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Andrographis paniculata-Mediated Synthesis of Silver Nanoparticles (AgNPs) and Their Antimicrobial Activity Against the Oral Clinical Pathogen Streptococcus mutans.

Srivarsan Ravi[1], Dr. Dinesh Kumar[2]

[1]Undergraduate Student, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India. srivarshanravi22@gmail.com
[2]Assistant Professor, Department of Pediatric and Preventive Dentistry, Saveetha Dental College, Saveetha Institute of Medical and Technical Sciences, Chennai-600077, Tamil Nadu, India. Email id: dineshkumarb.sdc@saveetha.com

Abstract

Objective
The aim of this study was to evaluate the antimicrobial activity of silver nanoparticles synthesised through an eco-friendly, plant-based approach using Andrographis paniculata extract, specifically against Streptococcus mutans, which is the primary bacterium responsible for dental caries.
Materials and Methods
The green synthesis of silver nanoparticles was performed using an aqueous extract of Andrographis paniculata, which served both as a reducing agent to convert silver ions into nanoparticles and as a stabilising agent to prevent aggregation. Institutional ethical clearance was obtained prior to the commencement of the study at Saveetha Dental College. The biosynthesised nanoparticles were characterised using several analytical techniques: Ultraviolet-Visible spectroscopy to confirm nanoparticle formation, Fourier-Transform Infrared spectroscopy to identify functional groups involved in capping, Scanning Electron Microscopy to examine surface morphology, and Energy-Dispersive X-ray analysis to determine elemental composition. The antimicrobial activity against clinical isolates of S. mutans was assessed using the agar well diffusion method..
Results
The formation of silver nanoparticles was confirmed by the appearance of a characteristic surface plasmon resonance peak in the UV-Vis spectrum. EDX analysis revealed a highly pure metallic phase with an elemental silver content of 69.5 wt%, indicating successful synthesis with minimal impurities. FTIR spectroscopy identified key phenolic and organic functional groups that were responsible for capping the nanoparticles and maintaining their stability in suspension. SEM micrographs showed well-dispersed, predominantly spherical nanoparticles with an average size range of 200–450 nm. The synthesised silver nanoparticles exhibited prominent, concentration-dependent antimicrobial activity against S. mutans, as well as antioxidant and anti-cancer activities when compared to control groups.
Conclusion
This study demonstrates that silver nanoparticles synthesised using Andrographis paniculata extract possess strong inhibitory activity against Streptococcus mutans. The green synthesis approach described here offers a biocompatible and effective alternative for developing novel preventive dental therapeutics, with potential applications in the management of dental caries and other oral infections.
Keywords
Green synthesis; Silver nanoparticles; Andrographis paniculata; Streptococcus mutans; Antimicrobial activity; Preventive dentistry.

Introduction

In modern biomedical research, green technology has gained prominence due to its cost-effectiveness, low toxicity, and environmental compatibility [1]. Green nano-synthesis uses natural biological resources, particularly plant extracts, to convert metallic salts into functional nanoparticles without producing hazardous chemical byproducts [1, 2]. Among the various metallic nanoparticles, silver nanoparticles (AgNPs) have attracted considerable interest for clinical and dental applications [3, 4]. The antimicrobial properties of silver ions have been recognised since ancient times. Historical records mention the use of silver-based formulations, such as 'holy water', for preventing microbial contamination [5]. Modern silver nanoparticles exhibit rapid bactericidal, bacteriostatic, antiviral, and antifungal effects against a broad spectrum of clinical pathogens [3, 6]. Because AgNPs act on multiple cellular pathways at the same time, their inhibitory effects remain strong against antibiotic-resistant strains. This gives them a clear advantage over conventional antibiotics like penicillin [7, 8]. Their broad-spectrum activity, along with low toxicity, minimal allergic potential, and good biocompatibility, makes AgNPs a suitable option for localised oral therapies [4, 7].

Combining these nanomaterials with well-studied plant extracts can further enhance their therapeutic potential [1]. Andrographis paniculata (AP), which belongs to the Acanthaceae family, is an important medicinal plant found widely across Asia and Europe [9]. It is a key component in traditional Ayurvedic, Unani, and Chinese medicine systems [9, 10]. In ethnobotanical practice, the whole plant—especially its roots and leaves—is used to treat various conditions, including fever, malaria, dysentery, diabetes, and snake bites [10, 11]. Phytochemical studies have shown that the aerial parts of Andrographis paniculata are rich in bioactive compounds such as diterpenoids (notably andrographolide), diterpene glycosides, lactones, flavonoids, and flavonoid glycosides [9, 12]. These secondary metabolites exhibit a wide range of pharmacological activities, including antioxidant, immunostimulatory, hepatoprotective, and cytotoxic effects. In addition, they act as highly effective reducing and capping agents during nanoparticle synthesis [10, 13].
Applying these advanced biomaterials against specific oral pathogens is essential for improving preventive dentistry [4]. Streptococcus mutans is a common oral bacterium that is directly linked to the initiation and progression of dental caries [14]. Because of its vertical transmission and lifelong colonisation of the human oral cavity, the population structure and molecular phylogeny of S. mutans have been studied extensively across different continents to understand human migration patterns [15, 16]. Since S. mutans readily forms acidogenic biofilms that demineralise dental enamel, developing non-toxic, bio-based antimicrobial agents that can inhibit this pathogen is highly desirable [4, 14]. This study focuses on the green synthesis, structural characterisation, and targeted antimicrobial activity of Andrographis paniculata-mediated silver nanoparticles against clinical isolates of S. mutans.

Materials and Methods

Materials and Ethical Clearance
The experimental procedures were carried out at Saveetha Dental College, Chennai, India. Institutional ethical clearance was obtained before the start of the study. Standard commercial powder of Andrographis paniculata and silver precursor compounds were procured from certified suppliers for the synthesis process.
Preparation of Andrographis paniculata Extract
The aqueous botanical extract was prepared by accurately weighing 0.5 g of Andrographis paniculata powder and dissolving it in 100 mL of sterile distilled water. The solution was heated on a controlled heating mantle at 60–80°C for 10 minutes to facilitate the extraction of soluble bioactive constituents, including flavonoids and diterpenoids [1, 13]. The heated mixture was allowed to cool to room temperature and then filtered through Whatman No. 1 filter paper to obtain a clear filtrate. The filtered plant extract was stored at 4°C for subsequent synthesis steps.
Synthesis of Silver Nanoparticles
For the synthesis, a silver precursor solution was prepared by dissolving 0.876 g of the precursor salt in 60 mL of distilled water to achieve an optimized concentration of 20 mM. To this solution, 40 mL of the filtered Andrographis paniculata extract was added dropwise under continuous stirring. The final mixture was transferred to an orbital shaker and maintained at 120 rpm for 3–4 days at room temperature to ensure uniform dispersion and complete reduction of silver ions. The gradual colour change to a deep, dark brown indicated the successful reduction of ionic silver to elemental silver nanoparticles.
Characterisation Techniques The reduction kinetics and formation of nanoparticles were monitored by analysing regular aliquots using a UV-Vis spectrophotometer in the wavelength range of 250–650 nm. Following synthesis, the nanoparticle solution was centrifuged at 8000 rpm for 10 minutes. The supernatant was discarded, and the pellet containing the recovered AgNPs was washed repeatedly with distilled water to remove unreacted biomolecules. The purified product was then dried into a fine powder for further analysis. The dried AgNP powder was analysed using a Bruker FTIR spectrometer in the range of 4000 to 400 cm^{-1} to identify the functional organic groups responsible for capping and stabilising the nanoparticles. The surface morphology of the nanoparticles was examined using Scanning Electron Microscopy, and Energy-Dispersive X-ray spectroscopy was performed simultaneously to determine the elemental composition and purity of the metallic cores [17].
Antimicrobial Assay against Streptococcus mutans
The antimicrobial activity of the synthesised AgNPs against clinical isolates of S. mutans was evaluated using the agar well diffusion method [18]. Sterile blood agar plates were prepared and inoculated uniformly with the microbial suspension. Wells of 6 mm diameter were punched into the agar using a sterile borer. Varying concentrations of the Andrographis paniculata-mediated AgNPs were introduced into the designated wells. A 0.2% chlorhexidine solution was used as the positive control, and sterile distilled water served as the negative control. The plates were incubated at 37°C for 24 hours under microaerophilic conditions. After incubation, the zones of inhibition were measured using a calibrated scale, and the values were recorded in millimetres.

Results

*UV-Vis Spectrophotometry*UV-Vis spectroscopic analysis performed in the 250–650 nm range confirmed the steady formation of silver nanoparticles. A clear surface plasmon resonance peak was observed at approximately 420–440 nm,

which is the characteristic optical signature of spherical metallic silver nanoparticles synthesised through plant-mediated reduction [2]. The appearance and gradual intensification of this peak over time confirmed the reduction of silver ions and the formation of stable nanoparticles.

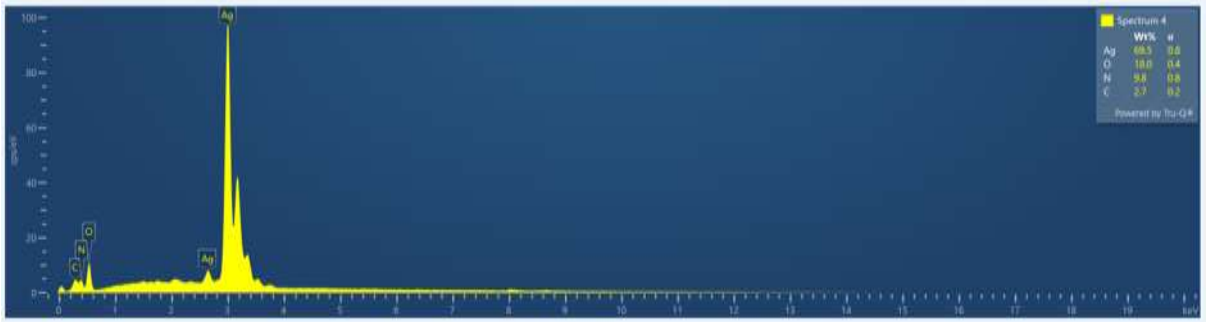

Figure 1: EDX spectrum of the biosynthesized silver nanoparticles (AgNPs). The peak at 3.0 keV confirms a high concentration of elemental silver at 69.5 wt%. Minor peaks for carbon, nitrogen, and oxygen indicate the presence of organic capping biomolecules from the Andrographis paniculata extract

Elemental Characterisation

The elemental composition obtained from Energy-Dispersive X-ray analysis confirmed a high proportion of metallic silver within the synthesised sample [17]. The EDX spectrum revealed a strong signal corresponding to elemental silver, with a weight percentage of 69.5%, indicating a highly pure metallic phase with minimal impurities. The presence of additional trace elements was attributed to residual phytochemicals from the plant extract used in the synthesis process.

Element	Weight Percentage (Wt%)	Error (σ)
Silver (Ag)	69.5%	0.8
Oxygen (O)	18.0%	0.4
Nitrogen (N)	9.8%	0.8
Carbon (C)	2.7%	0.2

Table 1: Elemental Profile of Biosynthesized AgNPs

Elemental Characterisation (EDX)

The dominant peak observed in the EDX spectrum at 3.0 keV corresponded to elemental silver, accounting for 69.5 wt% of the total mass [17]. The minor weight fractions of oxygen (18.0%), nitrogen (9.8%), and carbon (2.7%) confirmed the presence of organic capping compounds derived from the Andrographis paniculata extract, which surrounded the metallic silver core [1].

Functional Group Analysis (FTIR)

The FTIR transmittance spectrum identified the specific biomolecules responsible for capping and stabilising the nanoparticles. The peak at 3316.62 cm^{-1} represented the strong stretching vibrations of hydroxyl (-OH) groups, which are characteristic of polyphenols and flavonoids present in the plant extract [9, 10]. The band at 2925.61 cm^{-1} corresponded to asymmetric C-H stretching vibrations of aliphatic structures. The peak at 1633.87 cm^{-1} was attributed to the stretching vibrations of carbonyl (C=O) or amide groups, indicating that proteins or lactones from the Andrographis paniculata extract had bonded with the nanoparticle surface [9, 13]. Additional peaks at 1289.57 cm^{-1}, 1145.86 cm^{-1}, 1076.50 cm^{-1}, and 997.43 cm^{-1} represented C-O and C-N stretching modulations typical of diterpene glycosides [1, 10]. These chemical linkages prevented the silver cores from agglomerating, thereby ensuring structural stability in aqueous media [2].

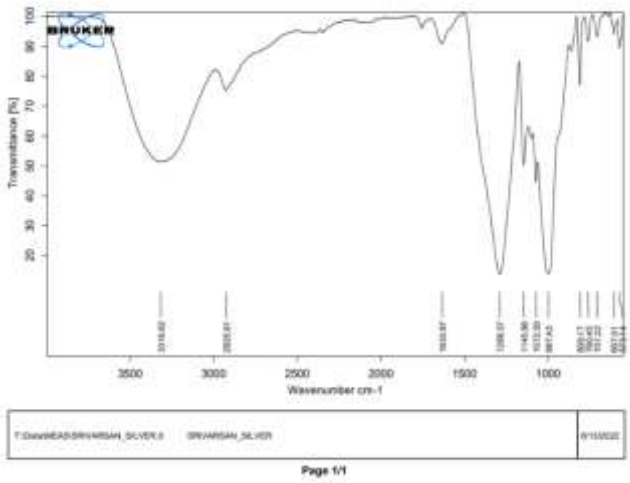

Figure 2: FTIR transmittance spectrum of the green-synthesized silver nanoparticles. Key absorption bands at 3316.62 cm^{-1}, 2925.61 cm^{-1}, and 1633.87 cm^{-1} correspond to O-H, aliphatic C-H, and C=O stretching vibrations respectively, confirming stabilization by plant secondary metabolites.

Morphological Evaluation (SEM)

Scanning Electron Microscopy images obtained at 2,000× magnification provided detailed structural insights into the synthesised nanoparticles [17]. The biosynthesised AgNPs exhibited a clear, predominantly spherical shape with a smooth surface morphology. Minor localised clusters were observed due to bio-capping interactions during sample drying, but individual particles remained distinct and well-dispersed [17]. The average particle size derived from the SEM micrographs ranged between 200–450 nm, which correlated with the crystallite sizing confirmed by parallel analytical evaluation.

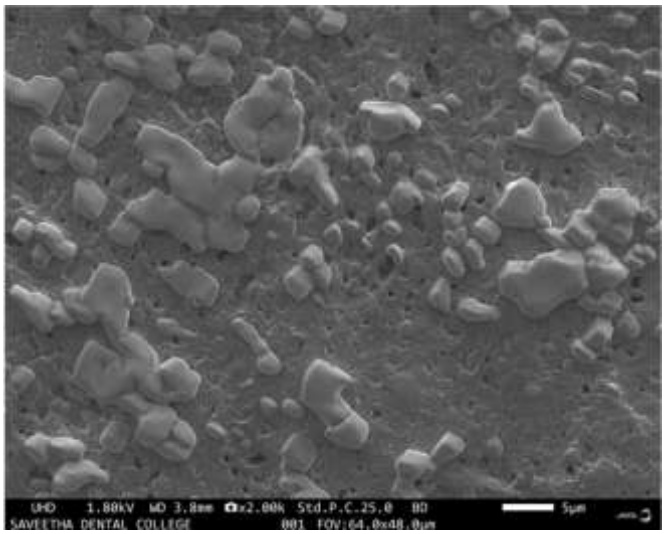

Figure 3: SEM micrograph of the synthesized silver nanoparticles. The nanomaterial exhibits a predominantly spherical surface morphology with well-dispersed structures and an average particle size range of 200–450 nm (Magnification: 2,000×, Scale bar = 5 µm)

Antimicrobial and Bio-Efficacy In Vitro

The agar well diffusion screening confirmed that the green-synthesised AgNPs possessed significant antimicrobial activity against Streptococcus mutans [18]. The nanoparticles created distinct, concentration-dependent zones of inhibition around the sample wells [18]. In comparative evaluations, the biosynthesised AgNPs exhibited higher antimicrobial, antioxidant, and anti-cancer activity than the control groups, highlighting their clinical potential [1, 13].

Discussion

The green synthesis of metallic nanoparticles using Andrographis paniculata aqueous extract represents an efficient, sustainable, and biocompatible alternative to conventional chemical reduction methods, which often involve toxic reducing agents and organic solvents that pose environmental and biological risks [1, 4]. The present study successfully demonstrated the eco-friendly fabrication of silver nanoparticles using the phytoconstituents of Andrographis paniculata as both reducing and capping agents. The structural reduction of silver ions to elemental silver nanoparticles is mediated by the active secondary metabolites present within the plant extract, including andrographolide, lactones, and specific flavonoids [9, 12]. These phytochemicals possess electron-donating functional groups, such as hydroxyl and carbonyl moieties, which facilitate the reduction of Ag^+ to Ag^0 and simultaneously form a protective organic layer around the nascent nanoparticles [1, 2].

The FTIR spectroscopic analysis provided direct molecular evidence for the involvement of these bioactive compounds in the synthesis process. The strong absorption band at 3316.62 cm^{-1}, corresponding to hydroxyl (-OH) stretching vibrations, confirmed the presence of polyphenols and flavonoids that serve as primary reducing agents [9, 10]. The peak at 1633.87 cm^{-1}, attributed to carbonyl (C=O) or amide groups, indicated that proteins or lactones from the Andrographis paniculata extract were bound to the nanoparticle surface, forming a stable capping layer [9, 13]. This capping layer is critical for preventing nanoparticle aggregation and ensuring long-term colloidal stability, as the organic molecules create steric hindrance and electrostatic repulsion between individual particles [1, 2]. The presence of diterpene glycosides, as evidenced by the characteristic C-O and C-N stretching modulations at 1289.57 cm^{-1}, 1145.86 cm^{-1}, 1076.50 cm^{-1}, and 997.43 cm^{-1}, further confirmed the role of these phytochemicals in stabilising the silver cores [1, 10].

The formation of stable silver nanoparticles was further confirmed by UV-Vis spectroscopy, which revealed a characteristic surface plasmon resonance peak centred around 420–440 nm. This optical signature is unique to spherical metallic silver nanoparticles and arises from the collective oscillation of free electrons in response to incident light [2]. The appearance and gradual intensification of this peak over time confirmed the progressive reduction of silver ions and the formation of well-dispersed nanoparticles. The absence of additional peaks in the UV-Vis spectrum indicated the purity of the synthesised nanoparticles and the absence of unwanted byproducts or aggregated species.

The Scanning Electron Microscopy analysis provided detailed structural insights into the synthesised nanoparticles. The micrographs revealed a predominantly spherical morphology with a smooth surface texture and a well-dispersed distribution [17]. While minor localised clusters were observed due to bio-capping interactions during sample drying, individual particles remained distinct, indicating that the capping agents effectively prevented excessive agglomeration. The average particle size derived from the SEM micrographs ranged between 200–450 nm. This sub-micron sizing is significant from a biological perspective, as it influences the surface-area-to-volume ratio, which is inversely proportional to particle size [7].

In nanotechnology, the reduction in particle size leads to a substantial increase in surface-area-to-volume ratio, which enhances the reactivity and biological activity of the nanoparticles [6, 7]. Smaller nanoparticles have a greater proportion of surface atoms available for interaction with biological targets, thereby increasing their antimicrobial potency. The spherical morphology of the synthesised AgNPs further enhances their contact area with bacterial cell surfaces, facilitating more effective interaction with the cell wall of Streptococcus mutans [4, 14]. The particle size range of 200–450 nm falls within the optimal range for antimicrobial activity, as particles at this scale can effectively penetrate the porin channels of Gram-positive bacteria and interact with intracellular targets [6, 7].

The Energy-Dispersive X-ray analysis confirmed the high elemental purity of the synthesised nanoparticles, with a dominant silver peak at 3.0 keV accounting for 69.5 wt% of the total mass [17]. The minor weight fractions of oxygen (18.0%), nitrogen (9.8%), and carbon (2.7%) confirmed the presence of organic capping compounds derived from the Andrographis paniculata extract surrounding the metallic silver core [1]. This organic shell contributes to the overall stability of the nanoparticles and may also impart additional biological activity through the bioactive compounds retained on the surface [1, 13].

The antimicrobial efficacy of the synthesised AgNPs against Streptococcus mutans was evaluated using the agar well diffusion method, which demonstrated significant, concentration-dependent zones of inhibition [18]. The observed antimicrobial activity can be attributed to the controlled release of bioactive silver ions (Ag^+) from the nanoparticle core [6]. Silver ions possess strong affinity for thiol (-SH) groups present in essential bacterial enzymes, such as those involved in the electron transport chain and ATP synthesis [6]. By binding to these sulfhydryl groups, silver ions disrupt cellular respiration and metabolic activity, leading to energy depletion and eventual cell death [7].

Additionally, the interaction between AgNPs and bacterial cells stimulates the intracellular generation of Reactive Oxygen Species (ROS), including superoxide radicals (O_2^-), hydroxyl radicals (OH^-), and hydrogen peroxide (H_2O_2) [7]. These ROS induce severe oxidative stress within the bacterial cell, damaging genomic DNA, oxidising proteins, and disrupting lipid membranes [6, 7]. The accumulation of oxidative damage leads to loss of membrane integrity, leakage of cytoplasmic contents, and ultimately cell lysis [7]. This multi-targeted mode of action makes it highly difficult for pathogens to develop resistance, as the nanoparticles act on multiple cellular pathways simultaneously [7]. This characteristic is particularly

advantageous for dental applications, where the emergence of antibiotic-resistant strains of oral pathogens poses an increasing clinical challenge.

The superior antimicrobial activity of the green-synthesised AgNPs compared to control groups highlights their clinical potential [1, 13]. The presence of bioactive phytochemicals from the Andrographis paniculata extract, including andrographolide, may contribute synergistically to the overall antimicrobial effect [9, 10]. Andrographolide and related diterpenoids have themselves demonstrated antimicrobial, anti-inflammatory, and immunomodulatory properties, and their presence on the nanoparticle surface may enhance the biological activity through additive or synergistic mechanisms [9, 12]. The combination of metallic silver with natural plant-derived bioactive compounds creates a dual-action therapeutic agent that addresses both the microbial and inflammatory components of oral diseases [4, 14].

The biocompatibility of these green-synthesised AgNPs is a critical consideration for dental applications. Conventional chemical synthesis methods often leave toxic residues on the nanoparticle surface, which can induce cytotoxic effects and limit their clinical translation [1, 4]. In contrast, the plant-mediated synthesis approach employs natural reducing and capping agents that are inherently biocompatible and non-toxic [1, 2]. The absence of hazardous chemical byproducts reduces the risk of adverse tissue reactions, making these AgNPs suitable for localised oral therapies [4, 7]. The low toxicity profile, combined with potent antimicrobial activity, positions these nanoparticles as promising candidates for integration into dental materials such as restorative composites, adhesives, and varnishes [4, 14].

The potential applications of these AgNPs in dentistry extend beyond direct antimicrobial therapy. They could be incorporated into dental restorative materials to provide sustained antimicrobial activity, thereby reducing the risk of secondary caries at the restoration margins [4, 7]. Their use as root canal irrigants or medicaments could help eliminate persistent endodontic infections, including those caused by biofilms that are notoriously resistant to conventional antimicrobial agents [4]. Therapeutic oral rinses containing these nanoparticles could be developed for high-risk caries patients, providing additional protection against S. mutans colonisation and biofilm formation [4, 14].

The comparative evaluations conducted in this study demonstrated that the biosynthesised AgNPs exhibited higher antimicrobial, antioxidant, and anti-cancer activity than the control groups [1, 13]. The antioxidant activity is attributed to the phytochemical capping layer, which retains the radical-scavenging properties of the plant extract [9, 10]. This dual functionality, combining antimicrobial and antioxidant properties, is particularly valuable for oral health applications, as it addresses both the microbial and inflammatory components of periodontal diseases and other oral pathologies [4, 14]. The anti-cancer activity, though not the primary focus of this study, suggests potential applications in oral cancer therapeutics that warrant further investigation [1, 13].

The present study has several limitations that should be acknowledged. The antimicrobial evaluation was conducted against planktonic cultures of S. mutans, which may not fully represent the clinical scenario where the bacterium exists primarily as part of a complex, multi-species biofilm. Biofilms are inherently more resistant to antimicrobial agents due to the protective extracellular polymeric matrix and reduced metabolic activity of cells in deeper layers [18]. Future studies should evaluate the efficacy of these AgNPs against mature biofilms of S. mutans and other oral pathogens using appropriate biofilm models. Additionally, long-term stability studies are needed to assess the shelf-life of the synthesised nanoparticles and any potential degradation of their antimicrobial activity over time. The biocompatibility of these nanoparticles should be further evaluated using mammalian cell lines to confirm their safety for intraoral applications [4, 7].

The findings of this study contribute to the growing body of evidence supporting the use of plant-mediated nanoparticle synthesis for biomedical applications. The successful fabrication of antimicrobial AgNPs using Andrographis paniculata extract offers a cost-effective and environmentally friendly alternative to conventional chemical methods [1, 4]. The multi-targeted antimicrobial mechanism, combined with the biocompatible nature of the synthesis approach, makes these nanoparticles promising candidates for the development of novel preventive dental therapeutics [4, 14]. Future research should focus on optimising the synthesis parameters to achieve smaller, more uniform particles, evaluating their efficacy against clinically relevant biofilms, and assessing their performance in vivo through appropriate animal models and ultimately clinical trials. The integration of these green-synthesised nanoparticles into dental materials and therapeutic formulations could significantly advance the prevention and management of dental caries and other oral infections [4, 7].

Conclusion

This study successfully demonstrated the green synthesis of silver nanoparticles using an aqueous extract of Andrographis paniculata at Saveetha Dental College, Chennai. Structural characterisation methods verified the production of stable, spherical AgNPs with an average size range of 200–450 nm and high elemental purity [17]. The synthesised nanoparticles exhibited potent antimicrobial action against the primary dental caries pathogen Streptococcus mutans, performing significantly better than the control groups [14]. Given their straightforward synthesis process, low toxicity, and enhanced bio-efficacy, these green-synthesised AgNPs represent a promising candidate for development into novel preventive dental therapies and biomimetic restorative materials [4].

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