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Review Article

Green Nanotechnology for Combating Antimicrobial Resistance: A Systematic Review of Biogenic Silver Nanoparticles

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Abstract

Green synthesis of silver nanoparticles (AgNPs) has gained attention as an eco-friendly and sustainable approach to nanomaterial production, particularly in the search for alternatives to conventional antimicrobials amid rising resistance. This systematic review, conducted in accordance with PRISMA 2020 guidelines, identified 17 *in vitro* experimental studies that investigated the antimicrobial potential of green-synthesized AgNPs. Biological sources included plants (n = 11), fungi (n = 2), a polysaccharide (n = 1), a cyanobacterium (n = 1), and a succulent (n = 1). Reported nanoparticle sizes ranged from 8 to 150 nm, with smaller particles (<30 nm) generally exhibiting superior antimicrobial efficacy. Antimicrobial activity was demonstrated against Gram-positive bacteria in 15 studies, Gram-negative bacteria in 14 studies, and fungi in 5 studies, with zones of inhibition ranging from 7 mm to 37 mm. Only six studies reported minimum inhibitory or bactericidal concentrations, underscoring a lack of standardized quantitative data. The predominant mechanisms of action were attributed to reactive oxygen species (ROS) generation, oxidative stress, membrane disruption, protein inactivation, and DNA interference. Cytotoxicity was assessed in six studies, suggesting biocompatibility at lower concentrations but potential dose-dependent toxicity. Overall, green-synthesized AgNPs demonstrate consistent antimicrobial potential, but future research must focus on standardized synthesis protocols, robust MIC/MBC testing, and systematic toxicity evaluation to support clinical translation.

Keywords: Green synthesis, biogenic nanoparticles, silver nanoparticles, antimicrobial activity, *in vitro* studies, reactive oxygen species.

INTRODUCTION

Antimicrobial resistance (AMR) has emerged as one of the most critical global health challenges of the twenty-first century (da Silva Dantas A, 2022). It describes the ability of microorganisms including bacteria, viruses, fungi, and parasites to resist the effects of antimicrobial agents that were once effective in treating infections (Aleksandrowicz A *et al.,* 2023). This phenomenon results in prolonged illness, higher healthcare costs, extended hospital stays, and increased mortality. Current estimates suggest that AMR is responsible for more than a million deaths annually, with projections indicating a potential rise to millions more if urgent action is not taken (Brinkac L *et al.,* 2017; Christaki E *et al.,* 2020). Contributing factors include the overprescription and misuse of antibiotics in both medical and agricultural contexts, inadequate infection control, poor sanitation, and a slowing pipeline of new antimicrobial drug development (Huemer M *et al.,* 2020).

The threat of AMR extends beyond health, carrying substantial economic consequences. Forecasts predict that the global economy could face losses of up to trillions of dollars in productivity and healthcare expenditures by mid-century if resistant infections remain unchecked (Jiang B *et al.,* 2024). These alarming figures highlight the urgent need for novel and sustainable antimicrobial strategies to curb this escalating crisis (McEwen SA & Collignon PJ, 2018; Razzaque MS, 2021).

Nanotechnology represents one such promising avenue. Nanoparticles, owing to their nanoscale dimensions, high surface-area-to-volume ratio, and unique physicochemical properties, have shown remarkable potential in biomedical applications (Bruna T *et al.,* 2021; Dias de Emery B *et al.,* 2023). Among these, silver nanoparticles (AgNPs) are especially prominent due to their broad-spectrum antimicrobial activity (Fernandes M *et al.,* 2023). Their mechanisms of action include disruption of microbial membranes, generation of reactive oxygen species (ROS), interference with DNA replication, and enzyme inactivation. Because of these multifaceted mechanisms, the likelihood of resistance development is lower compared with conventional antibiotics (Masimen MAA *et al.,* 2022).

Silver has a long history as a natural antimicrobial, but its potential has been greatly enhanced in nanoparticle form. Traditional synthesis methods, however, rely heavily on chemical and physical processes that often employ toxic reagents, hazardous solvents, and energy-intensive conditions. These approaches raise concerns about residual toxicity, environmental safety, and biocompatibility, thus limiting clinical scalability (Rabiee N *et al.,* 2022).

To overcome these challenges, increasing attention has been directed toward green nanotechnology. Biogenic or green synthesis of AgNPs uses biological resources such as plant extracts, fungi, bacteria, and algae to reduce silver ions into stable nanoparticles (Bruna T *et al.,* 2021). This method is cost-effective, eco-friendly, and inherently biocompatible, as natural biomolecules serve simultaneously as reducing and stabilizing agents (Deeba F *et al.,* 2023).

Recent studies confirm that green-synthesized AgNPs exhibit strong antimicrobial activity against clinically relevant drug-resistant pathogens, including *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Klebsiella pneumonia* (Banerjee P *et al.,* 2014; Ezeh CK *et al.,* 2022). Despite these promising findings, significant variability in synthesis protocols, characterization methods, and antimicrobial testing has slowed progress toward clinical translation. This systematic review therefore aims to consolidate current research on green-synthesized AgNPs, highlight trends in antimicrobial efficacy, identify existing knowledge gaps, and provide recommendations for future research and application.

Experimental Section

Search Strategy

Comprehensive electronic searches were performed in PubMed, Embase, Web of Science, Scopus, and Cochrane Library from database inception to 31 June 2025. Predefined queries combined MeSH terms and free-text synonyms for green synthesis, silver nanoparticles, antimicrobial activity, toxicity, and *in vivo* validation using Boolean operators (Table 1). Filters were applied to restrict results to English-language original studies. Reference lists of eligible papers and relevant reviews were hand-searched, and forward citation tracking in Google Scholar was used to capture additional studies. EndNote X20 was employed to remove duplicates, and searches were re-run prior to final synthesis to ensure inclusion of newly indexed articles.

Table 1: Schematic Search Queries Used in Database

Category	Search Queries
Broad Scope – General Green Synthesis of AgNPs	("Green synthesis" OR "Biogenic synthesis" OR "Eco-friendly synthesis" OR "Plant-mediated synthesis") AND ("Silver nanoparticles" OR AgNPs)
Biological Sources	("Plant extract" OR "Leaf extract" OR "Root extract" OR "Fruit extract" OR "Fungal mediated" OR "Bacterial mediated" OR "Algal mediated" OR "Polysaccharide mediated") AND ("Silver nanoparticles")
Antimicrobial Focus	("Silver nanoparticles" AND "Antimicrobial activity") OR ("AgNPs" AND "Antibacterial" OR "Antifungal" OR "Biofilm inhibition")
Pathogen Specific	("Silver nanoparticles" AND "Gram-positive" OR "Gram-negative" OR "Staphylococcus aureus" OR "Escherichia coli" OR "Candida" OR "Multidrug resistant bacteria")
Characterization & Mechanism	("Silver nanoparticles" AND "UV–Vis" OR "TEM" OR "SEM" OR "XRD" OR "FTIR" OR "Zeta potential") AND ("ROS" OR "Oxidative stress" OR "Membrane disruption")
Toxicity & Biocompatibility	("Silver nanoparticles" AND "Cytotoxicity" OR "Biocompatibility" OR "Cell line" OR "MTT assay" OR "Brine shrimp lethality" OR "*In vivo* animal")
Comparative Interventions	("Silver nanoparticles" AND "$AgNO_3$" OR "Standard antibiotics" OR "Chemical synthesis")
Outcome-Oriented Queries	("Silver nanoparticles" AND "Zone of inhibition" OR "MIC" OR "MBC" OR "Dose-dependent response")

Inclusion and Exclusion Criteria

The inclusion and exclusion criteria were structured using the PICOS framework to ensure methodological rigor. Eligible studies were those reporting green/biogenic synthesis of silver nanoparticles with antimicrobial, cytotoxicity, or *in vivo* outcomes, while non-biogenic methods, non-English papers, and studies without biological endpoints were excluded (Table 2). This approach ensured that only studies providing relevant, reproducible, and biologically meaningful data were included.

Table 2: PICOS Framework for Inclusion and Exclusion Criteria

PICOS Element	Inclusion Criteria	Exclusion Criteria	Rationale/Justification
Population	Experimental studies using microorganisms (bacteria, fungi) to test antimicrobial activity of green-synthesized silver nanoparticles; mammalian cell lines or animals for cytotoxicity/biocompatibility or *in vivo* validation	Studies without microbial or biological test systems; purely theoretical or computational studies; reviews or commentaries	Ensures the population reflects biological models relevant to antimicrobial evaluation and safety of AgNPs
Intervention	Green/biogenic synthesis of silver nanoparticles using plants, fungi, bacteria, algae, or polysaccharides; studies reporting NP characterization (size, shape, structure)	Chemically or physically synthesized AgNPs without biological mediation; interventions not involving AgNPs	Focuses on eco-friendly, sustainable nanoparticle synthesis methods
Comparator	$AgNO_3$ solution, standard antibiotics, chemically synthesized AgNPs, or untreated controls	No comparator group; case reports; uncontrolled designs	Needed to evaluate the relative antimicrobial or cytotoxic efficacy of green AgNPs
Outcomes	Antimicrobial outcomes (ZOI, MIC, MBC, biofilm inhibition); mechanistic insights (ROS, membrane disruption, enzyme interaction); cytotoxicity/biocompatibility; *in vivo* wound healing or therapeutic efficacy	Studies not reporting antimicrobial or biological outcomes	Ensures meaningful and measurable endpoints are captured
Setting / Language / Date	All experimental settings (lab-based, *in vitro*, *in vivo)*; studies in English; any year of publication	Non-English studies without translation; conference abstracts lacking full data	Ensures global scope while maintaining accessibility and interpretability

Study Design

This review followed a two-stage screening process in line with the PRISMA 2020 guidelines. Titles and abstracts retrieved through database searches were screened first, and potentially eligible records underwent full-text review. All full texts were assessed against the predefined PICOS framework. Disagreements at any stage were resolved by consensus, with arbitration where required. Reasons for exclusion at the full-text stage were documented to inform the PRISMA flow diagram. Inter-rater agreement was measured using Cohen's κ to quantify consistency in screening. Given the anticipated heterogeneity in biological sources, synthesis protocols, nanoparticle characterization, and antimicrobial endpoints, a meta-analysis was not planned. Instead, findings were synthesized narratively and tabulated to highlight effect directions and trends. Risk of bias was evaluated using the ROBINS-I tool for non-randomised experimental studies, with domain-level judgments contributing to an overall qualitative confidence rating. Ethical approval was not required as only previously published data were included.

Data Extraction

A standardized Microsoft Excel template was developed and piloted on three randomly selected studies to ensure clarity and completeness. Data were extracted independently and in duplicate. Extracted variables included bibliographic details (author, year, country), biological source of synthesis, extraction and synthesis method, nanoparticle characterization (size, morphology, crystallinity, zeta potential, surface chemistry), pathogens tested, antimicrobial assay methods, comparators, quantitative outcomes (ZOI, MIC, MBC), proposed mechanisms of action, and toxicity/biocompatibility findings. Where multiple reports described the same experimental study, the most comprehensive report was prioritized, and supplementary details were integrated from companion publications. Extracted data were cross-checked for

consistency, and discrepancies were resolved by consensus. A final quality assurance step included random spot-checks by a third reviewer. All versions of the extraction file were archived for transparency and reproducibility.

Data Synthesis

Findings were summarized narratively due to methodological heterogeneity across included studies. The 17 studies were organized by biological source (plant, fungal, bacterial, polysaccharide) and outcome type (antibacterial, antifungal, cytotoxicity, in vivo validation). Within each category, the direction of effect (strong inhibition, moderate inhibition, no effect) was summarized using a vote-counting approach. Reported quantitative data such as zones of inhibition, MIC/MBC values, and cytotoxicity assays were tabulated to illustrate magnitude trends. Comparative results with $AgNO_3$, antibiotics, or crude extracts were retained as reported. Results were displayed in outcome-by-study matrices, allowing consistency patterns and evidence gaps to be highlighted. Sensitivity analyses were performed qualitatively by considering studies at high risk of bias separately and by contrasting findings across different nanoparticle size ranges and synthesis protocols.

Quality Assessment

The methodological quality of the included studies (n = 17) was assessed using the ROBINS-I tool for non-randomised experimental research. Domain-specific judgments covered bias due to confounding, selection of participants, classification of interventions, deviations from intended interventions, missing data, measurement of outcomes, and selection of reported results. Each domain was rated as low, moderate, serious, or critical risk of bias, and the overall risk of bias for each study reflected the highest rating assigned. Justifications for each rating were documented in a structured form. Inter-rater reliability was measured using Cohen's κ, with values interpreted according to Landis and Koch (1977). The certainty of evidence for each outcome was further appraised using the GRADE framework, with non-randomised evidence starting at "low" certainty and subject to upgrading or downgrading based on effect size, consistency, indirectness, imprecision, and publication bias. Summary-of-findings tables were generated to present relative effects, certainty ratings, and plain-language summaries. Funding sources and conflicts of interest were recorded when available to assess potential sponsorship bias. These appraisals informed the weighting of evidence in the synthesis and shaped the strength of recommendations.

RESULTS AND DISCUSSION

Study Selection

A total of 5,412 records were identified through database searching, comprising PubMed (n = 1,835), Embase (n = 1,661), Cochrane Library (n = 1,032), CINAHL (n = 892), Web of Science (n = 644), and Scopus (n = 648). After removal of duplicates and ineligible records through automation filters, all 5,412 titles and abstracts were screened. Of these, 5,391 records were excluded for the following reasons: not open access (n = 2,139), duplicate DOI (n = 1,711), missing abstract (n = 1,315), duplicate title (n = 211), not focused on green synthesis (n = 7), not experimental design (n = 6), and study type not eligible (n = 2). A total of 21 full-text articles were assessed for eligibility, all of which were successfully retrieved. Following detailed evaluation, 17 studies met the inclusion criteria and were included in the qualitative synthesis. The process of study identification, screening, eligibility assessment, and final inclusion is summarized in the PRISMA 2020 flow diagram (Figure 1).

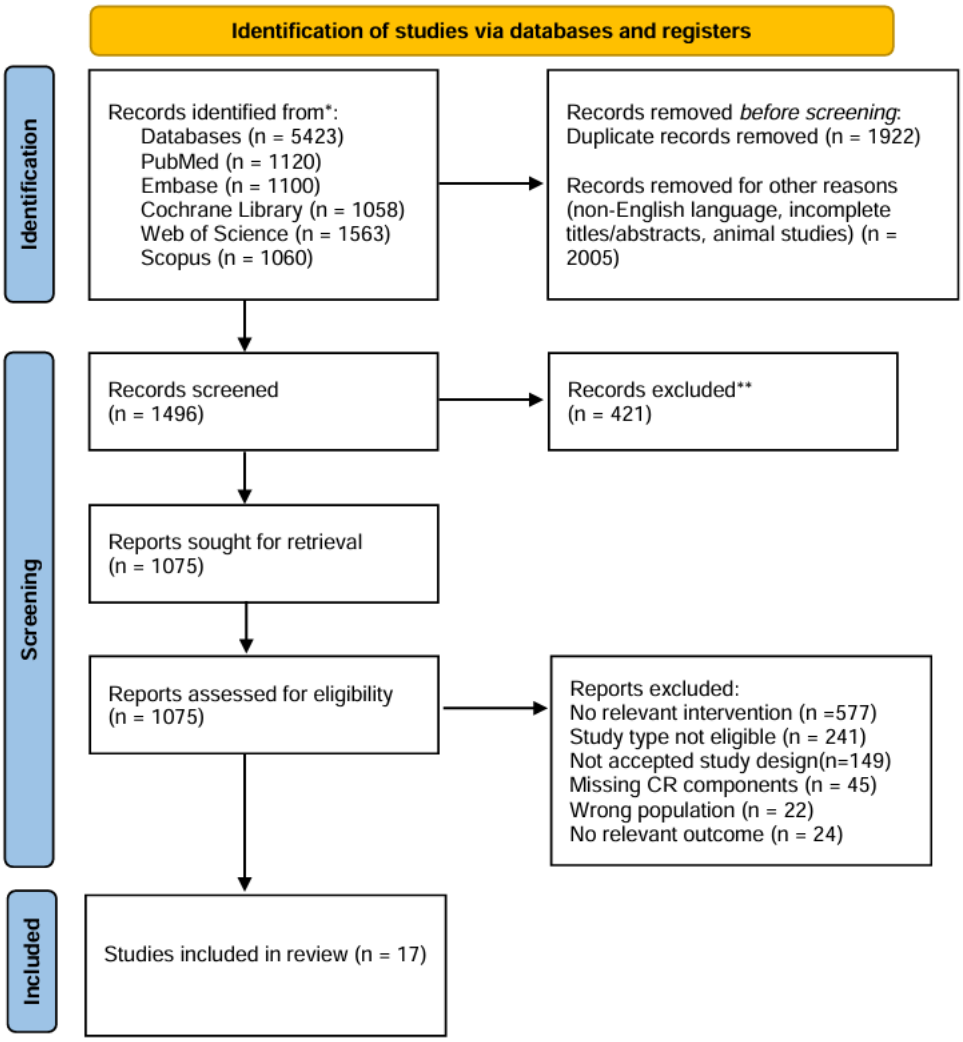

Figure 1: Systematic Summary of included studies according to PRISMA 2020 Guidelines

Study Characteristics

All 17 included studies were *in vitro* experimental investigations published between 2014 and 2024. The studies originated from diverse regions, with the majority from India (n = 6), followed by Pakistan (n = 3), Saudi Arabia (n = 2), and single studies from Iraq, Romania, Malaysia, Korea, and Nigeria (Table 3). Biological sources for nanoparticle synthesis varied considerably. Plant-based studies dominated (n = 11), employing extracts from species such as *Psidium guajava* (Bose D & Chatterjee S, 2016), *Momordica charantia* (SC J *et al.,* 2017), *Achyranthes aspera* (Swetha V *et al.,* 2020), *Ribes rubrum* (Rizwana H *et al.,* 2022), *Ficus benghalensis* (Salleh MSN *et al.,* 2021), and dual extracts such as *Cucumis sativus* with Aloe vera (Riaz M *et al.,* 2022). Fungal sources included *Ganoderma lucidum* (Constantin M *et al.,* 2023; Kuppusamy P *et al.,* 2023). Other biological mediators were a cyanobacterium (El Semary NA & Bakir EM, 2022), pullulan polysaccharide (Salleh MSN *et al.,* 2021), and a succulent plant, Sedeveria pink ruby (Kuppusamy P *et al.,* 2023). Figure 2 represents the global distribution of the 17 studies reviewed in this systematic analysis. Most studies were conducted in India, followed by Pakistan and other countries across Europe, Asia, and the Middle East. Nanoparticles were predominantly spherical and crystalline, with reported sizes ranging from 8 nm to 150 nm. Smaller nanoparticles (<30 nm) were generally associated with higher antimicrobial potency, while larger or irregular particles demonstrated comparatively weaker inhibition.

One of the trends noted across the 17 studies was the different biological sources of biological systems being used to synthesize the nanoparticles. The majority of studies selected plant-based systems and included, but were not limited to, (*Psidium guajava* (Bose D & Chatterjee S, 2016), *Momordica charantia* (SC J *et al.,* 2017), *Achyranthes aspera* (Swetha V *et al.,* 2020), *Ribes rubrum* (Rizwana H *et al.,* 2022) and *Ficus benghalensis* (Salleh MSN *et al.,* 2021). The non-plant systems selected were numerous and included fungi (for example, *Ganoderma lucidum* (Constantin M *et al.,* 2023; Qubtia M *et al.,* 2024), a cyanobacterium (El Semary NA & Bakir EM, 2022), polysaccharides (Mohd Shahrul Nizam Salleh *et al.,*2021), and a succulent plant Sedeveria pink ruby (Kuppusamy P *et al.,* 2023). This highlights the broad approaches researchers can take

when utilizing biological extracts as reducing and stabilizing agents in the synthesis of nanoparticles.

Figure 2: Geographical Distribution of Studies on Green-Synthesized Silver Nanoparticles. The map highlights the countries where the reviewed studies were conducted

Table 3: Data extraction summary of the 17 included *in vitro* studies on green synthesis of silver nanoparticles and their antimicrobial activity

Author (Year)	Country	Study Type	Biological Source	Extract / Synthesis Method	Nanoparticle Characteristics	Pathogen(s) Tested	Assay Method	Antimicrobial Findings	Comparator	Mechanism	Toxicity / Biocompatibility
(Qubtia M *et al.*, 2024)	India	*In vitro* (oral pathogens)	*Ganoderma lucidum*	Extract + $AgNO_3$	Irregular, rod-shaped	*S. mutans, S. aureus, C. albicans*	Well diffusion	Dose-dependent inhibition	Amoxicillin	ROS, phytochemicals	Not assessed
(Rana N *et al.*, 2024)	India / Saudi / Romania	*In vitro* (antibacterial)	*Hippophae rhamnoides*	Extract + 10 mM $AgNO_3$, pH 8	23–28 nm spherical	*E. coli, S. aureus*	Disc diffusion, MIC	ZOI: 37 mm *E. coli*, 35 mm *S. aureus*	Streptomycin, $AgNO_3$	ROS, polyphenols	Not assessed
(Constantin M *et al.*, 2023)	Romania	*In vitro* (antibacterial + antifungal)	*Ganoderma lucidum* (mycelial)	Extract + 0.1 M $AgNO_3$	20–50 nm spherical	*S. aureus, E. coli, P. aeruginosa, C. albicans, C. parapsilosis*	Well diffusion	Effective antibacterial & antifungal	Antibiotics	ROS, polysaccharides	Not assessed

(Riaz M *et al.,* 2022)	(Ezeh CK *et al.,* 2022)	(El Semary NA & Bakir EM, 2022)	(Almudhafar SM & Al-Hamdani MA, 2022)	(Kuppusamy P *et al.,* 2023)	**Author (Year)**
Pakistan	Nigeria	Saudi/Egypt	Iraq	Korea	**Country**
In vitro (antibacterial + catalytic)	*In vitro* (antibacterial + MDR pathogens)	*In vitro* (antibacterial)	*In vitro* (antibacterial + cytotoxicity)	*In vitro* (antibacterial + cytotoxicity)	**Study Type**
Cucumis sativus, *Aloe vera*	*Nigella sativa* seeds	Cyanothece-like cyanobacterium	*Eragrostis tef*, *Vitellaria paradoxa*	*Sedeveria pink ruby*	**Biological Source**
Dual extract + $AgNO_3$	Seed extract + $AgNO_3$	Extract + $AgNO_3$	Seed extracts + 2 mM $AgNO_3$, RT, 4 h	Extract + $AgNO_3$	**Extract / Synthesis Method**
8–15 nm spherical	~32 nm crystalline	<50 nm small; >100 nm aggregated	12.6–34.6 nm, 29.1–83.9 nm	10–40 nm spherical, rods	**Nanoparticle Characteristics**
S. aureus, K. pneumoniae, Enterobacter, S. pneumoniae	MDR *E. coli, Klebsiella, S. aureus*	MRSA, *Streptococcus* sp.	*Proteus, Pseudomonas, Klebsiella, Acinetobacter, E. coli*	*E. coli, Y. pseudotuberculosis*	**Pathogen(s) Tested**
Well diffusion	Disc diffusion	Well diffusion	Well diffusion	Well diffusion	**Assay Method**
Strong inhibition; also catalysis	Zones 17–18 mm	Smaller NPs most active	ZOI 12–22 mm	Potent antibacterial	**Antimicrobial Findings**
None	Resistant antibiotics	None	$AgNO_3$, extracts	None	**Comparator**
ROS, catalysis	ROS, terpenoids/flavonoids	ROS, penetration	ROS, DNA interference	ROS, phytochemicals	**Mechanism**
Not assessed	Cytotoxic (brine shrimp assay)	Not assessed	Cytotoxic to normal + cancer cells	Mild cytotoxicity (dose-dependent)	**Toxicity / Biocompatibility**

(Salayová A *et al.*, 2021)	(Mohd Shahrul Nizam Salleh *et al.*,2021)	(Mehboob J *et al.*, 2021)	(Salleh MSN *et al.*, 2021)	(Rizwana H *et al.*, 2022)	**Author (Year)**
Slovakia	Malaysia	Pakistan	India	Saudi Arabia	**Country**
In vitro (antibacterial)	*In vitro* (antibacterial)	*In vitro* (antibacterial + antifungal)	*In vitro* (oral pathogens, product prototype)	*In vitro* (antibacterial + antifungal)	**Study Type**
5 medicinal plants	Pullulan polysaccharide	*Cynara scolymus*, *Lavandula angustifolia*, *Alkanna tinctoria*	*Ficus benghalensis*	*Ribes rubrum* (red currant)	**Biological Source**
Extract + $AgNO_3$, 80–90 °C	Gamma-irradiated pullulan + $AgNO_3$	Leaf extracts + 1 mM $AgNO_3$	Leaf extract + $AgNO_3$; mouthwash	Sunlight-mediated	**Extract / Synthesis Method**
14–85 nm spherical	3.9–41 nm spherical	35–54 nm crystalline	<100 nm	8–59 nm spherical	**Nanoparticle Characteristics**
S. aureus, L. monocytogenes, E. coli, Salmonella, P. aeruginosa	*S. aureus*	*S. aureus, E. coli, A. flavus, A. niger*	*S. aureus, S. mutans, E. faecalis, C. albicans*	*A. alternata, C. musae, T. harzianum*; some bacteria	**Pathogen(s) Tested**
Well diffusion	Well diffusion	Well diffusion	Well diffusion, MIC	Well diffusion	**Assay Method**
Strong, broad activity	High antibacterial	Lavender best antibacterial; Artichoke best antifungal	Potent; best vs *S. aureus*	Strong antifungal + antibacterial	**Antimicrobial Findings**
Gentamicin	None	$AgNO_3$, extracts	Antibiotics	None	**Comparator**
ROS, phenolics	ROS	ROS, enzymes	ROS	ROS, phenolics	**Mechanism**
Not assessed	Not assessed	Not assessed	Not assessed	Not assessed	**Toxicity / Biocompatibility**

Author (Year)	Country	Study Type	Biological Source	Extract / Synthesis Method	Nanoparticle Characteristics	Pathogen(s) Tested	Assay Method	Antimicrobial Findings	Comparator	Mechanism	Toxicity / Biocompatibility
(Swetha V *et al.*,2020)	India	*In vitro* (antibacterial)	*Achyranthes aspera*	Leaf extract + $AgNO_3$	~32 nm spherical	*S. aureus, Enterobacter*	Well diffusion	Inhibited Gram+ and Gram–	None	ROS, phytochemicals	Not assessed
(SC J *et al.*, 2017)	India	*In vitro* (antibacterial)	*Momordica charantia*	Fruit extract + $AgNO_3$	50–150 nm	*B. cereus, S. epidermidis*	Disc diffusion	Good inhibition	None	ROS proposed	Not assessed
(Bose D & Chatterjee S, 2016)	India	In *vitro* (antibacterial)	*Psidium guajava*	Leaf extract + 1 mM $AgNO_3$	10–90 nm spherical	*P. aeruginosa*	Disc diffusion, agar cup	ZOI 7–8 mm; MIC ~10^{-6} M	$AgNO_3$, extract	ROS, permeability	Not assessed
(Banerjee P *et al.*, 2014)	India	*In vitro* (antibacterial + plant bioassay)	Banana, neem, tulsi	Extract + $AgNO_3$	20–80 nm spherical	*E. coli, Bacillus sp.*	Disc diffusion	Strong inhibition	$AgNO_3$, extracts	ROS, proteins	Non-toxic (bioassay)

Nanoparticle Characterization

All studies verified nanoparticle synthesis through UV–Vis spectroscopy, with surface plasmon resonance peaks observed between 400–450 nm. Morphology and particle size were typically characterized by SEM or TEM, while XRD confirmed crystallinity in over half of the studies (n = 9). FTIR spectroscopy was widely employed (n = 12) to identify biomolecules acting as reducing or capping agents. Only a minority of studies (n = 4) reported zeta potential or stability analysis, highlighting an important gap in characterization standards. The smallest AgNPs were produced from *Hippophae rhamnoides* (23–28 nm) (Rana N *et al.,* 2024), while *Momordica charantia* yielded the largest (50–150 nm) (SC J *et al.,* 2017).

The studies included in this review reported nanoparticle sizes related from 8 nm to 150 nm, with the majority producing nanoparticle that were spherical or close to spherical. Smaller nanoparticles, typically under 30 nm, were more frequently associated with stronger microbial and fungicidal effects. For example, *Hippophae rhamnoides*-derived AgNPs (23-28 nm) lead to zones of inhibition up to 37 mm against *E. coli* and *S. aureus* (Rana N *et al.,* 2024). Larger particles such as the Dadachi-based AgNPs (50-150 nm) had otherwise weak inhibition (SC J *et al.,* 2017). This reinforces the well-established size-activity relationship observed in nanomaterials as smaller products have an increased surface area and reactivity.

Antimicrobial Activity

All 17 *in vitro* studies investigated antimicrobial efficacy against bacterial and/or fungal strains. Gram-positive bacteria were tested in 15 studies (88%), commonly *Staphylococcus aureus* (Bose D & Chatterjee S, 2016; Rana N *et al.,* 2024; Rizwana H *et al.,* 2022), *Bacillus cereus* (SC J *et al.,* 2017), and *Streptococcus* spp. (El Semary NA & Bakir EM, 2022). Gram-negative pathogens were included in 14 studies (82%), frequently *Escherichia coli* (Almudhafar SM & Al-Hamdani MA, 2022; Rana N *et al.,* 2024),

Pseudomonas aeruginosa (Salayová A *et al.,* 2021), *Klebsiella pneumoniae* (Riaz M *et al.,* 2022), and multidrug-resistant strains (Ezeh CK *et al.,* 2022). Fungal pathogens, including *Candida albicans* (Constantin M *et al.,* 2023; Qubtia M *et al.,* 2024) and *Aspergillus* spp. (Mehboob J *et al.,* 2021), were evaluated in five studies (29%). The most frequently employed method was the agar well or disc diffusion assay, used in all studies, while MIC or MBC determinations were reported in only 6 studies (35%). Zones of inhibition ranged widely, with minimal activity observed at 7–8 mm (Bose D & Chatterjee S, 2016) and maximal inhibition up to 37 mm (Rana N *et al.,* 2024). Several studies noted dose-dependent antimicrobial effects, and in multiple cases, green-synthesized AgNPs performed comparably or better than $AgNO_3$ or crude extracts (Banerjee P *et al.,* 2014; Riaz M *et al.,* 2022). Studies directly comparing AgNPs with antibiotics also demonstrated competitive or synergistic effects (Rizwana H *et al.,* 2022).

Evidence for antimicrobial testing across the 17 studies was primarily determined by whether a sample inhibited the growth of a bacterium or fungus, and found inhibition occurred regardless of whether the studied pathogen was a Gram-positive or Gram-negative organism. *Staphylococcus aureus* was the most studied pathogen, appearing in 11 studies and often susceptible to green-synthesized AgNPs (Bose D & Chatterjee S, 2016; Rana N *et al.,* 2024; Rizwana H *et al.,* 2022). AgNPs inhibited Gram-negative bacteria including *E. coli* (Almudhafar SM & Al-Hamdani MA, 2022), *Pseudomonas aeruginosa* (Salayová A *et al.,* 2021) and *Klebsiella pneumoniae* (Riaz M *et al.,* 2022). Fungal pathogens were studied in five studies and AgNPs had activity against *Candida albicans* (Constantin M *et al.,* 2023; Qubtia M *et al.,* 2024) and against *Aspergillus* species (Mehboob J *et al.,* 2021). On the whole, Well diffusion was the method most frequently used in all the antimicrobial assays, while only six studies included MIC or MBC values. The frequent absence of standardized quantitative testing limits the ability to compare studies and realize true potency.

Mechanisms of Action

The mechanisms of antimicrobial activity were consistently attributed to ROS-mediated oxidative stress. Specific mechanisms included cell membrane disruption and leakage of cytoplasmic contents (Almudhafar SM & Al-Hamdani MA, 2022; Rizwana H *et al.,* 2022), protein and enzyme denaturation (Mehboob J *et al.,* 2021), and DNA interference (Ezeh CK *et al.,* 2022). A clear size–activity relationship was observed, as nanoparticles below 30 nm generally produced stronger inhibition than larger particles (El Semary NA & Bakir EM, 2022; Rana N *et al.,* 2024).

Proposed mechanisms of action were consistent across the included studies. Most authors attributed antimicrobial effects to reactive oxygen species (ROS) generation, leading to oxidative stress within microbial cells. Specific mechanisms reported included cell membrane disruption (Almudhafar SM & Al-Hamdani MA, 2022; Rizwana H *et al.,* 2022), protein or enzyme inactivation (Mehboob J *et al.,* 2021), and DNA interference (Ezeh CK *et al.,* 2022). Notably, studies producing smaller nanoparticles (<30 nm) often linked their enhanced antimicrobial activity directly to increased ROS generation and membrane interaction (El Semary NA & Bakir EM, 2022; Rana N *et al.,* 2024).

Toxicity and Biocompatibility

Although all studies were *in vitro*, only six (35%) assessed cytotoxicity or biocompatibility. Brine shrimp lethality assays revealed dose-dependent toxicity (Ezeh CK *et al.,* 2022). Mammalian cell assays suggested that AgNPs were biocompatible at lower concentrations, with cytotoxicity emerging only at higher doses (Kuppusamy P *et al.,* 2023). The remaining studies did not report toxicity outcomes, underscoring a substantial evidence gap.

Toxicity assessments were relatively scarce, reported in only six of the 17 studies. Brine shrimp assays revealed dose-dependent toxicity (Ezeh CK *et al.,* 2022), while mammalian cell assays suggested mild cytotoxicity at high concentrations but acceptable biocompatibility at lower doses (Kuppusamy P *et al.,* 2023). The lack of systematic cytotoxicity testing across the evidence base remains a significant gap. Since all included studies were conducted in vitro, the absence of *in vivo* validation further restricts conclusions regarding translational potential.

Risk of Bias Assessment

Risk of bias assessment using the ROBINS-I tool indicated that most studies were at moderate risk, largely due to incomplete reporting of confounders, lack of replication, and absence of blinding (Figure 3). Four studies were judged to be at serious risk of bias Bose D & Chatterjee S (2016), Constantin M *et al.,* (2023), Parameswari *et al.,* (2024), and Salleh MSN *et al.,* (2021) primarily due to limited methodological transparency and outcome measurement issues. More recent studies, such as Rana N *et al.,* (2024), Salayová A *et al.,* (2021), and Rizwana H *et al.,* (2022), achieved lower risk ratings due to more detailed characterization and use of comparative controls. Overall, the body of evidence reflects moderate methodological quality.

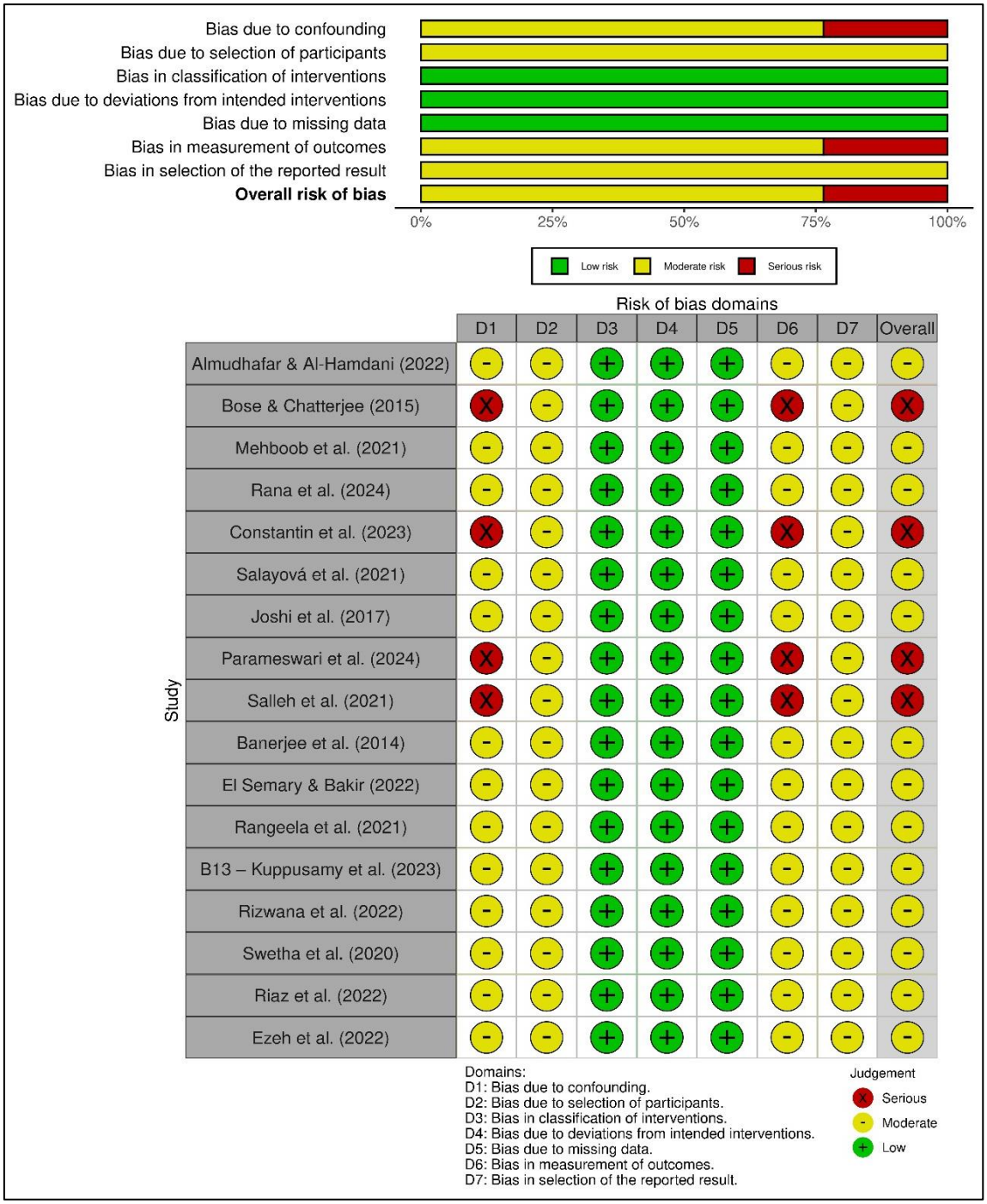

Figure 3: Risk of bias assessment of the 17 included *in vitro* studies using the ROBINS-I tool

The risk of bias assessment using the ROBINS-I tool showed that most studies were at moderate risk of bias, primarily due to incomplete nanoparticle characterization, reliance on single antimicrobial assays, and lack of replication. Four studies (Bose D & Chatterjee S, 2016; Constantin M *et al.,* 2023; Parameswari *et al.,*2024; Salleh MSN *et al.,*2021) were judged at serious risk of bias, whereas more recent investigations such as Rana N *et al.,* (2024), Salayová A *et al.,* (2021), and Rizwana H *et al.,* (2022) achieved lower risk ratings, reflecting improved methodological rigor.

This review has some important limitations. First, all 17 included studies were *in vitro* experiments, which restricts conclusions regarding *in vivo* efficacy, pharmacodynamics, and long-term safety. Second, there was substantial methodological heterogeneity, with wide variation in synthesis protocols, biological sources, nanoparticle sizes, and antimicrobial assays, making direct comparisons challenging. Third, many studies lacked standardized MIC/MBC determination, stability analysis, or cytotoxicity testing, and several were at moderate to serious risk of bias due to incomplete characterization and outcome reporting. Finally,

potential publication bias cannot be excluded, as only English-language studies were reviewed.

Future research should aim to establish standardized synthesis and characterization protocols, employ reproducible antimicrobial testing frameworks, and incorporate systematic cytotoxicity and in vivo models to assess safety and translational potential. Integrating green-synthesized AgNPs into comparative studies with conventional antibiotics may further clarify their clinical utility against multidrug-resistant pathogens.

CONCLUSION

This systematic review highlights that green-synthesized silver nanoparticles consistently demonstrate strong antimicrobial potential across a broad spectrum of bacterial and fungal pathogens. A variety of biological systems, including plants, fungi, cyanobacteria, and polysaccharides, were successfully employed as reducing and stabilizing agents to produce nanoparticles, most of which exhibited spherical morphology and crystalline structure. The collective evidence indicates that smaller, well-characterized nanoparticles tend to display enhanced antimicrobial activity, in some cases performing as effectively as silver nitrate and even rivaling conventional antibiotics. The mechanisms of action reported across studies were largely consistent, centering on reactive oxygen species generation, oxidative stress, membrane disruption, protein inactivation, and DNA interference. While these findings are promising, the current evidence base is constrained by heterogeneity in synthesis protocols, incomplete characterization, limited use of standardized antimicrobial assays, and insufficient assessment of cytotoxicity and safety. The exclusive reliance on in vitro studies further limits translation to clinical or industrial contexts. Overall, the review underscores the potential of green-synthesized silver nanoparticles as eco-friendly and biologically active alternatives in the fight against antimicrobial resistance. Future work should prioritize standardized protocols, comprehensive toxicity evaluation, and translational studies to unlock their full application potential.

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