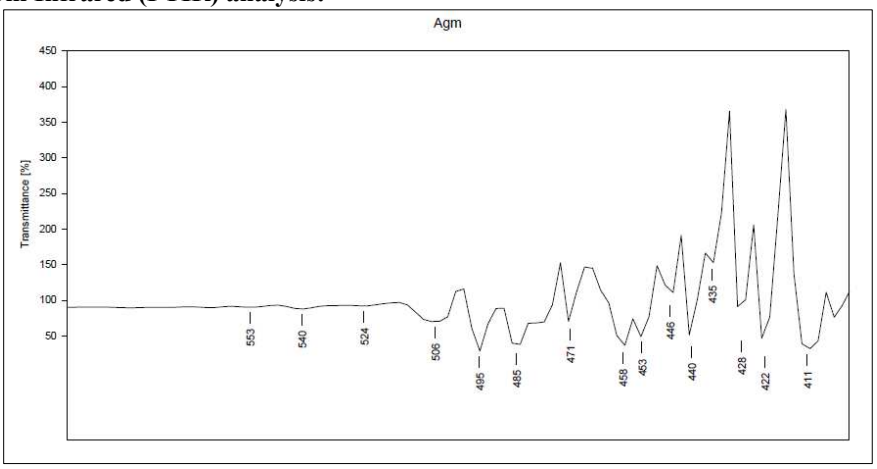


Figure 3: FTIR Analysis of AgNPs Synthesized from Wrightia tinctoria Leaf Extract

The FTIR spectrum showed bands associated with oxygen- and other heteroatom-containing functional groups. These observations suggest that phytochemical constituents of Wrightia tinctoria may have participated in the reduction process and/or remained associated with the nanoparticle preparation. However, FTIR alone cannot establish the identity of the individual phytochemicals or conclusively confirm Ag–O bond formation. The possible contribution of plant-derived surface-associated constituents is therefore considered a proposed mechanism and requires further phytochemical and surface-specific investigation.

X-ray Diffraction:

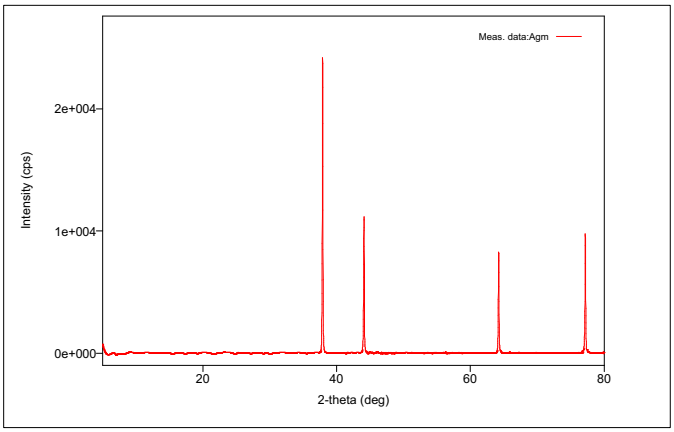


Figure 4: XRD pattern of synthesised Silver nanoparticles

The XRD pattern exhibited distinct diffraction peaks at 2θ values around 38.2°, 44.3°, 64.4°, and 77.5°, corresponding to the (111), (200), (220), and (311) lattice planes, respectively, and these positions are consistent with the face-centered cubic structure of metallic silver. No distinct additional reflections attributable to a separate crystalline silver oxide phase were observed within the obtained pattern. Therefore, the XRD data support the predominance of crystalline metallic silver in the analyzed sample; however, XRD alone cannot exclude a minor or poorly crystalline oxidized surface phase. The estimated crystallite size was approximately 79 nm. This value represents a coherent diffraction-domain estimate and should not be considered equivalent to the primary particle dimensions observed by TEM.

Energy Dispersive X-ray Analysis (EDX): The EDX spectrum (Figure 5) showed a strong characteristic silver (Ag) signal at approximately 3 keV, together with signals corresponding to oxygen (O), magnesium (Mg), and phosphorus (P). The EDX data support the presence of silver as a major constituent of the recovered material. The oxygen signal may originate from oxygen-containing surface species, residual plant-derived material, surface oxidation, or the analytical substrate, and therefore cannot by itself be used to confirm Ag2O formation. Similarly, the Mg and P signals may be associated with residual inorganic or plant-derived constituents. Accordingly, the EDX results were interpreted as elemental evidence rather than definitive phase identification.

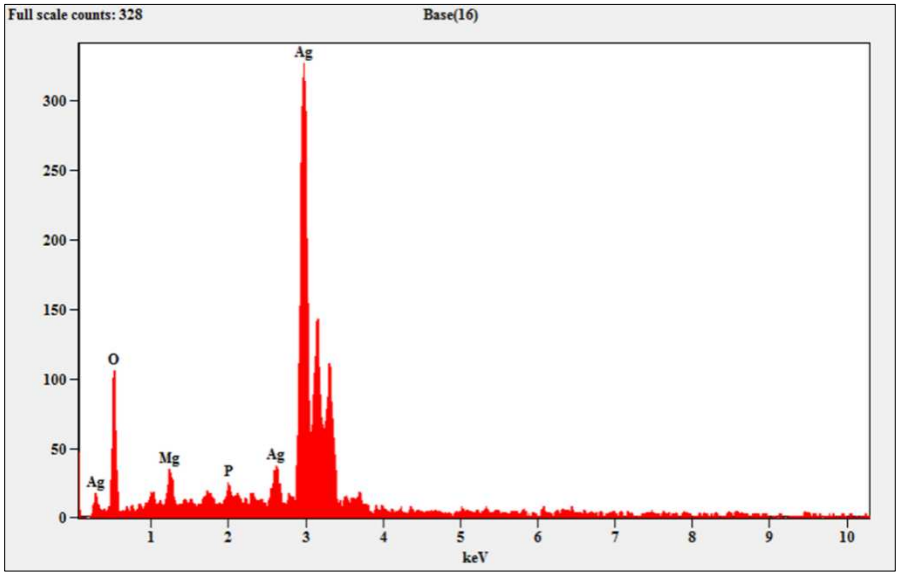


Figure 5: EDAX spectrum of *Wrightia tinctoria*-mediated AgNPs

Table 1: Quantitative Results for elemental composition of AgNPs

Element line	Weight %	Weight % Error	Atom %
O K	28.37	± 1.85	69.62
Mg K	2.74	± 0.24	4.43
P K	0.97	± 0.19	1.23
P L	---	---	---
Ag L	67.91	± 1.51	24.72
Ag M	---	---	---
Total	100.00		100.00

The quantitative elemental composition is presented in Table 1. Silver accounted for 67.91 ± 1.51 wt%, while oxygen accounted for 28.37 ± 1.85 wt%. The presence of oxygen should be interpreted cautiously because EDX cannot distinguish whether oxygen originates from surface-associated organic/inorganic species, oxidation, residual plant material, or the substrate. Thus, the elemental analysis supports the presence of silver and other elements but does not by itself establish a pure Ag or Ag2O phase. Phase assignment is based primarily on the XRD pattern.

Scanning Electron Microscopy:

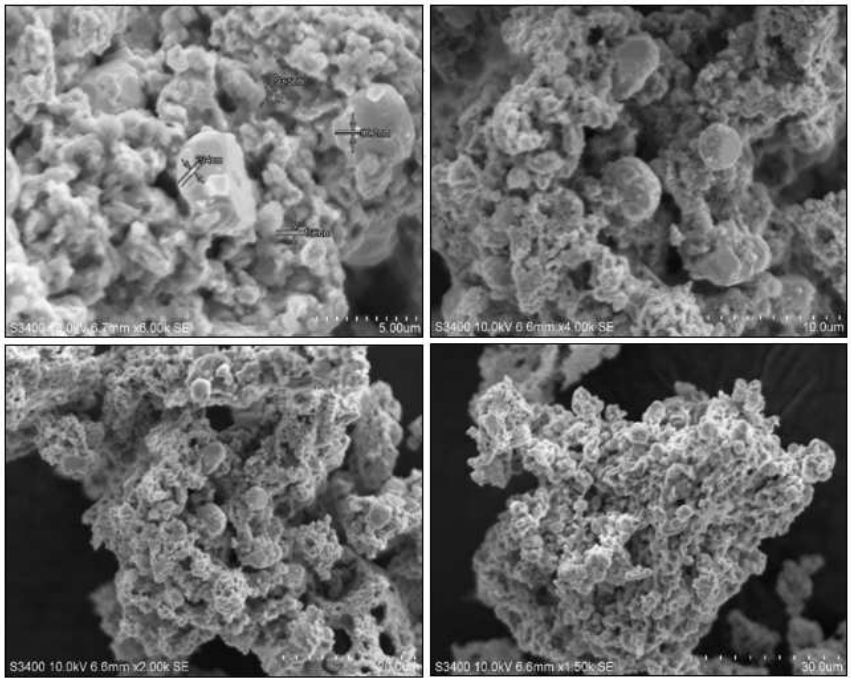


Figure 6: SEM micrographs of AgNPs using *Wrightia tinctoria* leaf extract

The SEM micrographs showed that nanosized particles with mostly quasi-spherical and irregular shapes formed successfully. The nanoparticles had a tendency to form aggregated clusters, and were a consequence of higher surface energy and intermolecular interaction between the capping agents, phytochemicals extracted from the leaf extract of *Wrightia tinctoria*. The particle size has been measured directly from the SEM images, ranging from 93.5 to 234 nm with an average of ~156 nm. The relatively smooth particle surfaces and distinguishable particle boundaries are good evidences of good crystallinity, as confirmed by the XRD analysis where sharp diffraction peaks are observed. The higher resolution in SEM than from the estimated crystallite size from XRD (~79 nm) indicates that each particle consists of several crystallite domains fused together.

Transmission Electron Microscopy:*w*

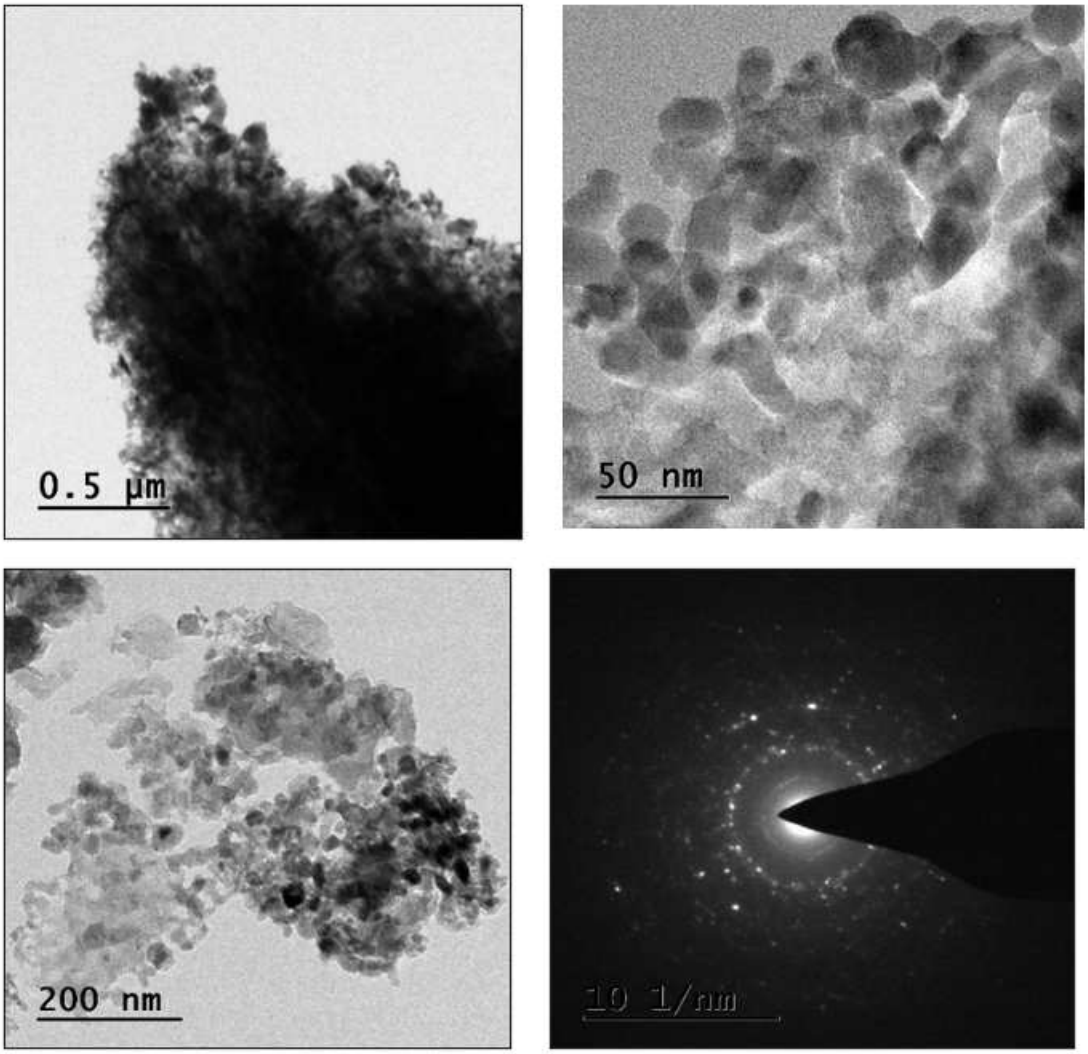


Figure 7: TEM micrographs of AgNPs using *Wrightia tinctoria* leaf extract

The TEM micrograph confirmed the successful formation of nanosized silver nanoparticles with predominantly spherical to quasi-spherical morphology. The nanoparticles were uniformly distributed with moderate agglomeration, which is commonly observed in biosynthesized nanoparticles due to their high surface energy and interactions among phytochemical capping agents derived from *Wrightia tinctoria*. The majority of the nanoparticles exhibited diameters in the range of approximately 15–35 nm, with an estimated average size of 25–30 nm. Individual nanoparticles possessed smooth surfaces and distinct boundaries, indicating controlled nucleation and growth during biosynthesis.

TEM revealed primary particles of 15–35 nm whereas SEM demonstrated larger aggregates (~156 nm), indicating that biosynthesized nanoparticles exist as aggregates composed of multiple crystalline primary particles. Such behaviour is commonly reported for phytochemical-capped AgNPs due to hydrogen bonding and intermolecular interactions among adsorbed biomolecules.

Particle Size and Zeta Potential Analysis: The particle size and zeta potential of the synthesized silver nanoparticles (AgNPs) were determined using Dynamic Light Scattering (DLS) and electrophoretic mobility measurements**.** The analysis provides insight into the hydrodynamic diameter**,** stability, and surface charge of the nanoparticles dispersed in solution.

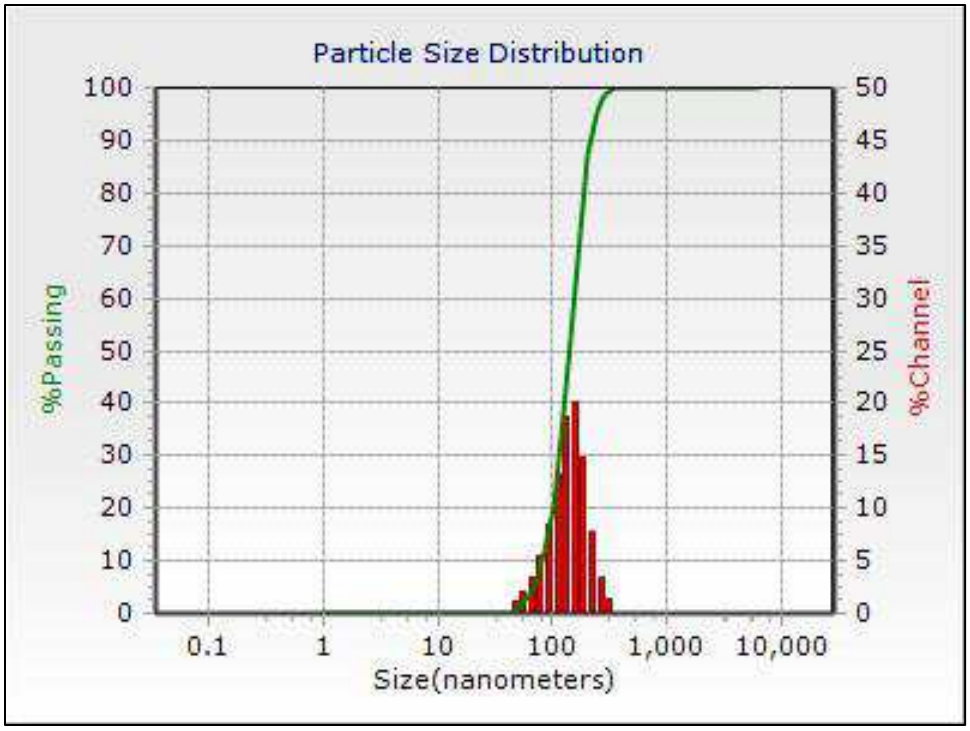


Figure 8: **Particle size distribution curve of silver nanoparticles synthesized using *Wrightia tinctoria* leaf extract**

Dynamic light scattering (DLS) analysis showed a hydrodynamic size distribution of approximately 90–250 nm in double-distilled water. The larger hydrodynamic dimensions compared with TEM-derived primary particle sizes are consistent with the influence of hydration layers and/or particle aggregation in suspension. Measurements were performed in triplicate. Zeta-potential analysis revealed a mean surface charge of −39.6 mV. This relatively high negative surface charge may contribute to electrostatic stabilization under the measurement conditions; however, a single zeta-potential measurement does not establish long-term colloidal stability under biological or application-specific conditions. Stability over time and in relevant biological media requires further investigation.

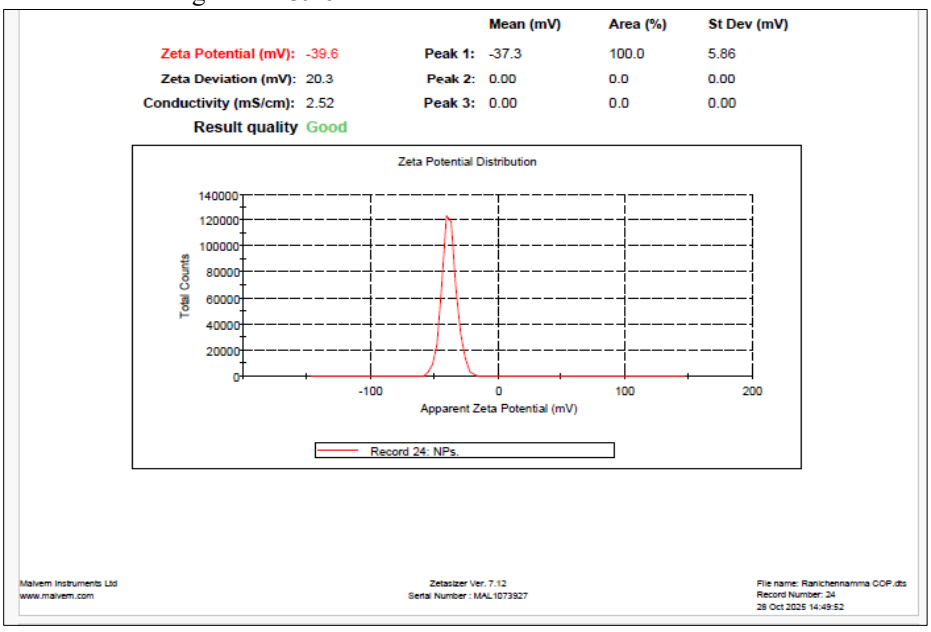


Figure 9: **Zeta Potential Distribution of Prepared Nanoparticles**

Cytotoxicity Assessment in HaCaT Cells: The cytotoxicity of synthesized AgNPs and crude extract was evaluated in HaCaT keratinocytes using the MTT assay following 24 h exposure. Cells were treated with AgNP concentrations of 10–320 µg/mL. AgNP treatment produced a concentration-dependent reduction in the reported cell viability values, whereas the crude extract showed comparatively higher viability. The reported IC50 value of AgNPs was 6.68 µg/mL. Because this estimated IC50 is below the lowest concentration tested (10 µg/mL), it should be interpreted cautiously and should ideally be confirmed using additional concentrations around the 50% response range and an appropriate nonlinear dose–response model. In addition, silver nanoparticles can potentially interfere with colorimetric assays; therefore, nanoparticle-only assay controls and a complementary viability assay would strengthen confirmation of the observed cytotoxic response. The cytotoxicity observed in HaCaT cells should be regarded as a safety-relevant preliminary finding and not as evidence of therapeutic benefit.

Table 2: Cytotoxic effect of silver nanoparticles on HaCaT cells

Sr. No.	Concentration ($\mu g/ml$)	Mean Cell Viability of Sample II (Silver Nanoparticles) %	Mean Cell Viability of Sample II (*Wrightia tinctoria* leaf extract) %
1	10	25.19±2.30	93.21±2.34
2	20	23.21±2.11	87.21±2.11
3	40	22.87±3.58	76.37±2.69
4	80	20.34±2.87	60.5±2.47
5	160	15.24±2.92	56.37±1.98
6	320	12.33±2.34	49.33±1.51

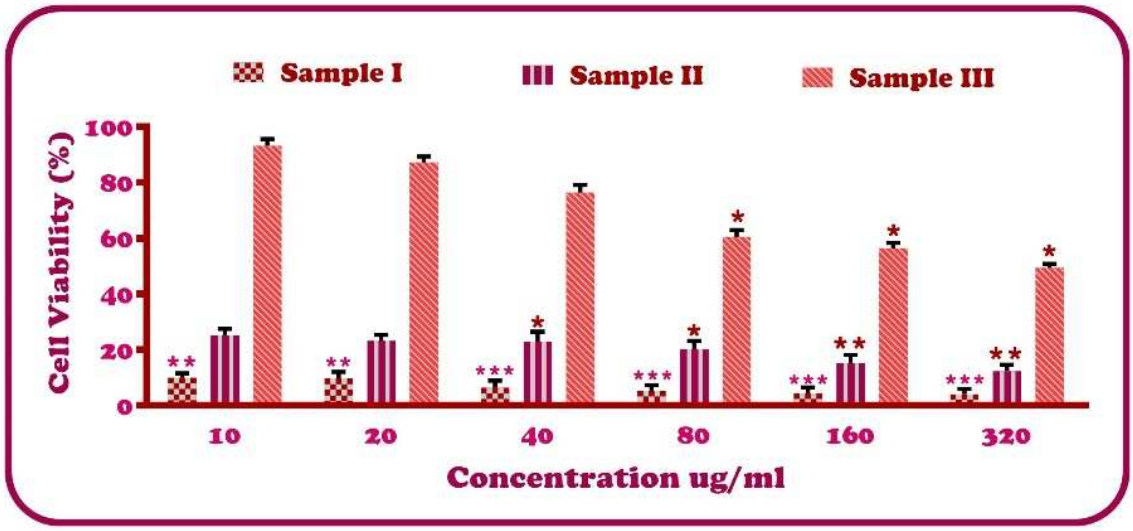


Figure 10: **Effect of silver nanoparticles on HaCaT cell viability**

CONCLUSION:

The present study demonstrated the successful green synthesis of silver nanoparticles (AgNPs) using the aqueous leaf extract of Wrightia tinctoria, highlighting the potential of plant-derived phytoconstituents in nanoparticle fabrication. Comprehensive physicochemical characterization using UV–Visible spectroscopy, FTIR, XRD, SEM, TEM, EDX, particle-size analysis, and zeta-potential measurements provided evidence of nanoparticle formation and their structural and morphological characteristics. The synthesized AgNPs exhibited a characteristic surface plasmon resonance absorption peak at 455 nm, diffraction patterns consistent with crystalline face-centered cubic metallic silver, and an estimated crystallite size of approximately 79 nm. TEM analysis revealed predominantly spherical to quasi-spherical nanoparticles measuring 15–35 nm, whereas SEM and DLS indicated the presence of larger aggregates. The zeta potential of −39.6 mV suggested a highly negative surface charge, potentially contributing to electrostatic stabilization. FTIR analysis indicated the presence of functional groups potentially associated with

plant-derived constituents, while EDX confirmed the presence of silver along with other elements.

Preliminary in vitro evaluation using the MTT assay revealed concentration-dependent cytotoxicity in HaCaT human keratinocyte cells, with an IC_{50} value of 6.68 µg/mL. Although these findings demonstrate the biological activity of the synthesized nanoparticles, their cytotoxic effects on normal keratinocytes warrant careful consideration regarding dermatological safety. Overall, the study establishes a preliminary foundation for further investigation of Wrightia tinctoria-mediated AgNPs. Additional studies focusing on nanoparticle stability, cellular uptake, mechanisms of cytotoxicity, antioxidant and antimicrobial activities, inflammatory pathways, and selective effects on disease-relevant cells are necessary. Comprehensive in vivo safety and efficacy evaluations are also essential before establishing their potential suitability for biomedical applications, particularly in psoriasis and other dermatological conditions.

REFERENCES:

1 Abass AM, Abdoon FM. Synthesis, Characterization, and Applications of Metal Oxides of ZnO, CuO, and CeO2 Nanoparticles: A Review. J Appl Organomet Chem 2024;4:349–66. https://doi.org/10.48309/JAOC.2024.471305.1220.

2 Koul B, Poonia AK, Yadav D, Jin JO. Microbe-Mediated Biosynthesis of Nanoparticles: Applications and Future Prospects. Biomol 2021, Vol 11, Page 886 2021;11:886. https://doi.org/10.3390/BIOM11060886.

3 Wilcoxon JP. Nanoparticles—Preparation, Characterization and Physical Properties. Front Nanosci 2012;3:43–127. https://doi.org/10.1016/B978-0-08-096357-0.00005-4.

4 Mota WS, Severino P, Kadian V, Rao R, Zielińska A, Silva AM, et al. Nanometrology: particle sizing and influence on the toxicological profile. Front Nanotechnol 2025;7:1479464. https://doi.org/10.3389/FNANO.2025.1479464/FULL.

5 Galúcio JMP, Souza SGB de, Vasconcelos AA, Lima AKO, Costa KS da, Braga H de C, et al. Synthesis, Characterization, Applications, and Toxicity of GreenSynthesized Nanoparticles. Curr Pharm Biotechnol 2021;23:420–43. https://doi.org/10.2174/1389201022666210521102307.

6 Khan Y, Sadia H, Ali Shah SZ, Khan MN, Shah AA, Ullah N, et al. Classification, Synthetic, and Characterization Approaches to Nanoparticles, and Their Applications in Various Fields of Nanotechnology: A Review. Catal 2022, Vol 12, Page 1386 2022;12:1386. https://doi.org/10.3390/CATAL12111386.

7 Villagrán Z, Anaya-Esparza LM, Velázquez-Carriles CA, Silva-Jara JM, Ruvalcaba-Gómez JM, Aurora-Vigo EF, et al. Plant-Based Extracts as Reducing, Capping, and Stabilizing Agents for the Green Synthesis of Inorganic Nanoparticles. Resour 2024, Vol 13, Page 70 2024;13:70. https://doi.org/10.3390/RESOURCES13060070.

8 Nuruzzaman M, Liu Y, Rahman MM, Dharmarajan R, Duan L, Uddin AFMJ, et al. Nanobiopesticides: Composition and preparation methods. Nano-Biopesticides Today Futur Perspect 2019:69–131. https://doi.org/10.1016/B978-0-12-815829-6.00004-8.

9 Ijaz I, Gilani E, Nazir A, Bukhari A. Detail review on chemical, physical and green synthesis, classification, characterizations and applications of nanoparticles. Green Chem Lett Rev 2020;13:59–81. https://doi.org/10.1080/17518253.2020.1802517.

10 Khan F, Shariq M, Asif M, Siddiqui MA, Malan P, Ahmad F. Green Nanotechnology: Plant-Mediated Nanoparticle Synthesis and Application. Nanomater 2022, Vol 12, Page 673 2022;12:673. https://doi.org/10.3390/NANO12040673.

11 Huston M, Debella M, Dibella M, Gupta A. Green Synthesis of Nanomaterials. Nanomater 2021, Vol 11, Page 2130 2021;11:2130. https://doi.org/10.3390/NANO11082130.

12 Cardoso B, Nobrega G, Afonso IS, Ribeiro JE, Lima RA. Sustainable green synthesis of metallic nanoparticle using plants and microorganisms: A review of biosynthesis methods, mechanisms, toxicity, and applications. J Environ Chem Eng 2025;13:116921. https://doi.org/10.1016/J.JECE.2025.116921.

13 Peralta-Videa JR, Huang Y, Parsons JG, Zhao L, Lopez-Moreno L, Hernandez-Viezcas JA, et al. Plant-based green synthesis of metallic nanoparticles: scientific curiosity or a realistic alternative to chemical synthesis? Nanotechnol Environ Eng 2016 11 2016;1:4-. https://doi.org/10.1007/S41204-016-0004-5.

14 Edo GI, Mafe AN, Ali ABM, Akpoghelie PO, Yousif E, Isoje EF, et al. Green Biosynthesis of Nanoparticles Using Plant Extracts: Mechanisms, Advances, Challenges, and Applications. BioNanoScience 2025 152 2025;15:267-. https://doi.org/10.1007/S12668-025-01883-W.

15 Namakka M, Rahman MR, Said KAM Bin, Abdul Mannan M, Patwary AM. A review of nanoparticle synthesis methods, classifications, applications, and characterization. Environ Nanotechnology, Monit Manag 2023;20:100900. https://doi.org/10.1016/J.ENMM.2023.100900.

16 Keshavamurthysetty PH, Patel DH. Effect of Process Variables on the Development and

Characterization of Nanocellulose as Novel Biopolymer. Indian J Pharm Educ Res 2023;57:s32–40. https://doi.org/10.5530/ijper.57.1s.5.

17 Shahzad Shirazi M, Moridi Farimani M, Foroumadi A, Ghanemi K, Benaglia M, Makvandi P. Bioengineered synthesis of phytochemical-adorned green silver oxide (Ag2O) nanoparticles via Mentha pulegium and Ficus carica extracts with high antioxidant, antibacterial, and antifungal activities. Sci Reports 2022 121 2022;12:21509-. https://doi.org/10.1038/s41598-022-26021-4.

18 El-Ghmari B, Farah H, Ech-Chahad A. A New Approach for the Green Biosynthesis of Silver nanoparticles Ag2O, Characterization and Catalytic Application. Bull Chem React Eng Catal 2021;16:651–60. https://doi.org/10.9767/BCREC.16.3.11577.651-660.

19 Gungure AS, Jule LT, Nagaprasad N, Ramaswamy K. Studying the properties of green synthesized silver nanoparticles in the application of organic dye degradation under visible light. Sci Reports 2024 141 2024;14:26967-. https://doi.org/10.1038/s41598-024-75614-8.

20 Pawar O, Deshpande N, Dagade S, Waghmode S, Nigam Joshi P. Green synthesis of silver nanoparticles from purple acid phosphatase apoenzyme isolated from a new source Limonia acidissima. J Exp Nanosci 2016;11:28–37. https://doi.org/10.1080/17458080.2015.1025300.

21 Pasieczna-Patkowska S, Cichy M, Flieger J. Application of Fourier Transform Infrared (FTIR) Spectroscopy in Characterization of Green Synthesized Nanoparticles. Molecules 2025;30:684. https://doi.org/10.3390/MOLECULES30030684.