Int.J.Curr.Microbiol.App.Sci (2026) *15*(1): 272-280

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 15 Number 1 (2026)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2026.1501.033

Multi-Facet Phyto-Assisted Synthesis of Silver Nanoparticles; Characterization and Evaluation of Antioxidant and Anticancer Activities Using the Leaf Source of *Kydia calycina* a Traditional Medicinal Tree Taxon

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Keywords

Silver nanoparticles (AgNPs), *Kydia calycina,* antibacterial activity, antioxidant and anticancer activities

Article Info

Received:
18 November 2025
Accepted:
15 December 2025
Available Online:
10 January 2026

A B S T R A C T

Silver nanoparticles (AgNPs) represent one of the most promising and extensively studied nanomaterials due to their unique physicochemical properties and wide-ranging applications in the nanotechnology domain. They have demonstrated significant utility in biomedical fields, catalysis, antimicrobial activity, bio sensing, electronics, optical devices, bio-labelling, and agriculture. Among various synthesis approaches, green synthesis has emerged as a safe, cost-effective, and environmentally friendly method for the production of AgNPs. The antioxidant and anti-cancer efficacy of AgNPs is primarily attributed to the release of silver ions, which interact with injected cells. The present study aimed to Phytosynthesis of silver nanoparticles using aqueous leaf extract of *Kydia calycina* as a natural reducing and stabilizing agent, providing a cost-effective and non-hazardous alternative to conventional methods. Silver nanoparticles were synthesized by adding 1 mM silver nitrate ($AgNO_3$) solution to the aqueous leaf extract of *Kydia calycina*. The antibacterial activity of the synthesized AgNPs was evaluated anti-oxidant and anticancer activities. Characterization of the synthesized nanoparticles was performed using UV–Visible spectroscopy and Fourier Transform Infrared Spectroscopy (FT-IR). The synthesized AgNPs exhibited significant antioxidant and anti-cancer activity, and the standard was taken ascorbic acid. Characterization studies confirmed the successful formation, stability, and crystalline nature of the nanoparticles. The results suggest that the bioactive phytochemicals present in *Kydia calycina* leaf play a crucial role in nanoparticle synthesis and stabilization. Green-synthesized AgNPs demonstrate considerable potential for biomedical applications, particularly as antioxidant and anticancer agents. This approach offers a sustainable pathway for developing novel nanomaterials for future biotechnological and pharmaceutical applications.

Introduction

Nanoparticles (NPs) have emerged as versatile materials with wide-ranging applications in fields such as antimicrobial research, agriculture, biosensor development, drug delivery systems, and biomedicine (Altammar, KA, 2023). Their diverse applicability is largely attributed to their unique physicochemical properties, including high surface area-to-volume ratio, tunable size, and enhanced reactivity. Among the various classes of nanoparticles, metal-based NPs have gained considerable attention due to their remarkable functional properties. In particular, silver nanoparticles (AgNPs) are among the most extensively studied and utilized nanomaterials in contemporary nanotechnology, owing to their strong antimicrobial activity, chemical stability, and broad-spectrum biological applications, and water purification systems (Fahim *et al.,* 2024). However, conventional methods for synthesizing AgNPs frequently entail the use of hazardous chemicals and result in the generation of toxic by-products (Abishad *et al.,* 2022). In response to the limitations associated with conventional chemical and physical synthesis methods, there has been a growing interest in the green synthesis of silver nanoparticles. This eco-friendly approach utilizes biological systems such as plant extracts, microorganisms (bacteria and fungi), or enzymes as both reducing and stabilizing (capping) agents during nanoparticle formation. Such biologically mediated synthesis not only minimizes the use of hazardous chemicals and reduces environmental impact but also improves the biocompatibility and functional properties of the nanoparticles.

Furthermore, the presence of bioactive phytochemicals on the nanoparticle surface can enhance their stability and therapeutic potential, making them highly suitable for diverse biomedical applications (Pandiyan I *et al.,* 2023), and drug delivery systems to environmental detoxification and renewable energy (More *et al.,* 2023). Green synthesis has emerged as an environmentally benign and sustainable alternative to conventional nanoparticle fabrication methods, employing biological entities such as plants, bacteria, fungi, and algae to mediate nanoparticle formation (Bhardwaj *et al.,* 2020). Among these approaches, plant-mediated synthesis has attracted considerable attention owing to its simplicity, cost-effectiveness, and scalability. This method is particularly advantageous due to the abundance of diverse bioactive metabolites present in plant extracts, including flavonoids, alkaloids, terpenoids, and phenolic compounds, which act as natural reducing and stabilizing agents during nanoparticle synthesis (Gupta *et al.,* 2023). Several studies have demonstrated that plant extracts employed in the synthesis of silver nanoparticles (AgNPs) i.e., *Walsura trifoliata* (Subbaiah VK *et al.,* 2026); *Boswellia ovalifoliolata* (2010); *Withania somnifera* (Venkata Subbaiah KP and Savithramma **N)** *Syzygium Alternifolium* (Wt.) Walp. (Yugandhar Pand Nataru Savithramma, 2015), *Plumbago zeylanica L.* (Venkata Subbaiah KP *et al.,* 2013) *Adansonia digitata L.* (Maruthi Kesava Kumar *et al.,* 2016), *Azadirachta indica*, *Ocimum sanctum*, and *Artemisia scoparia* have been extensively studied for their effectiveness in synthesizing silver nanoparticles (AgNPs), owing to their abundant bioactive compounds.

This approach not only minimizes environmental impact but also enhances the biocompatibility and functional properties of the synthesized nanoparticles. Despite the advantages of green synthesis, concerns regarding the potential toxicity of silver nanoparticles (AgNPs) to human health and the environment remain significant. Therefore, to ensure their safe and effective application; future research should prioritize comprehensive biocompatibility evaluations, including cytotoxicity and genotoxicity assessments. Such studies are essential to establish the safety profile of these nanoparticles and to support their responsible use in biomedical and environmental applications (Kirubakaran *et al.,* 2025). In this context, *Kydia calycina medicinal plant known for its pharmacological potential, emerges as an ideal candidate for green nanoparticle synthesis,* and serve dual functions as reducing and capping agents, primarily due to the presence of bioactive compounds such as flavonoids, phenolics, alkaloids, and proteins.

Kydia calycina, a member of the Malvaceae family, commonly known as Pulao, Boranga, or Pula in English language, for this is a medicinal plant widely recognized for its traditional therapeutic applications. It has been reported to possess various pharmacological properties, including treatment for skin diseases, hyperglycaemia, hyperlipidemia, and also exhibits analgesic, anti-inflammatory, anticancer, antioxidant, antiulcer, antifungal, immunomodulatory, febrifuge, and wound healing activities. The plant is widely distributed across India and is commonly found in deciduous forests and the sub-Himalayan regions. It is also utilized by tribal communities in Rajasthan, Maharashtra, and Himachal Pradesh due to its nutritional and medicinal importance, particularly in arid regions. *Kydia calycina* is a small

evergreen tree that grows in a wide range of habitats. Although many plant species have been extensively studied for synthesis of Nano particles, *K. calycina* remains relatively unexplored. Therefore, the present study was undertaken to find out the efficacy for the phyto synthesized NPs for anti-oxidant and anticancer activities. The leaves of *K. calycina* used for the above purpose without causing harm to other parts of the plant.

Material and Methods

Fresh leaves of *Kydia calycina* were collected from the Srisailam region of the Nallamala Forest, Andhra Pradesh. The collected samples were transported to the laboratory in sterile polythene bags to prevent contamination. The leaves were first washed thoroughly under running tap water to remove surface dirt and contaminants, followed by rinsing with distilled water to obtain clean plant material. Excess moisture and dust particles were removed using a sterile cotton cloth. The cleaned leaves were then cut into small pieces to facilitate the extraction process. Subsequently, the plant material was shade-dried for a period of 14–21 days to reduce moisture content before further experimental use.

Plant Extract Preparation

A total of 25 g of dried plant material was accurately weighed and transferred into a 250 ml conical flask. To this, 100 ml of Milli-Q water was added. The mixture was shaken thoroughly for 5 minutes to ensure proper mixing and then heated in a water bath at 100°C for 20 minutes. After heating, the extract was allowed to cool to room temperature and subsequently filtered using Whatman No. 1 filter paper to remove solid residues. The filtrate (plant extract) was collected and stored in an amber-coloured bottle at room temperature until further use in the synthesis process.

Preparation of 1mM Silver Nitrate solution

10 g Silver nitrate was procured from Sigma-Aldrich, Bangalore, India. A 1 mM Ag $(NO_3)_2$ solution was prepared by dissolving the required amount of Silver nitrate in 100 ml of Milli-Q water in a sterile Erlenmeyer conical flask. The prepared solution was stored in an amber-coloured bottle and kept under refrigerated conditions until further use in the synthesis.

Phyto-synthesis of Silver noparticles (AgNPs)

A volume of 20 ml of aqueous leaf extract of *Kydia calycina* was added to the freshly prepared 1 mM silver nitrate ($AgNO_3$) solution. The reaction mixture was then heated in a water bath at 60–80°C. A visible color change from light yellow to dark brown indicated the successful formation of silver nanoparticles due to the reduction of silver ions by the plant extract. The synthesized nanoparticle solution was subsequently used for further characterization and for evaluating its antioxidant and anticancer activities.

Characterization

The synthesized Silver nanoparticles (AgNPs) were characterized using advanced analytical techniques. The optical properties of the nanoparticles were analyzed using a UV–Visible spectrophotometer (NanoDrop) in the wavelength range of 190–750 nm. Fourier Transform Infrared (FTIR) spectroscopy was employed to identify the functional groups responsible for the reduction and stabilization of AgNPs. The particle size distribution and surface charge were determined using Dynamic Light Scattering (DLS) with a Malvern Zeta analyzer. The crystalline nature of the nanoparticles was examined using X-ray diffraction (XRD) (Shimadzu XRD-6000). Additionally, the morphology and size of the nanoparticles were studied using Transmission Electron Microscopy (TEM) (Hitachi HF-3300, 300 kV).

DPPH antioxidant Activity

The antioxidant activity of AgNPs was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay, following the standard protocol described by Subramanian *et al.,* (2013). A 1 mM DPPH stock solution was prepared by dissolving 4 mg of DPPH in 100 mL of methanol. For the assay, 2 mL of the DPPH solution was mixed with 1 mL of methanolic AgNPs at two different concentrations (50 and 100 µg/mL). The reaction mixture was incubated at room temperature for 30 minutes in the dark, after which the absorbance was measured at 517 nm against a blank. The percentage of radical scavenging activity (RSA) was calculated using the standard formula, and the antioxidant potential was expressed in terms of IC_{50} values. All experiments were performed in triplicate to ensure accuracy and reproducibility. The results demonstrated that AgNPs possess significant antioxidant activity.

Anticancer studies of PdNPs (Cell proliferation assay using SRB (Sulforhodomine-B)

Different human cancer cell lines, including MDA-MB-231, SK-OV-3, PC-3, PANC-1, and HeLa, were obtained from the American Type Culture Collection (ATCC). The cytotoxic activity of AgNPs was evaluated using the Sulforhodamine B (SRB) assay, a quantitative colorimetric method that measures cell survival and proliferation based on cellular protein content. Cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with non-essential amino acids and 10% fetal bovine serum (FBS). The PC-3 cell line was maintained separately in RPMI medium supplemented with glutamine and non-essential amino acids.

All cell lines were incubated at 37°C in a humidified atmosphere containing 5% CO_2. Sub-confluent cells grown in T75 flasks or 90 mm culture dishes were harvested by trypsinization and seeded into 96-well plates at a density of 1×10^4 cells/mL in complete medium, 24 hours prior to treatment. Subsequently, the cells were treated with varying concentrations of AgNPs (12.5–100 µg/mL) and incubated for 48 hours. All treatments were performed in triplicate to evaluate the cytotoxic potency of the nanoparticles.

Results and Discussion

Ultraviolet- Visible (UV-Vis) Spectroscopy analysis of AgNPs

Upon the addition of aqueous leaf extract of *Kydia calycina* to a 1 mM Silver nitrate ($AgNO_3$) solution, a distinct colour change from pale yellow to deep brown was observed, indicating the formation of Silver nanoparticles (AgNPs). This visual transition serves as a preliminary confirmation of nanoparticle synthesis due to the reduction of Ag^{2+} ions. UV–Visible spectroscopy further confirmed nanoparticle formation, showing a broad absorption peak at 406 nm (Fig. 1), which is attributed to the Surface Plasmon Resonance (SPR) of AgNPs. SPR arises from the collective oscillation of conduction electrons on the nanoparticle surface when excited by incident light. The results suggest that the *Kydia calycina* leaf extract acts as both a reducing and stabilizing agent, where phyto-constituents facilitate the reduction of Ag^{2+} ions and simultaneously cap the synthesized nanoparticles, enhancing their stability.

FT-IR

Green-synthesized nanoparticles were characterized using Fourier Transform Infrared (FT-IR) spectroscopy over a scan range of 4000–500 cm^{-1}. The analysis was carried out using an ALPHA ECO-ATR interferometer (Bruker, Ettlingen, Karlsruhe, Germany) to identify the functional groups of phytochemicals involved in the reduction, capping, and stabilization of the nanoparticles. The FT-IR spectrum of the synthesized AgNPs (Fig. 4) exhibited several characteristic absorption peaks. 3358.22- typically belongs to hydroxyl (-OH) groups or N-H stretching vibrations found in phenolic compounds, flavonoids, proteins, or terpenoids, which play a key role as reducing and stabilizing agents in nanoparticle synthesis. 1625.95- 1614/cm-1 correspond to C=C strong bond stretch α, β- unsaturated ketone. This suggests the involvement of proteins or biomolecules in capping and stabilization of nanoparticles. 1377.17/cm-O-H bonding (bending) of phenolic compounds or alcohols. 1444/cm-1 and 1404/cm-1 medium correspond to O-H bending carboxylic acid, 876/cm-1 strong C=C bending alkene vinylidene, 1112.39/cm-1 bond assigned C–O (esters, ethers, or alcohols) or C–N (amines) bonds. 876.81, 844.94-1 strong C=C bending alkene vinylidene, 776.97/cm-1- C–H out-of-plane bending vibrations of aromatic compounds, 616.58- strong bond belongs C=C bending alkene. These functional groups indicate the presence of plant-derived biomolecules contributing to nanoparticle stabilization. 509.66 cm^{-1} Assigned to C–Br stretching of alkyl halides or metal–ligand vibrations. 444.80/cm-1typically indicates the presence of Ag–O bonds or metal-oxygen (Ag–O) stretching vibrations (Fig. 2). These type of results accordance with previous study AgNPs synthesized from bark of *Ficus mollis* vahl.

Bark of *Ficus mollis* vahl

Application of the AgNPs

DPPH Anti-oxidant activity

The antioxidant potential of the biosynthesized AgNPs was systematically evaluated using the DPPH radical scavenging assay, a widely accepted method for assessing hydrogen atom or electron-donating capacity.

Table.1 DPPH activity of AgNPs from *Kydia calycina*

S. No.	Compounds	% of inhibition 50µ/mL	% of inhibition 100µ/mL
1	Ascorbic acid	62.48±0.44	87.54±0.4
2	EvL AgNPs	58.07±0.34	69.80±0.31
3	EvL-Plant aqueous extract	31.17±0.25	49.98±0.50

Figure.1 UV-graph Synthesis of AgNPs from *Kydia calycina* leaves acquired by UV-Vis Spectroscopic analysis

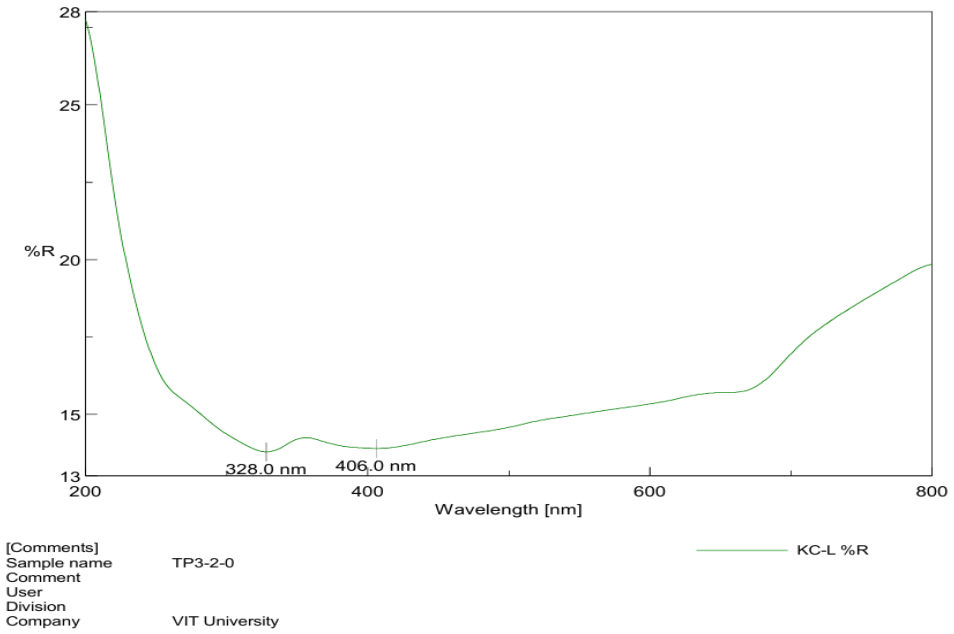


Figure.2 Fourier- Transform Infra-Red (FT-IR) spectra of bio-synthesized AgNPs from *Kydia calycina*

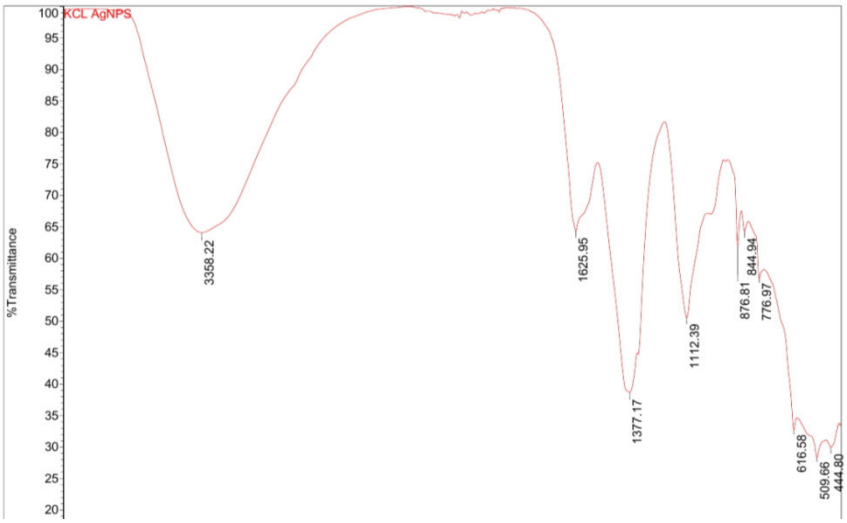


Figure.3 Graphical representation- DPPH anti-oxidants activity of AgNPs from *Kydia calycina*

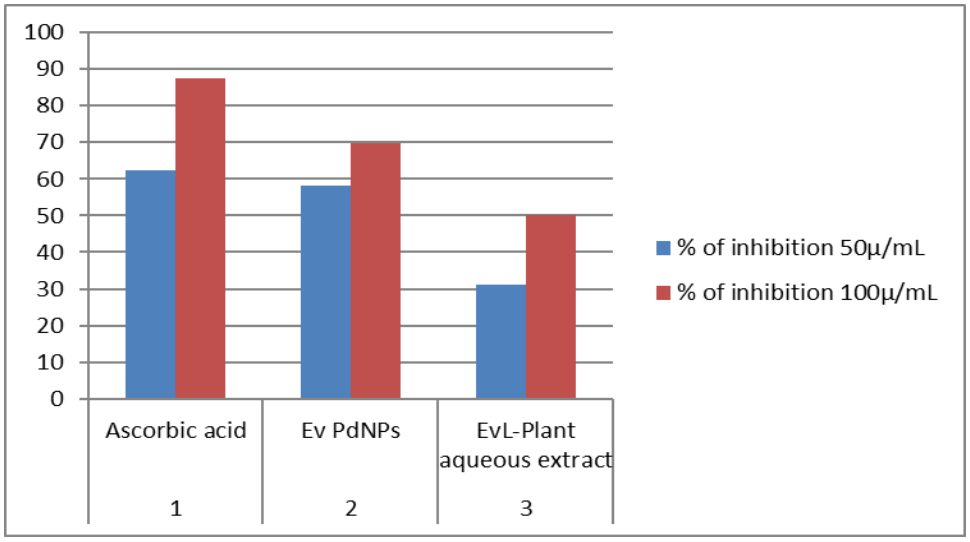
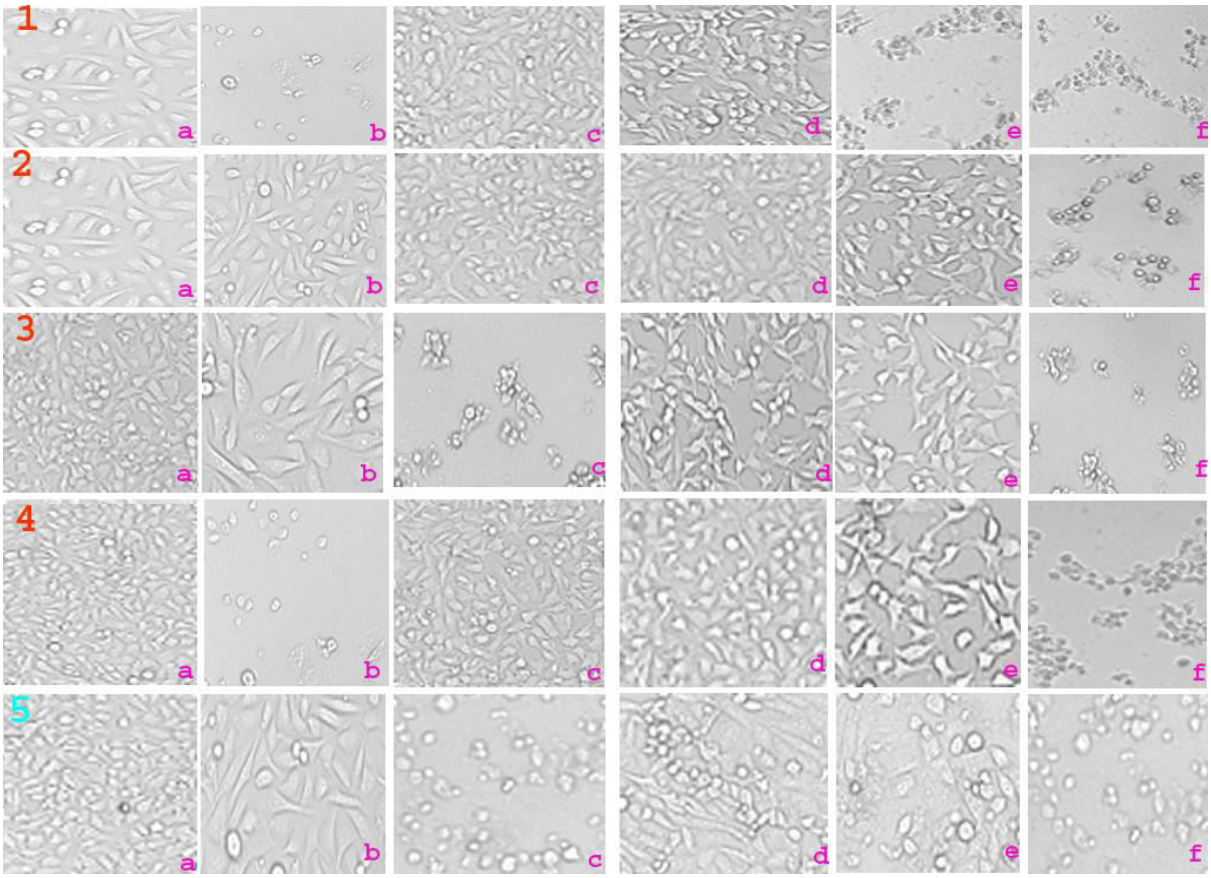
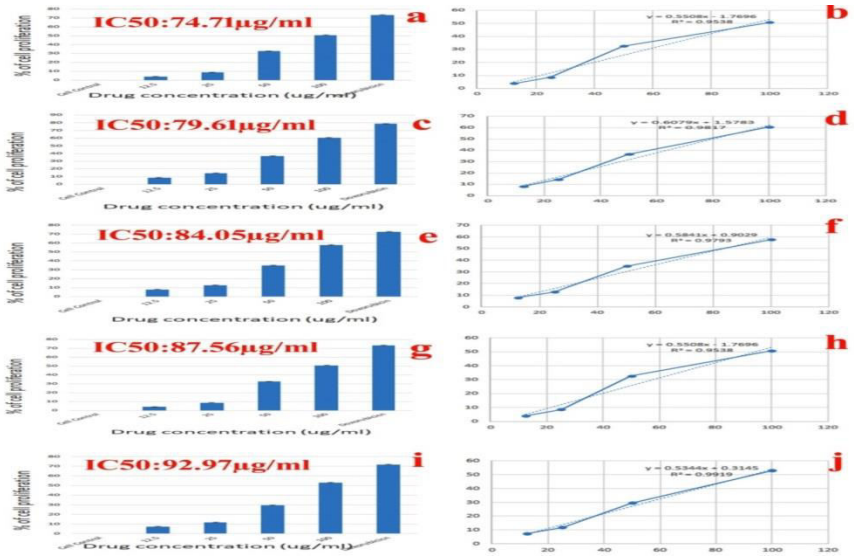


Figure.4 Anti-cancer activity on selected human cancer cell lines by AgNPs synthesized from *Kydia calycina*



A). PC-3, **B).** MDA-MB-231, **C).** SK-OV-3, **D).** PANC-1, **E).** HeLa
a) Cell control, b) Standard control, c) 12.5 µg/ml, d) 25 µg/ml, e) 50 µg/ml and f) 100 µg/ml

Figure.5 Graphical representation of anticancer activity by using AgNPs against five selected human cancer cell lines



a,b- PC-3; **c,d-** MDA-MB-231; **e,f-** SK-OV-3; **g,h-** PANC-1; **I,j-** HeLa;

The assay is based on the reduction of the stable DPPH radical (deep violet) to its non-radical form (yellow), resulting in a measurable decrease in absorbance. The IC_{50} values of the aqueous leaf extract of *Kydia calycina* and the corresponding AgNPs are presented in Table 1. A clear concentration-dependent increase in radical scavenging activity was observed, indicating enhanced antioxidant efficacy at higher nanoparticle concentrations. Notably, AgNPs exhibited significant scavenging activity, reaching a maximum of 58.070% at 100µg/mL, while a lower activity of 69.80% was recorded at 50µg/mL. The observed antioxidant activity can be attributed to the synergistic interaction between palladium nanoparticles and phyto-constituents such as flavonoids, tannins, and other polyphenolic compounds inherently present in *Kydia calycina*. These biomolecules are known to facilitate electron transfer and hydrogen donation, thereby neutralizing free radicals effectively. Furthermore, the adsorption of these phytochemicals onto the nanoparticle surface may enhance their stability and bioavailability, leading to improved antioxidant performance. The enhanced activity of AgNPs compared to the crude extract highlights the potential role of Nano structuring in amplifying biological properties (Fig. 3). Similar trends have been reported for AgNPs synthesized using *Cassia absus* seed extract, supporting the consistency of green-synthesized Silver nanoparticles as effective antioxidant agents. Same type of results observed in AgNPs synthesized from fruit material of *Walsura trifoliata* (Subbaiah VK *et al.,* 2026).

Anti-cancer study of AgNPs

Biologically synthesized AgNPs (*Kydia calycina* – mediated Silver nanoparticles) were evaluated for their anticancer potential against a panel of human cancer cell lines, including MDA-MB-231 (breast carcinoma), SK-OV-3 (ovarian carcinoma), PC-3 (prostate carcinoma), PANC-1 (pancreatic carcinoma), and HeLa (cervical carcinoma). Cytotoxic activity was assessed using the Sulforhodamine B (SRB) assay, a reliable colorimetric method for quantifying cell proliferation based on total cellular protein content. Cells were treated with varying concentrations of AgNPs (12.5, 25, 50, and 100 µg/mL), alongside untreated controls and a standard anticancer drug (doxorubicin). The results demonstrated a concentration-dependent inhibition of cell proliferation across all tested cell lines, indicating the potent cytotoxic potential of the biosynthesized nanoparticles (Fig. 4&5). Mechanistically, the enhanced cytotoxicity of AgNPs can be attributed to their small size and high surface reactivity, which facilitate efficient cellular uptake via endocytosis. Once internalized, the nanoparticles interact with intracellular biomolecules, leading to alterations in cellular structure and biochemical pathways. Furthermore, the non-aggregated nature of these nanoparticles promotes the generation of reactive

oxygen species (ROS), resulting in oxidative stress, DNA damage, and subsequent induction of apoptosis. Among the tested cell lines, AgNPs exhibited the highest cytotoxic activity against SK-OV-3 cells (63.63% inhibition at 100 µg/mL), followed by MDA-MB-231 cells (60.66% inhibition at 100 µg/mL). Notably, these cell lines also showed comparatively higher sensitivity even at lower concentrations, indicating selective efficacy of the nanoparticles. The observed results are consistent with previous reports on Anti-cancer potential of biologically synthesized AgNPs using with *Lantana camera* leaf extract (Leena Hublikar *et al.,* 2023)

In conclusion, a green and sustainable approach was successfully employed for the synthesis of Silver nanoparticles using an aqueous leaf extract of *Kydia calycina*, thereby eliminating the need for hazardous chemicals. The formation of AgNPs was initially confirmed by a visible colour change from pale yellow to deep brown upon the addition of Ag+2 to the plant extract. Comprehensive physicochemical characterization was carried out using UV–Visible spectroscopy and FT-IR. UV–Vis analysis revealed a characteristic absorption peak at 406 nm, confirming nanoparticle formation. FT-IR results confirmed the involvement of phyto constituents in the reduction and stabilization processes. Biological evaluation revealed that AgNPs possess significant antioxidant activity in the DPPH assay. The anticancer potential of AgNPs was demonstrated against multiple human cancer cell lines, including MDA-MB-231, SK-OV-3, PC-3, PANC-1, and HeLa, with enhanced cytotoxicity observed at higher concentrations. Overall, the synthesized KcL-AgNPs were found to be small, spherical, stable, and biologically active. The plant-mediated synthesis approach offers several advantages, including environmental safety, cost-effectiveness, minimal waste generation, and enhanced biocompatibility. These findings highlight the promising potential of AgNPs as multifunctional nanomaterials for biomedical applications, particularly in antioxidant systems, and cancer treatment. Furthermore, their nanoscale size and surface functionality make them attractive candidates for future drug delivery applications.

Author Contributions

P. Earnest Vijayanand - Conceptualization, Resources, Investigation, writing original draft, Methodology. Ankanna Sade - Data curation, Formal analysis, Validation; K. Venkata Subbaiah – Project administration, Supervision, Writing-review and editing. Savithramma Nataru– Project administration, Supervision, Writing-review and editing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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How to cite this article:

Earnest Vijayanand P., Ankanna Sade, Venkata Subbaiah K. and Savithramma Nataru. 2026. Multi-Facet Phyto-Assisted Synthesis of Silver Nanoparticles; Characterization and Evaluation of Antioxidant and Anticancer Activities Using the Leaf Source of *Kydia calycina* a Traditional Medicinal Tree Taxon. *Int.J.Curr.Microbiol.App.Sci.* 15(1): 272-280. **doi:** https://doi.org/10.20546/ijcmas.2026.1501.033