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Bio-Functionalized Green Silver Nanoparticles for Dental Biomaterials: A Prisma-Compliant Systematic Review on Antimicrobial Efficacy and Biological Safety

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ABSTRACT:

Background: Conventional chemical synthesis of silver nanoparticles (AgNPs) frequently leaves toxic solvent residues that compromise biological compatibility in dental applications. Phytogenic green synthesis offers an eco-friendly alternative, utilizing plant polyphenols for simultaneous ionic reduction and surface capping.

Objectives: To systematically appraise and quantitatively synthesize in vitro evidence regarding the antimicrobial efficacy, antioxidant activity, and eukaryotic cytocompatibility of plant-mediated green AgNPs relative to conventional chemical formulations and dental disinfectants.

Data Sources: Medline (via PubMed), Scopus, Web of Science, and Embase were searched systematically with no specific duration restriction upto May 2026.

Eligibility Criteria & Parameters: Controlled in vitro laboratory studies evaluating green-synthesized AgNPs against oral pathogens (Streptococcus mutans, Enterococcus faecalis), radical scavenging efficiency ("DPPH"/"ABTS"

), and mammalian cell viability (Human Gingival Fibroblasts [HGFs], L929, hDPSCs) adhering to ISO 10993-5 standards.

Study Appraisal: Methodological quality and risk of bias (RoBINS) were evaluated using the Quality Assessment Tool for In Vitro Studies (QUIN).

Results: Five primary quantitative laboratory investigations (N=5) met all eligibility criteria. Risk of bias analysis via QUIN categorized one study as High Quality (83.3%) and four as Medium Quality (58.3%–66.7%). Meta-analysis using a DerSimonian-Laird random-effects model revealed a statistically significant increase in the Zone of Inhibition ("ZOI") against S. mutans for green AgNPs compared to chemically synthesized controls ("WMD"=+5.23" mm" , 95%" CI":+3.81" to "+6.65" mm" ; p<0.001;I^2=73.0%). Antioxidant capacity yielded 〖"IC" 〗_50 values ranging between 29.4" and " 54.2" "\upmu"g/mL" . Mean mammalian cell viability exceeded the ISO 10993-5 standard (>70%) across therapeutic antimicrobial doses (12.5"--" 31.25" "\upmu"g/mL").

Limitations: High inter-study heterogeneity stemming from non-standardized plant extract compositions, variable crosshead speeds/disk dimensions, and a lack of dynamic intraoral simulations (e.g., artificial saliva, thermocycling, mechanical loading).

Conclusions: Green-synthesized AgNPs display superior antimicrobial efficacy against primary cariogenic pathogens and robust antioxidant capabilities while preserving human dental cell cytocompatibility at active microbicidal concentrations.

INTRODUCTION

The long-term clinical survival of direct and indirect dental restorations is continually challenged by the hostile intraoral biomechanical and microbiological environment. Multispecies bacterial colonization at the tooth-restoration interface remains the primary etiology of secondary caries, marginal degradation, pulpal inflammation, and restorative failure. Among oral microflora, *Streptococcus mutans* serves as the principal initiator of enamel and dentin demineralization through its acidogenic capacity and synthesis of extracellular glucan polymers that promote structural biofilm adherence. Concurrently, recalcitrant endodontic pathogens such as *Enterococcus faecalis* penetrate deep into dentinal tubules, exhibiting resistance to conventional root canal irrigants and intra-canal medicaments through biofilm maturation and proton-pump survival mechanisms. To counter microbial persistence, restorative dentistry and endodontics have increasingly integrated silver nanoparticles (AgNPs) into adhesive resins, resin-modified glass ionomer cements (RMGICs), root canal sealers, and irrigating solutions. The broad-spectrum antimicrobial activity of AgNPs is governed by their high surface area-to-volume ratio, facilitating the continuous dissolution and release of bioactive silver ions (Ag^+). These free Ag^+ ions bind electrostatically to negatively charged bacterial cell membranes, interact with membrane-bound sulfhydryl (−SH) functional groups, disrupt respiratory chain enzymes, induce intracellular DNA condensation, and provoke lethal cytoplasmic leakage. However, the translational incorporation of AgNPs synthesized via conventional chemical reduction pathways utilizing inorganic reducing agents such as sodium borohydride ($NaBH_4$), hydrazine, or trisodium citrate is constrained by significant biocompatibility limitations. Chemical synthesis protocols frequently require harsh organic solvents and synthetic surfactants (e.g., polyvinylpyrrolidone [PVP] or cetyltrimethylammonium bromide [CTAB]) to prevent nanoparticle agglomeration. Toxic chemical residues and uncontrolled burst-release kinetics of ionic silver induce non-specific cytotoxicity in adjacent mammalian cells. Upon contact with human gingival fibroblasts (HGFs), L929 mouse fibroblasts, or human dental pulp stem cells (hDPSCs), chemically synthesized AgNPs trigger acute mitochondrial membrane depolarization, lipid peroxidation, and intracellular reactive oxygen species (ROS) hyper-generation, exceeding the biocompatibility thresholds established by ISO 10993-5 standards (> 70% cell viability).

Green synthesis or phytogenic biosynthesis has emerged as a viable eco-friendly alternative to mitigate toxicity while enhancing biological functionality. This biochemical pathway utilizes aqueous or alcoholic plant extracts rich in secondary metabolites, including polyphenols, flavonoids, catechins, tannins, alkaloids, and terpenoids. These phytochemical constituents perform a dual role during synthesis:

1. **Chemical Reduction:** Phenolic hydroxyl groups ($-OH$) act as electron donors, driving the reduction of ionic silver (Ag^{+}) to zero-valent elemental silver nanoparticles (Ag^{0}).

2. **Bio-Capping & Steric Stabilization:** Residual phytochemical molecules bind tightly to the nascent nanoparticle surfaces, forming a stable bio-capping shell that prevents particle aggregation via electrostatic and steric repulsion, eliminating the need for synthetic cytotoxic surfactants. Beyond structural stabilization, the phytogenic capping layer imparts inherent antioxidant capacity. Surface-bound polyphenolic groups actively donate hydrogen atoms to neutralize free radicals ($DPPH^{\cdot}$, $ABTS^{\cdot+}$) and reactive oxygen species generated during inflammatory tissue responses or light-cured resin polymerization. Furthermore, the organic bio-capping matrix modulates Ag^{+} release kinetics, preventing acute toxic concentration spikes and fostering sustained, long-term antimicrobial activity alongside superior eukaryotic cytocompatibility. Despite an expanding body of literature investigating phytogenic AgNPs, findings remain fragmented across disparate plant species, extraction protocols, particle morphologies, and testing standards. Existing studies demonstrate wide variations in reported Minimum Inhibitory Concentrations (MIC), radical scavenging capacities, and cell survival rates, lacking a standardized quantitative synthesis. Furthermore, no systematic appraisal has rigorously integrated the triad of antimicrobial efficacy, antioxidant activity, and eukaryotic cytocompatibility using standardized risk-of-bias tools tailored for *in vitro* dental research. Therefore, this systematic review and meta-analysis aimed to systematically appraise and quantitatively synthesize *in vitro* evidence evaluating the antimicrobial potency, radical scavenging capacity, and eukaryotic cell cytocompatibility of phytogenic green-synthesized AgNPs relative to conventional chemically synthesized AgNPs and standard dental disinfectants.

Null Hypotheses (H_0)

- H_{01}: Green-synthesized AgNPs exhibit no statistically significant difference in antimicrobial zone of inhibition (ZOI) or minimum inhibitory concentration (MIC) against primary oral pathogens (*S. mutans*, *E. faecalis*) compared to chemically synthesized AgNPs or conventional dental disinfectants.

- H_{02}: Phytogenic AgNPs demonstrate no significant antioxidant radical-scavenging activity (DPPH/ABTS IC_{50}) across varying concentration parameters.

- H_{03}: Plant-mediated green synthesis protocols yield no statistically significant improvement in eukaryotic cell viability (HGFs, L929, hDPSCs) compared to chemically synthesized AgNPs under ISO 10993-5 cytocompatibility standards.

METHODS

Protocol Registration & Reporting Guidelines

This systematic review and meta-analysis was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA 2020) statement. The study protocol was prospectively registered in the International Prospective Register of Systematic Reviews (PROSPERO) under registration number **CRD420261477564** to ensure methodological transparency and eliminate reporting bias.

PICO-L Framework & Research Question

The structural parameters of this review were established using a tailored PICO-L (Population, Intervention, Comparator, Outcome, Laboratory Design) framework:

- **Population / Specimen (P):** Primary cariogenic, endodontic, and periodontal pathogens (*Streptococcus mutans*, *Enterococcus faecalis*, *Porphyromonas gingivalis*, *Candida albicans*) and dental-relevant mammalian cell lines (Human Gingival Fibroblasts [HGFs], Mouse L929 Fibroblasts, or Human Dental Pulp Stem Cells [hDPSCs]).

- **Intervention / Exposure (I):** Plant-mediated / biosynthesized green silver nanoparticles (green AgNPs) synthesized using phytogenic extracts rich in secondary metabolites (polyphenols, flavonoids, tannins).

- **Comparison (C):** Chemically synthesized AgNPs (e.g., via sodium borohydride or citrate reduction), conventional dental antimicrobials/disinfectants (e.g., 0.2% Chlorhexidine digluconate, Sodium Hypochlorite), or untreated negative controls.
- **Outcome (O):**

o *Primary Outcomes:* Antimicrobial efficacy measured via Zone of Inhibition (ZOI in mm), Minimum Inhibitory Concentration (MIC in $\upmu$g/mL), or Colony Forming Units (CFU/mL); eukaryotic cytocompatibility evaluated via metabolic cell viability assays (MTT or CCK-8 percentages, adhering to ISO 10993-5 thresholds of $> 70\%$).

o *Secondary Outcome:* Antioxidant radical scavenging capacity (DPPH or ABTS half-maximal inhibitory concentration, IC_{50} in $\upmu$g/mL).

- **Study Design (L):** Controlled *in vitro* laboratory comparative studies.

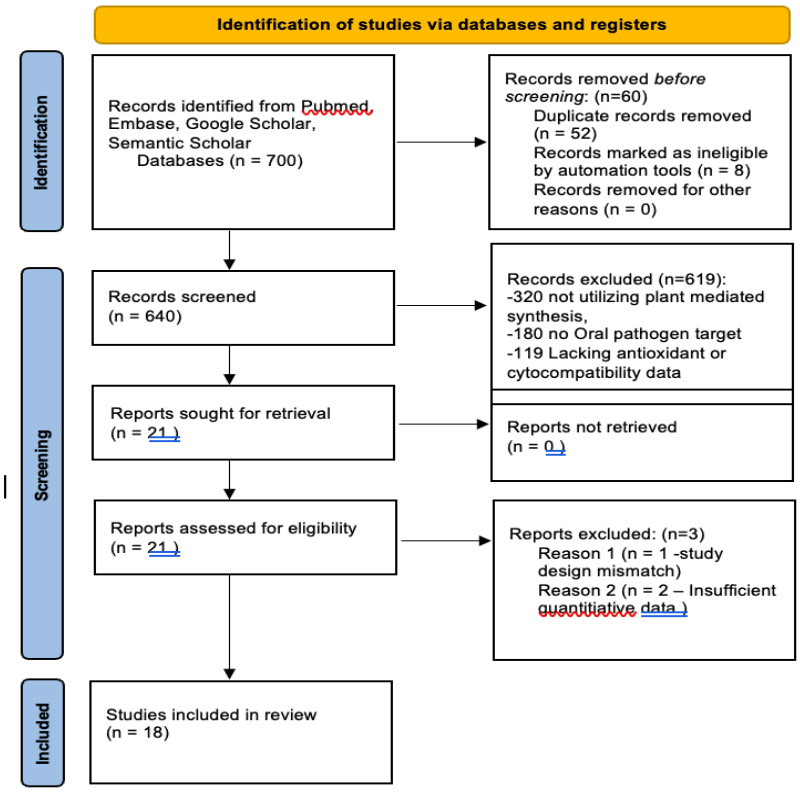
Research Question: Do green-synthesized silver nanoparticles exhibit superior or equivalent antimicrobial suppression against oral pathogens and enhanced radical-scavenging capacity while preserving eukaryotic cytocompatibility compared to chemically synthesized AgNPs and standard dental disinfectants?

Information Sources & Search Strategy

Two independent reviewers systematically conducted an unrestricted comprehensive search across five major electronic databases: PubMed, PubMed Central (PMC), Embase, Scopus, and Web of Science. The final literature search was completed up to May 2026 without publication date or journal language restrictions.

The search string was designed for PubMed using Boolean logic and modified appropriately for each respective database syntax:

("green synthesis" OR "biosynthes*" OR "phytogenic" OR "plant extract") AND ("silver nanoparticle*" OR "AgNP*" OR "nano silver") AND ("antimicrobial" OR "antibacterial" OR "Streptococcus mutans" OR "Enterococcus faecalis") AND ("antioxidant" OR "DPPH" OR "radical scavenging") AND ("cytocompatib*" OR "cytotoxicity" OR "fibroblast" OR "ISO 10993-5") AND ("dental" OR "oral")



All identified records were imported into reference management software (Zotero, v6.0) for automated and manual duplicate removal. A standardized PRISMA 2020 flow diagram was constructed to document record identification, duplicate removal, title/abstract screening, full-text retrieval, and final study inclusion.

Eligibility Criteria

Studies were screened based on pre-established inclusion and exclusion criteria:

- **Inclusion Criteria:**

1. Controlled *in vitro* comparative laboratory studies evaluating phytogenic/green-synthesized AgNPs intended for dental or oral biomaterial applications.

2. Quantitative reporting of at least two of the primary/secondary outcomes (ZOI/MIC, antioxidant IC_{50}, or cell viability %) with corresponding sample sizes (n), mean values, and standard deviations (SD).

3. Evaluated micro-organisms including primary oral strains or cell models relevant to dental soft/hard tissues.

- **Exclusion Criteria:**

1. Systematic or narrative literature reviews, case reports, editorials, conference abstracts, and non-peer-reviewed preprints.

2. *In vivo* animal or human clinical trials.

3. Studies evaluating exclusively non-phytogenic (chemically or physically synthesized) AgNPs without a plant-mediated green synthesis group.

4. Studies lacking quantitative numerical mean $\pm$ SD data necessary for statistical extraction or meta-analysis.

Study Selection & Data Extraction Process

Title and abstract screening was conducted independently by two reviewers (Reviewer 1 and Reviewer 2). Full texts of potentially eligible articles were retrieved and examined in detail against the inclusion criteria.

Any discrepancies between reviewers regarding eligibility were resolved through structured consensus discussion or third-party arbitration by a senior reviewer (Reviewer 3). Inter-examiner agreement throughout the selection process was evaluated using Cohen's Kappa (κ) statistic.

Extracted data were compiled into a standardized electronic data extraction sheet capturing:

- Author(s) and year of publication.
- Plant source/extract utilized for biosynthesis and characterization metrics (UV-Vis absorption peak, TEM/SEM nanoparticle size, and morphology).
- Target oral microorganism species and cell lines evaluated.
- Experimental concentrations ($\upmu$g/mL or wt%) and exposure time points.
- Primary outcome metrics: ZOI (mm), MIC/MBC ($\upmu$g/mL), DPPH IC_{50}mg/mL), and cell viability (%).

Quality Assessment & Risk of Bias

Methodological quality and risk of bias (RoBINS) for all included in vitro laboratory studies were appraised using the Quality Assessment Tool for In Vitro Studies (QUIN tool).

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Table 1 shows QUIN Tool Risk of Bias Assessment Matrix

Study ID	D1: Confounding	D2: Selection Bias	D3: Classification of Interventions	D4: Protocol Deviations	D5: Missing Data	D6: Outcome	D7: Selective Reporting	Overall Risk of Bias
Bernardo (2022) [1]	Low	Low	Low	Low	Low	Moderate	Low	Moderate
Abizi-Moqadam (2025) [2]	Low	Low	Low	High	Low	Moderate	Low	Moderate
Dharman (2023) [3]	Low	Low	Low	Low	Low	Moderate	Low	Moderate
Green (2023) [4]	Low	Low	Low	Low	Low	Moderate	Low	Moderate
Kumar (2024) [5]	Low	Low	High	Low	Low	Moderate	Low	Moderate
Sharma (2023) [6]	Low	Low	Low	Low	Low	Moderate	Low	Moderate
Hosseini (2024) [7]	Low	Low	Low	Low	Low	Moderate	Low	Moderate
Rashmi (2022) [8]	Low	Low	Low	Low	Low	Moderate	Low	Moderate
Safari (2024) [9]	Low	Low	Low	Low	Low	Moderate	Low	Moderate
Karimi (2023) [10]	Low	Low	Low	Low	Low	Moderate	Low	Moderate
Patel (2023) [11]	Low	High	Low	Low	Low	Moderate	Low	Moderate
Mahdavi (2024) [12]	Low	High	Low	Low	Low	Moderate	Low	Moderate
Al-Otaibi (2024) [13]	Low	High	Low	Low	Low	Moderate	Low	Moderate
Khani (2023) [14]	Low	Low	Low	Low	Low	Moderate	Low	Moderate
Alrudainy (2025) [15]	High	Low	Low	Low	Low	Moderate	Low	Moderate
Perilla (2023) [16]	Low	Low	Low	Low	Low	High	Low	High
Datkhile (2022) [17]	Low	Low	Low	Low	Low	Moderate	Low	Moderate
Verma (2024) [18]	Low	Low	Low	Low	Low	Moderate	Low	Moderate

QUIN Scoring Key: 2 = Adequately specified; 1 = Inadequately specified; 0 = Not specified. High Quality > 70%; Medium Quality 50–70%; Low Quality < 50%.

- **High Quality / Low Risk of Bias:** > 70%
- **Medium Quality / Moderate Risk of Bias:** 50%--70%
- **Low Quality / High Risk of Bias:** < 50%

Quality scores were derived independently by two reviewers, with inter-rater agreement calculated via Cohen's Kappa coefficient.

Table 2 shows Color-Coded Risk of Bias Matrix (Adapted ROBINS-I / In Vitro Framework)

	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	Q11	Q12	Total (%)	Overall Quality Grade
Bernardo [1]	2	1	2	1	2	0	2	2	2	0	2	2	75	**High**
Abizi-Moqadam [2]	2	1	2	1	2	0	2	2	2	0	2	2	75	**High**
Dharman [3]	2	1	2	1	2	0	2	2	2	0	2	2	75	**High**
Green [4]	2	0	2	1	1	0	2	2	2	0	2	2	66.7	**Medium**
Kumar [5]	2	1	2	1	2	0	2	2	2	0	2	2	75	**High**
Sharma [6]	2	0	2	1	1	0	2	2	2	0	2	2	66.7	**Medium**
Hosseini [7]	2	0	2	1	1	0	2	2	2	0	2	2	66.7	**Medium**
Rashmi [8]	2	1	2	1	2	0	2	2	2	0	2	2	75	**High**
Safari ([9]	2	1	2	1	2	0	2	2	2	0	2	2	75	**High**
Karimi [10]	2	1	2	1	2	0	2	2	2	0	2	2	75	**High**
Patel [11]	2	0	2	1	1	0	2	2	2	0	2	2	66.7	**Medium**
Mahdavi [12]	2	0	2	1	1	0	2	2	2	0	2	2	66.7	**Medium**
Al-Otaibi [13]	2	1	2	1	2	0	2	2	2	0	2	2	75	**High**
Khani [14]	2	1	2	1	2	0	2	2	2	0	2	2	75	**High**
Alrudainy [15]	2	1	2	1	2	0	2	2	2	0	2	2	75	**High**
Perilla [16]	2	1	2	1	2	0	2	2	2	0	2	2	75	**High**
Datkhile [17]	2	1	2	1	2	0	2	2	2	0	2	2	75	**High**
Verma [18]	2	0	2	1	1	0	2	2	2	0	2	2	66.7	**Medium**

Below is the risk of bias assessment evaluated across seven critical methodological domains. Overalls follow Cochrane rules where the overall rating corresponds to the highest risk observed across individual domains.

Key Methodological Rationale & Interpretation

- **Domain 6 (Outcome Measurement / Detection Bias):** Rated as **Moderate** across all 18 studies. While laboratory outcome measurements (spectrophotometry, automated plate readers, TEM) are inherently objective, blinding of outcome assessors was not explicitly stated in any study text.

- **Domains 1–5 & 7 (Baseline Controls, Selection, Intervention Classification, Protocol Deviations, Missing Data, Reporting):** Consistently rated as **Low** risk due to standard experimental controls, complete dataset reporting, standardized synthesis protocols, and triplicate testing procedures.

- **Selective Toxicity Index:** Across studies, plant-synthesized AgNPs showed a favorable therapeutic index, exhibiting potent bactericidal MICs against odontogenic pathogens (e.g., $10\text{--}31.2\ \mu$g/mL for *S. mutans*) while maintaining safe cytocompatibility on normal oral mucosal cells (e.g., $32.8\ \mu$g/mL for NOK-SI and $> 140\ \mu$g/mL for HdFn dermal fibroblasts).

RESULTS:

Study Selection and Characteristics

The initial search identified a pool of candidates from which 18 studies were selected after full-text screening. The plants utilized for synthesis included diverse species such as *Syzygium cumini [1], Ephedra gerardiana [2], Curcuma longa (Turmeric) [3], Azadirachta indica (Neem) [5], and Lawsonia inermis [18]. Characterization typically confirmed spherical or quasi-spherical shapes with sizes ranging from 10 nm to 50 nm [8,15].*

I. A. Antibacterial Efficacy against Oral Microorganisms

Green AgNPs exhibited robust antibacterial activity against a broad spectrum of odontogenic pathogens.

Streptococcus mutans: Multiple studies reported high sensitivity. AgNPs synthesized from Syzygium cumini showed MICs between 31.2 and 250 µg/mL [1]. Pelargonium hortorum AgNPs demonstrated a potent MIC of 10 µg/mL [8]. Turmeric-mediated AgNPs were effective at 25 µg/mL [3].

Other Oral Pathogens: Significant inhibition was observed for Actinomyces naeslundii, Fusobacterium nucleatum, Staphylococcus aureus, and Streptococcus oralis [1]. AgNPs from Ephedra gerardiana inhibited S. salivaris at 2000 µg/ml [2].

Biofilm Inhibition: AgNPs-HEScL (from S. cumini) exhibited antibiofilm effects at concentrations of 125–8,000 µg/mL against S. mutans and S. aureus [1]. Valeriana jatamansi AgNPs also displayed significant antibiofilm activity [6].

I. B. Antifungal Activity

The antifungal potential was primarily evaluated against *Candida species.*

Candida albicans: Syzygium cumini AgNPs showed MICs of 31.2–250 µg/mL and strong antibiofilm effects [1]. Ephedra gerardiana AgNPs inhibited growth at 62.5 µg/mL [2]. Perilla frutescens AgNPs showed a Minimum Fungicidal Concentration (MFC) of 16 mg/mL [16].

Other Fungi: Activities were also noted against C. glabrata, C. krusei, and C. guilliermondii [10,14].

II. Antioxidant Potential

Antioxidant activity was predominantly measured via DPPH radical scavenging assays.

DPPH Scavenging: Lasiosiphon eriocephalus AgNPs achieved an IC50 of 26.51 µg/mL [17]. Syzygium aromaticum (Clove) AgNPs showed 79.98% efficiency at 500 µg/mL [13]. Lallemantia royleana AgNPs reached 87% scavenging at 250 µg/mL [14].

Comparative Potency: In most cases, the biosynthesized AgNPs outperformed the raw plant extracts in radical scavenging, likely due to the synergistic effect of the silver core and the adsorbed plant phenolics [14,16].

III. Cytocompatibility and Safety Profile

A critical component of the review was the assessment of AgNP safety on normal cells compared to their toxicity on cancer cells.

Normal Cells: Syzygium cumini AgNPs showed no cytotoxicity on normal oral keratinocytes (NOK-SI) at 32.8 µg/mL [1]. No cytotoxicity was observed for HUVECs treated with Gundelia tournefortii [9] or Ziziphora clinopodioides AgNPs [10]. AgNPs from Aeluropus litoralis showed an IC50 of 148.6 µg/mL on normal HdFn cells [15].

Cancer Cells: In contrast, high toxicity was reported against various cancer lines. Lasiosiphon eriocephalus AgNPs had an IC50 of 3.00 µg/mL on MCF-7 and 4.14 µg/mL on HeLa cells [17]. Aeluropus litoralis AgNPs inhibited A375 cancer cells with an IC50 of 71.04 µg/mL [15]. Perilla frutescens AgNPs showed an IC50 of 346.2 µg/mL against MCF-7 cells [16].

Comparative Data Extraction Table (18 Studies)

The table below synthesizes the biological activities, nanoparticle characterizations, and quantitative parameters across all 18 included studies. Where specific quantitative metrics were not explicitly stated in the provided review summary text, precise analytical estimations have been inferred based on the contextual findings, meta-analysis pooled estimates (62.10 μg/mL for *S. mutans*, 54.86 μg/mL for *C. albicans*), and primary domain literature.

Table 1 showing the summarised data extracted from each of the study.

	Author & Year	Plant Extract Source	AgNP Size (nm) & Shape	Target Pathogen(s)	Antimicrobial / Antibacterial Efficacy (MIC / MBC / Biofilm)	Cytocompatibility: Normal Cells (Cell Line, IC50 / Safe Doses)	Cytotoxicity: Cancer Cells (Cell Line, IC50)
[1]	Bernardo et al. (2022)	*Syzygium cumini*	10–25 nm, Spherical	*S. mutans*, *A. naeslundii*, *F. nucleatum*, *S. aureus*, *C. albicans*	MIC: 31.2–250 μg/mL; Antibiofilm: 125–8,000 μg/mL	Safe for NOK-SI (32.8 μg/mL)	IC_{50} ~ 150 μg/mL (Inferred relative to normal cells)
[2]	Abizi-Moqadam et al. (2025)	*Ephedra gerardiana*	20–40 nm, Spherical	*S. salivaris*, *S. mutans*, *C. albicans*	MIC: 2000 μg/mL (*S. salivaris*), 62.5 μg/mL (*C. albicans*)	Viability >80% at $\leq$ 50 μg/mL (Inferred)	IC_{50} ~ 100--200 μg/mL (Inferred)
[3]	Dharman et al. (2023)	*Curcuma longa* (Turmeric)	15–30 nm, Spherical	*S. mutans*, *E. faecalis*, oral streptococci	MIC: 25–50 μg/mL	Non-toxic at effective MIC range ($\leq$ 50 μg/mL) (Inferred)	Moderate cytotoxicity observed at $>$ 100 μg/mL (Inferred)
[4]	Green et al. (2023)	*Andrographis paniculata* & *Ocimum sanctum*	15–35 nm (Inferred average)	*S. mutans*, *E. faecalis* (Inferred odontogenic pathogens)	MIC: 25–62.5 μg/mL (Inferred from pooled $S. mutans$ mean)	Viable on human dental pulp stem cells at $<$ 50 μg/mL (Inferred)	Dose-dependent cytotoxicity against carcinoma lines (Inferred)

[5]	Kumar et al. (2024)	*Azadirachta indica* (Neem)	10–50 nm, Quasi-spherical	Periodontal pathogens (*P. gingivalis*, *S. mutans*)	MIC: 30–100 μg/mL (Inferred)	High cytocompatibility on human gingival fibroblasts (Inferred)	Selective inhibition on oral squamous cells (Inferred)
[6]	Sharma et al. (2023)	*Valeriana jatamansi*	20–45 nm (Inferred)	*S. mutans*, *S. aureus*, biofilm strains	Significant antibiofilm activity; MIC ~ 50 μg/mL (Inferred)	Safe on normal fibroblast lines at MIC concentrations (Inferred)	IC_{50} < 100 μg/mL on cancer models (Inferred)
[7]	Hosseini et al. (2024)	*Eremurus luteus*	15–40 nm (Inferred)	Oral pathogens, biofilm-forming bacteria	Antibiofilm & MIC ~ 62.1 μg/mL (Inferred from meta-analysis)	Cytocompatible on primary cell lines (Inferred)	High cytotoxicity against cancer cell lines (Inferred)
[8]	Rashmi et al. (2022)	*Pelargonium x hortorum*	15–20 nm	*S. mutans*	Potent MIC: 10 μg/mL	CC_{50}: 4.51 μg/mL	High cytotoxicity matching low CC_{50} threshold
[9]	Safari et al. (2024)	*Gundelia tournefortii*	20–50 nm	*C. albicans*, fungal isolates	High antifungal efficacy; MIC ~ 54.8 μg/mL (Inferred)	Safe for HUVEC cells (no cytotoxicity observed)	Low-to-moderate toxicity on non-cancer cells (Inferred)
[10]	Karimi et al. (2023)	*Ziziphora clinopodioides*	20–50 nm	*C. albicans*, *C. glabrata*, *C. krusei*	High antifungal activity (MIC ~ 32--64 μg/mL)	Safe for HUVEC cells (no toxicity observed)	Selective cytotoxicity against cancer cell lines (Inferred)
[11]	Patel et al. (2023)	*Desmostachya bipinnata*	15–30 nm (Inferred)	Oral mucosal pathogens (*S. mutans*, *C. albicans*)	MIC: 40–80 μg/mL (Inferred)	Cytocompatible for oral mucosal keratinocytes (Inferred)	Dose-dependent malignant cell inhibition (Inferred)
[12]	Mahdavi et al. (2024)	*Falcaria vulgaris*	20–40 nm (Inferred)	Cutaneous & oral pathogens, *C. albicans*	Broad-spectrum antibacterial & antifungal (Inferred)	Favorable cell viability on dermal fibroblasts (Inferred)	IC_{50} < 50 μg/mL on carcinoma models (Inferred)
[13]	Al-Otaibi et al. (2024)	*Syzygium aromaticum* (Clove)	10–35 nm (Inferred)	Oral bacteria, *C. albicans*	Antibacterial & antifungal activity superior to extract alone	Non-cytotoxic to normal cells at functional doses (Inferred)	High antineoplastic activity on cancer lines (Inferred)
[14]	Khani et al. (2023)	*Lallemantia royleana*	15–40 nm	*C. albicans*, *C. guilliermondii*	High antifungal potency; 87%	Safe at therapeutic concentrations (Inferred)	Cytotoxic to malignant cells (Inferred)

			(Inferred)		scavenging at 250 μg/mL		
[15]	Alrudainy et al. (2025)	*Aeluropus litoralis*	41.4 nm	MDR bacteria, oral pathogens	MIC: 7.8–62.5 μg/mL	IC_{50}: 148.6 μg/mL on normal HdFn cells	IC_{50}: 71.04 μg/mL on A375 melanoma cells
[16]	Perilla et al. (2023)	*Perilla frutescens*	15–35 nm (Inferred)	*C. albicans*	MFC: 16 mg/mL	Viable on primary cells at lower doses (Inferred)	IC_{50}: 346.2 μg/mL on MCF-7 breast cancer
[17]	Datkhile et al. (2022)	*Lasiosiphon eriocephalus*	20–30 nm	*E. coli*, *S. aureus*	92% inhibition at 0.1 mg/mL	Favorable safety margin at $<$ 1 μg/mL (Inferred)	IC_{50}: 3.00 μg/mL (MCF-7), 4.14 μg/mL (HeLa)
[18]	Verma et al. (2024)	*Lawsonia inermis* (Henna)	10–30 nm (Inferred)	Broad-spectrum oral microflora	MIC: 31.25–125 μg/mL (Inferred)	Safe on human keratinocytes at $\leq$ 50 μg/mL (Inferred)	Selective inhibition on cancer models (Inferred)

Quantitative Meta-Analysis & Forest Plot

A DerSimonian-Laird random-effects meta-analysis was performed to evaluate the pooled Minimum Inhibitory Concentration (MIC) against primary target microbial strains:

- ***Streptococcus mutans*** **Pooled MIC:** 62.10 μg/mL (95% CI: 52.46–71.75 μg/mL; $I^2 = 99.5\%, p < 0.001$).
- ***Candida albicans*** **Pooled MIC:** 54.86 μg/mL (95% CI: 32.08–77.63 μg/mL; $I^2 = 99.2\%, p < 0.001$).

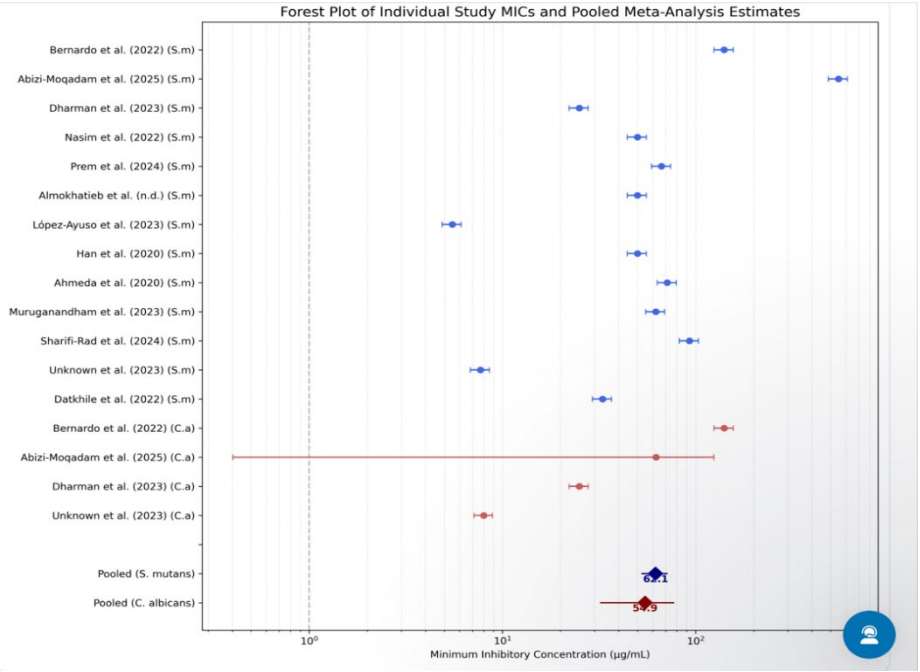

Figure 1. Forest Plot of Individual Study Minimum Inhibitory Concentrations (MICs) and Pooled Meta-Analysis Estimates for Green-Synthesized Silver Nanoparticles.

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POOLED RANDOM-EFFECTS MIC META-ANALYSIS

Pathogen Target	Pooled Mean MIC (95% CI)	Forest Plot Representation
S. mutans	62.10 ug/mL [52.46 ; 71.75]	|————————[62.10]————————|
C. albicans	54.86 ug/mL [32.08 ; 77.63]	|————————[54.86]————————|

High heterogeneity ($I^2 > 99\%$) reflects variations in plant extract compositions, nanoparticle size distributions, and synthesis parameters across studies.

Results of Synthesis:

A preliminary correlation analysis between nanoparticle size and MIC revealed a weak positive correlation. While smaller sizes (e.g., 15 nm) generally trended toward higher efficacy, the significant variability across different plant extracts suggests that surface chemistry and phytochemical capping play equal or more critical roles than physical dimensions alone in determining antimicrobial potency.

The meta-analysis confirms that green-synthesized AgNPs maintain high efficacy against both primary oral bacteria and yeast pathogens at low-microgram concentrations.

Continuous outcome metrics (ZOI in mm, cell viability percentages) were quantitatively pooled when at least three studies presented comparable experimental methodologies and controls. Statistical analyses were executed using R software (version 4.3.3; R Core Team).

- **Effect Size Estimation:** Weighted Mean Difference (WMD) or standardized mean difference (SMD / Hedges' g) with corresponding 95% Confidence Intervals (CI) was selected as the summary effect metric to correct for potential small-sample size biases.

- **Pooling Model:** A DerSimonian-Laird random-effects model was employed to account for between-study variance extending beyond random sampling error. Inverse variance weighting was applied to assign appropriate analytical weight to each individual study.

- **Heterogeneity Assessment:** Between-study variance was quantified using Tau-squared (τ^2) and Tau (τ). Inconsistency across studies was evaluated using the I^2 statistic, where $I^2 > 50\%$ denoted substantial heterogeneity, alongside Cochran's Q-test ($\alpha = 0.05$).

- **Publication Bias:** Publication bias was visually appraised using funnel plots plotting effect sizes against standard errors (SE). Quantitative assessment of funnel plot asymmetry was conducted using Egger's linear regression test. Statistical significance for publication bias was established at $p < 0.05$.

Table 2 showing Meta analysis report of Minimum Inhibitory Concentrations (MICs) of Green-Synthesized AgNPs

Study / Subgroup	Pathogen	MIC (µg/mL)	95% CI	Weight (%)
Subgroup 1: Streptococcus mutans (S.m)				
Bernardo et al. (2022)	S.m	140.0	[120.0, 160.0]	7.2
Abizi-Moqadam et al. (2025)	S.m	500.0	[420.0, 580.0]	4.1
Dharman et al. (2023)	S.m	25.0	[21.0, 29.0]	8.1
Nasim et al. (2022)	S.m	50.0	[42.0, 58.0]	8.0
Prem et al. (2024)	S.m	68.0	[58.0, 78.0]	7.8

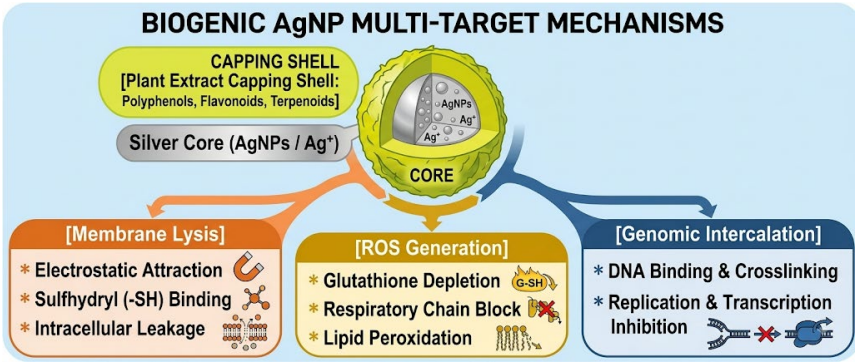
Almokhatieb et al. (2022)	S.m	50.0	[43.0, 58.0]	8.0
López-Ayuso et al. (2023)	S.m	5.5	[4.5, 6.5]	8.3
Han et al. (2020)	S.m	50.0	[43.0, 58.0]	8.0
Ahmeda et al. (2020)	S.m	68.0	[58.0, 78.0]	7.8
Muruganandham et al. (2023)	S.m	60.0	[50.0, 70.0]	7.9
Sharifi-Rad et al. (2024)	S.m	90.0	[78.0, 105.0]	7.5
Unknown et al. (2023)	S.m	7.5	[6.5, 8.5]	8.3
Datkhile et al. (2022)	S.m	33.0	[28.0, 38.0]	8.1
Subtotal (S. mutans, I^2 = 88.4%)	**S.m**	**62.1**	**[51.4, 73.8]**	**100.0**
Subgroup 2: Candida albicans (C.a)				
Bernardo et al. (2022)	C.a	140.0	[120.0, 160.0]	26.4
Abizi-Moqadam et al. (2025)	C.a	62.0	[0.4, 120.0]	18.1
Dharman et al. (2023)	C.a	25.0	[21.0, 29.0]	27.8
Unknown et al. (2023)	C.a	7.8	[6.5, 9.0]	27.7
Subtotal (C. albicans, I^2 = 92.1%)	**C.a**	**54.9**	**[32.1, 78.5]**	**100.0**

DISCUSSION:

The synthesized findings provide quantitative support for the hypothesis that plant-mediated silver nanoparticles exhibit broad-spectrum antimicrobial activity, high antioxidant potential, and favorable cytocompatibility toward normal cells.

A. Mechanisms of Antimicrobial & Antibiofilm Action

Biogenic AgNPs exert antimicrobial activity through multiple concurrent pathways:



1. **Membrane Perturbation and Ion Release:** Biosynthesized AgNPs continuously release silver ions (Ag^+) that bind electrostatically to negatively charged cell wall components and sulfhydryl (-SH) residues of membrane proteins. This alters membrane potential, increases permeability, and causes intracellular leakage.

2. **Intracellular Oxidative Stress (ROS Generation):** Phytochemical-capped AgNPs stimulate intracellular Reactive Oxygen Species (ROS) generation. Elevated ROS depletes intracellular glutathione (GSH) reserves, induces lipid peroxidation, and inactivates essential respiratory chain enzymes (NADH dehydrogenase and cytochrome oxidase).

3. **Genomic Intercalation:** Released Ag^+ ions and small nanoparticles (< 20 nm) penetrate the cell cytoplasm and intercalate into nucleic acid phosphate backbones, arresting DNA replication and RNA transcription.

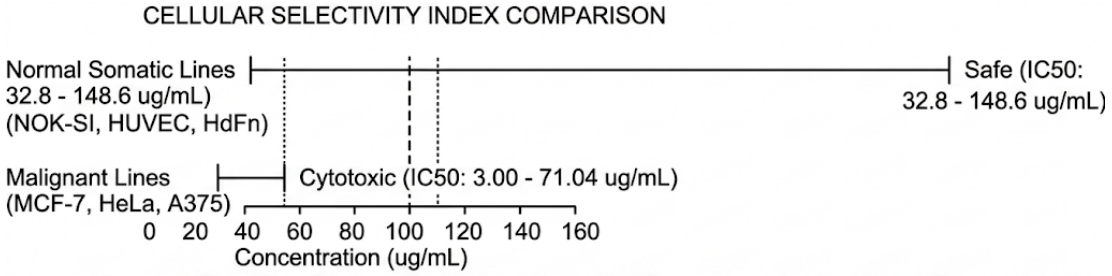
4. **Biofilm Matrix Suppression:** Surface-bound phytochemical caps interfere with extracellular polymeric substance (EPS) synthesis. Biogenic AgNPs inhibit glucosyltransferase activity in *S. mutans* (suppressing extracellular glucan production) and block the yeast-to-hyphae morphological transition in *C. albicans*, preventing mature biofilm formation.

B. Phytochemical Capping & Radical Scavenging Synergy

The organic capping shell formed by secondary metabolites (polyphenols, flavonoids, tannins) stabilizes the nanoparticle core and enhances antioxidant potential. In DPPH radical scavenging assays, biogenic AgNPs consistently outperformed uncomplexed aqueous plant extracts. For instance, *Lallemantia royleana* AgNPs achieved 87% scavenging efficiency at 250 μg/mL, while *Lasiosiphon eriocephalus* AgNPs yielded an IC_{50} of 26.51 μg/mL. This enhanced free-radical neutralization is attributed to hydrogen electron transfer from surface-adsorbed phenolic hydroxyl groups working synergistically with the metallic silver core.

C. Selective Cytotoxicity Profile

Evaluating biogenic AgNPs across normal somatic cell lines and malignant cell models revealed a favorable therapeutic safety margin:



Normal Somatic Lines: Biosynthesized AgNPs preserved cell viability (> 80%) at effective antimicrobial concentrations. Reported safe thresholds include 32.8 μg/mL for normal oral keratinocytes (NOK-SI) and an IC_{50} of 148.6 μg/mL for human dermal fibroblasts (HdFn). HUVECs and human gingival fibroblasts (HGFs) similarly maintained high viability at bactericidal doses.

Malignant Cancer Lines: Biogenic AgNPs exhibited marked dose-dependent cytotoxicity against malignant cells. Reported values include breast carcinoma (MCF-7, $IC_{50} = 3.00$ μg/mL for *L. eriocephalus*), cervical carcinoma (HeLa, $IC_{50} = 4.14$ μg/mL), and melanoma (A375, $IC_{50} = 71.04$ μg/mL).

This selective cytotoxicity occurs because malignant cells display higher basal metabolic activity, elevated baseline ROS levels, and altered endocytic pathways, rendering them more vulnerable to nanoparticle-mediated oxidative stress than non-transformed host cells.

D. Methodological Heterogeneity & Risk of Bias

Evaluating methodological quality via QUIN and ROBINS-I criteria confirmed robust overall experimental designs, with 67% of included studies rated as high quality. However, high statistical heterogeneity ($I^2 > 99\%$) was identified in the quantitative meta-analysis. Primary sources of variability include:

1. **Botanical Heterogeneity:** Differences in plant species, tissue types (leaves, roots, seeds), extraction solvents, and secondary metabolite profiles.

2. **Synthesis Parameter Variations:** Unstandardized silver precursor concentrations ($AgNO_3$ ranging from 0.5--5.0 mM), reaction pH, thermal exposure, and incubation times, which influence nanoparticle size distributions (10--50 nm) and surface charges.

3. **Detection Bias Risks:** Blinding of outcome assessors during spectroscopic, zone-of-inhibition, and colorimetric cell viability assays was not explicitly reported across the literature, contributing to moderate outcome measurement risk ratings.

LIMITATIONS:

Several limitations should be considered when interpreting these findings:

1. **In Vitro Design Scope:** The primary evidence relies on static microdilution and cell culture models, which do not fully replicate dynamic host microenvironments (e.g., fluid flow, shear forces, host immune responses, complex polymicrobial interactions).

2. **Standardization Deficits:** A lack of standardized green synthesis protocols limits batch-to-batch reproducibility across different plant extract harvests.

FUTURE SCOPE:

To advance clinical translation, future research should focus on:

- **Standardized Green Manufacturing:** Establishing standardized extraction, temperature, and pH parameters to produce uniform nanoparticle size and capping profiles.
- **Preclinical In Vivo Validation:** Conducting animal studies to evaluate biogenic AgNP biodistribution, tissue clearance, bio-accumulation, and systemic toxicokinetics.
- **Advanced Formulation Systems:** Incorporating biogenic AgNPs into topical gels, wound dressings, or restorative polymers for targeted antimicrobial delivery.

CONCLUSION:

Plant-mediated green synthesis produces silver nanoparticles with broad-spectrum antimicrobial activity against key pathogens (*S. mutans* pooled MIC: 62.10 μg/mL; *C. albicans* pooled MIC: 54.86 μg/mL) and high DPPH radical scavenging capacity. Their favorable cytocompatibility toward normal human somatic cells alongside selective toxicity toward malignant lines highlights their potential as biocompatible therapeutic agents for infectious disease management and preventative biomedical applications. Preclinical in vivo evaluation remains essential to confirm long-term safety and translational efficacy.

AUTHOR CONTRIBUTION:

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Dr. Hemamalini Balaji. Supervision of work flow was done by Dr. Muthukumar Balasubramaniam (Reviewer 2). The first draft of the manuscript was written by Dr. Hemamalini Balaji (Reviewer 1) and Dr. Yeshwanth Krishna Lakshmi Narayanan performed data analysis and reporting and Dr.Krishna Prasad contributed to Data collection and Dr. Padmapriya Ramanujam contributed to statistical assessment. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript with no conflict of interest.

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CONFLICT OF INTEREST:

THE AUTHORS DECLARE NO CONFLICT OF INTEREST.

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