IJRAR.ORG E-ISSN: 2348-1269, P-ISSN: 2349-5138

INTERNATIONAL JOURNAL OF RESEARCH AND ANALYTICAL REVIEWS (IJRAR) | IJRAR.ORG

An International Open Access, Peer-reviewed, Refereed Journal

BIOGENIC SYNTHESIS OF PVP-STABILIZED SILVER NANOPARTICLES VIA *TINOSPORA CORDIFOLIA* FOR POTENT ANTIBACTERIAL APPLICATIONS

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Abstract: The biological synthesis of metallic nanoparticles has emerged as a cornerstone of nanobiotechnology, offering a rapid, non-toxic, and economically viable alternative to hazardous chemical reduction methods. This research focuses on the phyto-mediated synthesis of silver nanoparticles (AgNPs) and their subsequent stabilization via polyvinylpyrrolidone (PVP) encapsulation, leveraging the bioactive potential of *Tinospora cordifolia* leaf extract. The secondary metabolites within the extract served as robust natural reducing and capping agents, while the addition of PVP ensured long-term colloidal stability and prevented particle agglomeration. Comprehensive characterization via UV-Vis spectrophotometry revealed a distinct surface plasmon resonance (SPR) peak at 369 nm, confirming the formation of silver nanostructures, while Fourier Transform Infrared Spectroscopy (FTIR) and X-ray Diffraction (XRD) validated the presence of functional groups and the highly crystalline nature of the particles. Notably, the PVP-AgNPs exhibited potent antimicrobial properties against a spectrum of pathogens, including *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. A significant synergistic effect was observed when these nanoparticles were integrated with the antibiotic Ofloxacin, suggesting a potential strategy to overcome multidrug resistance. These findings underscore the efficacy of *T. cordifolia*-derived PVP-AgNPs as high-performance, sustainable antimicrobial agents with the potential to revolutionize therapeutic approaches to microbial infections.

Keyword: *Tinospora cordifolia,* Green Synthesis, Silver Nanoparticles, PVP-Encapsulation, Antimicrobial Activity.

1 INTRODUCTION

Nanobiotechnology has transcended its role as a nascent field to become a transformative discipline, particularly through the integration of environmentally benign, sustainable methodologies [1–3]. This strategic shift toward "Green Chemistry" is necessitated by the urgent requirement to circumvent the ecological and biomedical limitations inherent in conventional synthesis [4,5]. Traditional physical and chemical approaches often entail high energy consumption and the use of hazardous reducing agents such as sodium borohydride ($NaBH_4$) and hydrazine, which leave toxic residues on the nanoparticle surface [6–8]. Such impurities not only pose environmental hazards but also critically compromise the biocompatibility and clinical safety of the resulting nanomaterials. In response, phyto-mediated synthesis has emerged as a superior, eco-conscious alternative, leveraging complex plant secondary metabolites as dual-function reducing and stabilizing agents [9–12]. These biomolecules, ranging from polyphenols to terpenoids, facilitate precise control over nanoparticle morphology while eliminating the need for synthetic stabilizers [13–16].

Among noble metal nanostructures, silver nanoparticles (AgNPs) remain the "gold standard" in antimicrobial research due to their exceptional localized surface plasmon resonance (LSPR) and potent bioactivity [17–18]. Their high surface-area-to-volume ratio enables intensive interaction with microbial cell walls, leading to membrane perforation, disruption of respiratory chains, and the lethal generation of reactive oxygen species (ROS). However, translating AgNPs from laboratory synthesis to therapeutic applications is frequently hindered by their high surface energy, which drives irreversible agglomeration [19]. To maintain colloidal integrity, this study utilizes Polyvinylpyrrolidone (PVP) as a strategic capping agent [20]. PVP provides a

robust steric barrier that prevents particle-to-particle contact, thereby ensuring long-term dispersion stability and enhancing the biological performance of the nanoparticles in complex physiological media.

Tinospora cordifolia (TC), traditionally known as *Guduchi* or *Giloy*, occupies a preeminent position in Ayurvedic medicine as a "Rasayana" or potent immunomodulator. Its leaf extract is a rich source of alkaloids (e.g., berberine), glycosides, and diterpenoid lactones [21–23]. These phytoconstituents possess electron-rich functional groups, specifically hydroxyl and carbonyl moieties, that act as potent catalysts for the reduction of silver ions (Ag^+ to Ag^0). Beyond their role in synthesis, the inherent pharmacological properties of TC, including its documented anti-inflammatory, antioxidant, and anticancer activities, endow the silver core with a biofunctional coating, creating a hybrid nanotherapeutic system with enhanced medicinal value [24–26].

The global escalation of Multi-Drug Resistant (MDR) pathogens has precipitated a clinical crisis, rendering conventional fluoroquinolones like Ofloxacin increasingly obsolete. As bacterial resistance mechanisms evolve, there is an urgent need for multifaceted antimicrobial strategies that employ physical disruption rather than single-target biochemical pathways [27]. The integration of *T. cordifolia*-derived biomolecules with silver nanoparticles presents a compelling synergistic approach. This synergy combines the plant's intrinsic medicinal properties with the broad-spectrum bactericidal action of silver, potentially circumventing common resistance mechanisms.

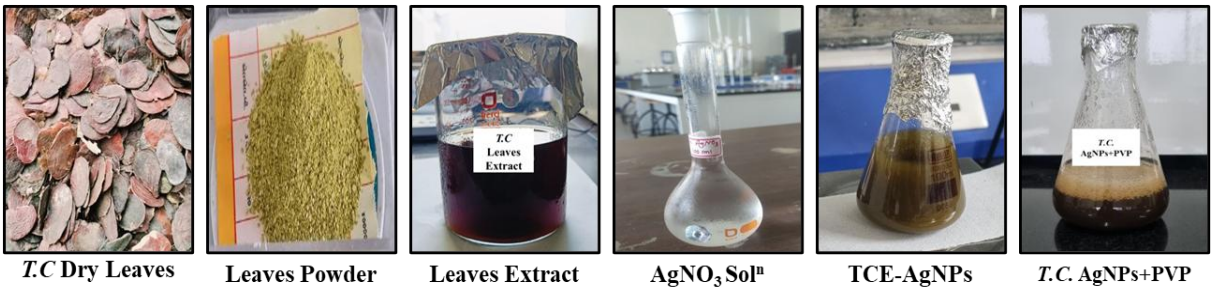
This research investigates the green synthesis of PVP-capped silver nanoparticles utilizing *Tinospora cordifolia* leaf extract as a primary bio-reductant. We report on the comprehensive physicochemical characterization of these particles and evaluate their comparative antimicrobial efficacy against clinically significant Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) pathogens. By aligning the principles of sustainable nanotechnology with advanced surface engineering, this study offers a promising avenue for developing high-performance, biocompatible nanotherapeutics to combat the rising tide of antibiotic-resistant infections [28].

2 MATERIAL AND METHODS

Materials: The biological precursor, fresh leaves of *Tinospora cordifolia*, was procured from a local commercial market in Gujarat, India. All chemical reagents utilized in this study were of analytical grade and used as received without further purification. Silver nitrate ($AgNO_3$), acting as the metal precursor, and Polyvinylpyrrolidone (PVP, MW 40,000), serving as the stabilizing agent, were both sourced from S.D. Fine-Chem Ltd. To ensure high-purity conditions and prevent ionic interference, double-distilled water was used to prepare all aqueous solutions and plant extractions.

Preparation of Plant extract: The aqueous extract of *Tinospora cordifolia* was prepared by first washing 10 g of fresh leaves with double-distilled water to remove surface impurities, then drying them on filter paper. The cleaned leaves were pulverized into a fine powder and subjected to Soxhlet extraction, in which 10 g of the plant material was extracted with 350 mL of double-distilled water. The extraction was maintained until the solvent siphoned from the thimble changed from a deep reddish-brown to colorless, indicating complete recovery of bioactive metabolites. The resulting crude extract was cooled, filtered through Whatman No. 1 filter paper, and stored in an airtight container at -4°C to preserve its phytochemical profile for subsequent nanoparticle synthesis.

Synthesis of AgNPs using *Tinospora cordifolia* leaf extract: The green synthesis of silver nanoparticles (AgNPs) was achieved by incorporating 30 mL of freshly prepared *Tinospora cordifolia* leaf extract into 90 mL of a 0.03 mM aqueous silver nitrate ($AgNO_3$) solution (Figure 1). The reaction was conducted in an Erlenmeyer flask at ambient temperature under continuous magnetic stirring for 2 hours. A distinct chromatic transition from light brown to dark brown was observed during the stirring period, providing visual confirmation of the successful bioreduction of silver ions (Ag^+) to metallic silver (Ag^0). This morphological transformation, mediated by the bioactive phytochemicals in the leaf extract, resulted in a stable colloidal suspension ready for subsequent characterization.



T.C Dry Leaves (A) | Leaves Powder (B) | Leaves Extract (C) | $AgNO_3$ Sol^n (D) | TCE-AgNPs (E) | *T.C.* AgNPs+PVP (F)

Figure 1: (A) Dry Leaves, (B) Leaves powder, (C) Leaves extract, (D) $AgNO_3$ Sol^n, (E) *T.C.* AgNPs, (F) AgNPs + PVP (Polyvinylpyrrolidone)

Synthesis of PVP encapsulated AgNPs: The PVP-encapsulated silver nanoparticles (PVP-AgNPs) were synthesized by first dispersing 0.50 g of PVP in 250 mL of double-distilled water under magnetic stirring for one hour at ambient temperature. Subsequently, 120 mL of the pre-synthesized AgNP solution was added dropwise to the polymeric matrix, resulting in a distinct chromatic shift from dark brown to light brown. After a 10-minute equilibration at 25°C, the stabilized colloidal mixture was centrifuged for 30 minutes to isolate the encapsulated nanostructures.

3 CHARACTERIZATION:

The optical properties of the synthesized AgNPs and PVP-functionalized AgNPs were analyzed using a Shimadzu UV-1800 spectrophotometer within a wavelength range of 200–800 nm. To identify the biomolecular functional groups involved in the reduction and stabilization processes, Fourier-transform infrared (FTIR) spectroscopy was performed at a resolution of 5 cm^{-1} across the 4000–400 cm^{-1} range. The crystalline nature and phase purity of the nanostructures were determined via X-ray diffraction (XRD) using a Rigaku D/max 40 kV analyzer. Furthermore, the detailed structural morphology and particle size distribution of both the bare and encapsulated nanoparticles were elucidated through High-Resolution Transmission Electron Microscopy (HR-TEM).

Antimicrobial Activity: The biological efficacy of PVP, AgNPs, and PVP-AgNPs was evaluated using the Agar well diffusion method against a panel of clinically significant pathogens, including Gram-negative (*Escherichia coli, Pseudomonas aeruginosa*) and Gram-positive (*Staphylococcus aureus*) bacterial strains. Nutrient agar plates were uniformly inoculated with the respective bacterial cultures to create a confluent lawn. Subsequently, 100 µL of each nanoparticle formulation was introduced into the wells. The plates were then incubated under aerobic conditions at 37°C for 24 hours. The antibacterial potency was quantified by measuring the diameter of the zones of inhibition (ZOIs) formed around each well, reflecting the microorganisms' susceptibility to the synthesized nanotherapeutics.

4 RESULTS AND DISCUSSION:

UV-Visible spectroscopy: The successful bioreduction of silver ions (Ag^+) into metallic nanoparticles (Ag^0) was confirmed by a distinct chromatic transition from light brown to dark brown. UV-Vis spectroscopic analysis, as illustrated in Figure 2, identified a characteristic Surface Plasmon Resonance (SPR) peak at 369 nm [29] for both AgNPs and PVP-AgNPs. This absorption maximum indicates stable, spherical nanostructures whose optical properties are fundamentally governed by their specific size and morphological distribution.

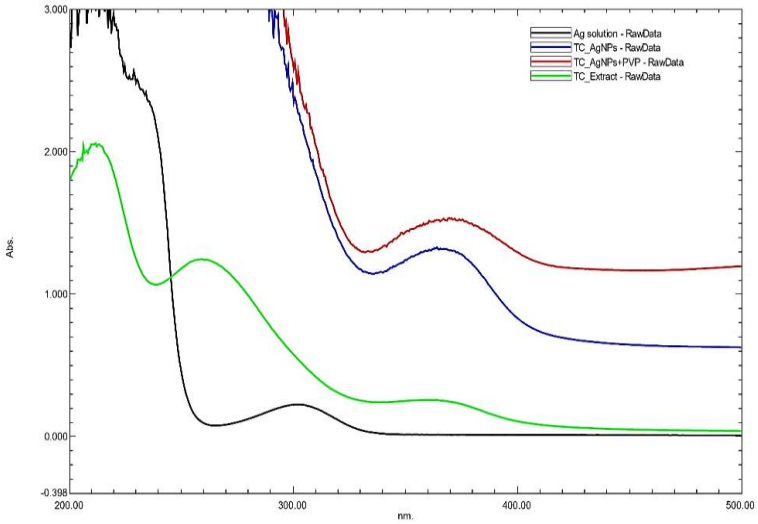


Figure 2: UV-Vis absorbance spectra of (**A**) Silver salt $AgNO_3$, (**B**) Plant extract, (**C**) Ag-NPs, (**D**) PVP-AgNPs

FTIR studies: Fourier transform infrared (FTIR) spectroscopy was performed to identify the functional groups involved in the bioreduction and stabilization of silver nanoparticles (Figure 3). As shown in the spectrum, the broad absorption band at 3410 cm^{-1} corresponds to the O-H stretching vibrations of polyphenols or water molecules, while the peaks at 3065 cm^{-1}, 2989 cm^{-1}, and 2882 cm^{-1} represent the C-H stretching of aliphatic groups from the *T. cordifolia* extract and PVP. A prominent peak at 1651 cm^{-1} is characteristic of the C=O (carbonyl) stretching in the pyrrolidone ring of PVP, confirming its role as a capping agent. Additionally, the vibrations at 1545 cm^{-1} and 1390 cm^{-1} are attributed to C=C aromatic stretching and C-N

stretching, respectively, while the peak at 1758 cm^{-1} indicates the presence of ester or acid groups. These results collectively verify that the bioactive phytochemicals and the PVP polymer successfully functionalized the AgNP surface, ensuring colloidal stability and preventing particle agglomeration.

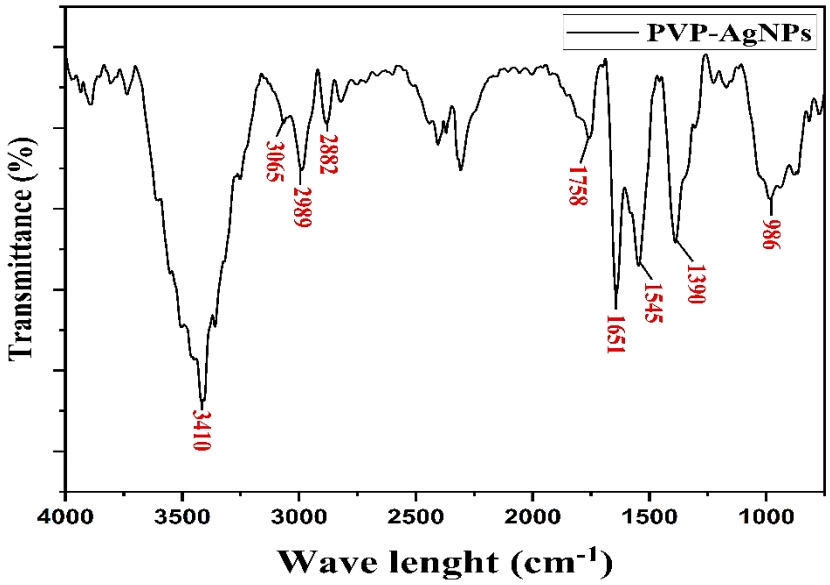


Figure 3: FTIR spectrum of PVP-AgNPs

XRD studies: The crystalline nature and phase purity of the PVP-AgNPs were evaluated through X-ray diffraction (XRD) analysis, as illustrated in the provided diffractogram. The characteristic Bragg reflections at 2θ values of 32.38°, 38.4°, 55.26°, 64.44°, and 77.18° correspond to the (111), (200), (220), (311), and (400) crystallographic planes, respectively, identifying a well-defined face-centered cubic (fcc) structure (JCPDS No. 04-0783) [30]. The high intensity and sharpness of the (200) and (111) peaks signify a high degree of crystallinity, while the presence of minor peaks at 28.16° (110) and 46.36° (211) likely originates from the bio-organic crystallization of *Tinospora cordifolia* phytoconstituents or trace silver oxide formed during the green synthesis process. These results confirm that the PVP encapsulation successfully stabilized the metallic silver cores without compromising their structural integrity or crystalline phase.

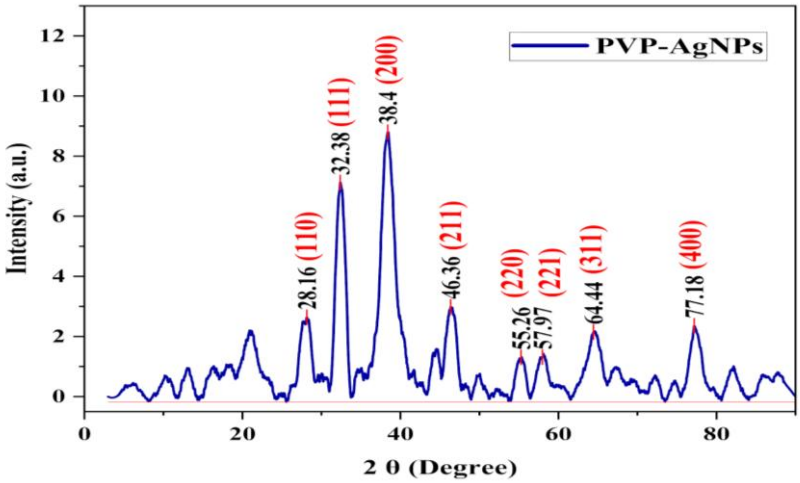


Figure 4: XRD Analysis of biosynthesized PVP-AgNPs.

Antimicrobial activity: The antimicrobial efficacy of the green-synthesized PVP-AgNPs was quantitatively assessed using a modified Agar well diffusion method, demonstrating substantial inhibitory potential across all tested bacterial pathogens at a standardized concentration of 100 µL. As evidenced by the measurable zones of inhibition (ZOIs), the nanoparticles exhibited high susceptibility to Gram-negative *P. aeruginosa* (25 mm) and *E. coli* (18 mm), and significant activity against Gram-positive *S. aureus* (16 mm). Compared with the standard antibiotic Ofloxacin, the maximum growth inhibition observed for the PVP-AgNPs was 28 mm against Gram-positive strains; however, the overall performance of the PVP-AgNPs indicates superior, more consistent antibacterial activity. These results underscore the potential of *Tinospora cordifolia*-mediated, polymer-stabilized silver nanostructures as a highly potent and sustainable alternative to conventional antibiotics in treating diverse microbial infections.

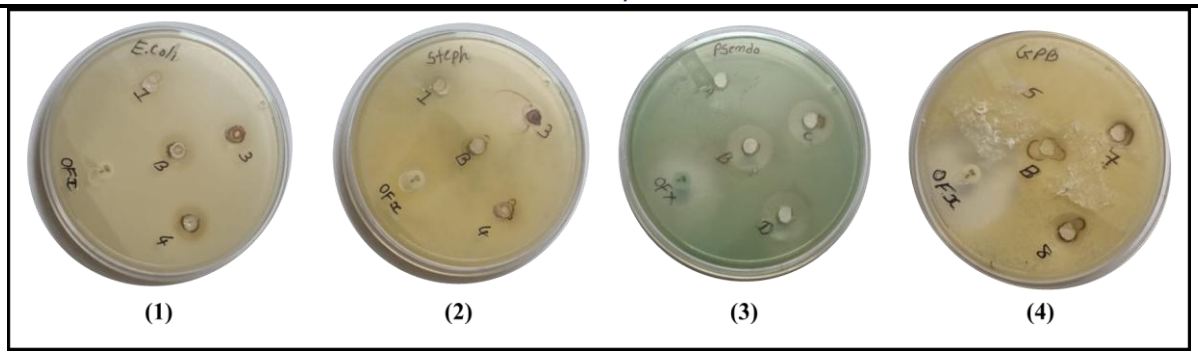


Figure 6. Antimicrobial activity of 1) *E. coli* 2) *Staphylococcus aureus* 3) *Pseudomonas aeruginosa* 4) GPB using *Tinospora cordifolia* leaf extract and silver nanoparticles.

Table 1: Antibacterial activity of plant extract, silver nanoparticles of *Calotropis procera* leaves and PVP-capped silver nanoparticles

Test Sample	Concentration (µL)	Zone of Inhibition (mm)			
		E. coli	*S. aureus*	*P. aeruginosa*	GPB
Plant Extract	100	09	10	12	11
Silver Metal Salt	100	13	11	14	12
AgNPs	100	14	14	16	15
Ofloxacin	100	17	13	20	28
PVP-AgNPs	100	18	16	25	22

5 CONCLUSION:

In conclusion, this study successfully demonstrated the green synthesis of silver nanoparticles (AgNPs) and their stable polyvinylpyrrolidone-encapsulated derivatives (PVP-AgNPs) using the bioactive aqueous extract of *Tinospora cordifolia*. Physicochemical characterization via UV-Vis spectroscopy and XRD confirmed the formation of highly crystalline, face-centered cubic (fcc) nanostructures with a characteristic surface plasmon resonance at 369 nm, while FTIR analysis verified the dual role of plant metabolites and PVP in the reduction and stabilization processes. The biological evaluation revealed potent, broad-spectrum antimicrobial efficacy against both Gram-positive (*S. aureus*) and Gram-negative (*E. coli*, *P. aeruginosa*) pathogens, with inhibition zones reaching up to 25 mm. These findings underscore the potential of *T. cordifolia*-mediated PVP-AgNPs as a biocompatible, cost-effective, and highly efficient alternative to traditional antibiotics, offering a promising sustainable solution for combating multi-drug resistant (MDR) infections in clinical settings.

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