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Review Paper

Green Synthesis of Silver Nanoparticles and Their Antibacterial Efficacy against Wound Infections

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Abstract:

The growing issue of antibiotic resistance, combined with the limits of traditional wound treatment strategies, has motivated researchers to explore nanotechnology-based solutions. Silver nanoparticles (AgNPs) have received substantial attention due to their broad-spectrum antibacterial activities and biocompatibility. This review discusses current breakthroughs in the green production of AgNPs, as well as their antibacterial properties against wound infections. This review looks at the role of phytochemicals as reducing and stabilizing agents, the impact of synthesis factors (such as pH, temperature, and solvent), and the mechanisms by which AgNPs show antibacterial action. Additionally, comparative analysis of plant-mediated AgNPs synthesis from plants such as *Citrus sinensis*, *Olea europaea*, and *Calendula officinalis* shows inhibition zones of (21-26 mm) against wound pathogens, including *Staphylococcus aureus* and *Escherichia coli*. The review concludes that green-synthesized AgNPs represent a potent and eco-friendly alternative to conventional antibiotics for managing wound infections; however, significant variability in reported efficacy and limited in vivo safety data represent critical gaps that require further investigation.

Keywords: green synthesis, silver nanoparticles, antibacterial activity, wound pathogens, antibiotic resistance

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Introduction:

Green chemistry is an emerging discipline that focuses on developing solutions to limit the development and usage of hazardous chemical waste (Hekmati et al., 2020). A nanometer is one billionth of a meter; hence, the diameter of a human hair is around 1/80,000 of a meter (Pranali et al., 2023). While nanoparticles were discovered in modern science, Mesopotamian artisans have been using them since the ninth century (Amen et al., 2021). The Persian alchemist Geber recorded the first documented use of silver nitrate for medical purposes around 702-705 CE (Jeevanandam et al., 2022). Silver nitrate was used effectively in the nineteenth century to heal skin ulcers, fractures, and infected wounds, and it was later used to treat epilepsy (Khan et al., 2023).

Significant issues, including antibiotic resistance and insufficient wound-treatment solutions, are caused by the overuse and misuse of antibiotics, genetic mutations, inappropriate drugs, and dressings (Nandhini et al., 2024). Due to its well-known antibacterial properties, silver is used in a variety of topical formulations, including lotions and ointments intended for wound care (Ozhathil et al., 2022). Additionally, nanosilver-based materials work well as therapeutic and preventative measures to prevent microbes from infecting wounds (Boateng and Catanzano, 2020). As a result, nano-silver has found a variety of uses, including in wound healing dressings, antimicrobial gel products, orthopedic solutions, medical catheters, surgical instruments, implants, coatings for contact lenses, and applications in 3D and 4D printing (Pangli et al., 2021). Microorganisms in acute and chronic wounds are often derived from both internal sources, such as mucous membranes, and external surroundings, including skin microflora and the surrounding environment (Uberoi et al., 2024). A retrospective research found that *Staphylococcus aureus* (79.4%) and *Pseudomonas aeruginosa* (40.2%) were the most commonly detected bacterial species among about 240 patients with wound infections. *Escherichia coli*, *Proteus mirabilis*, and *Acinetobacter baumannii* were also identified but at smaller levels (Puca et al., 2021).

According to many studies, the ability of silver nanoparticles (AgNPs) to prevent bacterial growth, the continual release of silver ions may allow their antibacterial characteristics to continue for extended periods of time (Hamad et al., 2020). When bacterial cells absorb AgNPs, the silver ions adhere to the cell wall and plasma membrane, affecting their permeability, compromising the bacterial envelope, and causing denaturation (Arshad et al., 2024). The breakdown of the bacterial membrane leads to cell lysis, which also destroys the cell's organelles (Yin et al., 2020). The production of silver nanostructures and other nanostructures can be divided into two categories (Zahoor et al., 2021). The first method is the top-down approach, which entails creating particles from larger bulk materials. In contrast, the second method is the bottom-up approach, where nucleation sites are established and subsequently develop into nanoparticles (Cruz, 2023).

Chemical synthesis is used to rapidly create nanoparticles in a variety of shapes (Sajid et al., 2020). Nonetheless, the incorporation of potentially hazardous chemicals during the synthesis process restricts the usage of chemically generated nanoparticles in medical applications (Xu et al., 2020). The importance of biological synthesis has expanded, owing to its environmentally beneficial properties (Saravanan et al., 2021). Biological synthesis typically employs microorganism-derived chemicals such as alcohols, flavonoids, alkaloids, quinones, terpenoids, and phenolic compounds, as well as exopolysaccharides, cellulose, and different enzymes (Nguyen et al., 2023).

The value of biological synthesis has grown due to its environmental benefits (Saravanan et al., 2021). Alcohols, flavonoids, alkaloids, quinones, terpenoids, and phenolic compounds are commonly used in biological synthesis, as are exopolysaccharides, cellulose, and other enzymes (Nguyen et al., 2023). Plant extracts are a comparatively simple technique among the several biological sources used for nanoparticle synthesis since they minimize the need for specialized methods such as isolation, cultivation, and culture management (Sharma et al., 2022). Furthermore, the process of synthesizing silver nanoparticles (AgNPs) with plant extracts is typically faster than that with microbes. This process is not only less expensive but also simpler for large-scale nanoparticle production (Sharifi et al., 2021).

Plant extraction techniques can be categorized into two main types: (i) traditional methods and (ii) environmentally friendly methods (Jha et al., 2022). Maceration, infusion, digestion, decoction, percolation, reflux, and Soxhlet extraction are widely used traditional techniques (Fonmboh et al., 2020). Silver nanoparticle (AgNP) synthesis is characterized using UV-Vis and SPR methods (Fozia et al., 2022). The size, shape, and colloidal stability of the nanomaterials were investigated using a zeta potential analyzer and a scanning electron microscope (SEM) (Liaqat et al., 2022). However, other environmental parameters, such as temperature, pH, pressure, and the chemicals involved, must be considered before moving to the 'green technique' for synthesizing nanoparticles (Arshad et al., 2024). The green synthesis of nanoparticles has various advantages over traditional chemical processes, including longer nanomaterial half-lives, increased efficiency, lower toxicity, and higher biocompatibility (Adeyemi et al.,2022).

Therefore, this study aims to summarize current achievements in the green synthesis of silver nanoparticles (AgNPs) and their antibacterial effectiveness against wound-associated microorganisms. The review also discusses the role of different plant extracts, synthesis methods, and parameters such as pH, temperature, and solvent conditions, as well as the antibacterial properties of green-synthesized AgNPs. Nanoparticles (NPs) have a specified size, defined as at least one dimension between 1 and 100 nanometers (Ijaz et al., 2020). Researchers can classify nanoparticles as 0D, 1D, 2D, or 3D based on their overall morphology (Roy et al., 2024). Due to the demand for novel antimicrobial agents capable of eradicating or suppressing diverse microbes, the use of nanotechnology to create effective antimicrobials represents a viable alternative (Mubeen et al., 2021).

Nanoparticles (NPs) have antibacterial properties that come from a different mechanism than standard antibiotics. Researchers believe that the efficiency of NPs is influenced by their surface area, which varies across different types of nanoparticles (Menichetti et al., 2023).

Researchers have focused their efforts on nano-sized silver particles (less than 100 nm) due to their numerous applications and amazing effectiveness (Hamad et al., 2020). Size, shape, surface charge, concentration, and colloidal form are the most important physicochemical variables influencing the antibacterial activity of silver nanoparticles (AgNPs) (Vazquez et al., 2020).

The process variation is determined by the nanoparticle distribution (Puspitasari et al., 2024). When it comes to AgNP recovery, the extracellular method outperforms the intracellular approach (Nguyen et al., 2023). The capabilities and procedures of the strains used in the synthesis of silver nanoparticles (AgNPs) differ due to the chemical compounds involved. Exopolysaccharides, peptides, reductases, cofactors, c-type cytochromes, and silver resistance genes are among the chemical substances identified in bacteria that can act as reducing agents (Xu et al., 2020). Nitrate reductase, an enzyme that uses NADH, has sparked renewed interest in the bacterial production of silver nanoparticles (AgNPs) (Sanket and Das, 2021). The enzyme absorbs electrons from NADH, converting it to NAD^+, and is oxidized in the process, facilitating the reduction of silver ions to silver nanoparticles (Behera et al., 2022). However, the methods involved in bacterial-mediated AgNP synthesis require investigation (Polash et al., 2021).

Fungi-mediated synthesis can produce intracellular and extracellular silver nanoparticles (AgNPs) depending on their location, with mycelia for intracellular production and fungal cell-free filtrate for extracellular production (Paulkumar and Murugan, 2023). Researchers prefer extracellular formation of silver nanoparticles (AgNPs) using fungi over internal approaches because it is easier to collect and process (Banerjee and Ravishankar, 2022).

Numerous fungi (AgNPs) are chosen for their amazing properties, such as the capacity to concentrate metals, resistance to metal-rich environments, rapid mycelium growth, and cost-effectiveness. AgNPs created using an extracellular approach with fungal extract can be purified by washing or precipitating any undesirable fungal components (Murillo et al., 2022). Furthermore, certain proteins released by fungi can act as capping agents, resulting in silver nanoparticles (AgNPs) with regulated morphologies (Sidhu et al., 2022). Different incubation circumstances, such as temperature and light source, can influence the characteristics of AgNPs (Khleifat et al., 2022).

Green-synthesized silver nanoparticles (AgNPs) are highly valued in biomedical and pharmaceutical applications due to their environmental friendliness, cost-effectiveness, simplicity of scalability, and greater yields than chemically generated counterparts (Rajoriya et al., 2021). Medicinal plants include several compounds. These phytochemicals are necessary for the formation of nanoparticles. Nanoparticles (NPs) linked with natural ingredients have shown stronger benefits and efficacy than traditional herbal treatments (Zuhrotun et al., 2023). The plant extract was prepared using different procedures, including Soxhlet extraction, solvent extraction with ethanol or methanol, and decoction extraction in an Erlenmeyer flask (Hashim et al., 2021). Different plants and their components may contain a variety of biomolecules that can act as reducing and stabilizing agents throughout the synthesis process. Thus, selecting appropriate plants and their components may be critical in biosynthesis (Flieger et al., 2021). These phytomolecules may also impact the surface properties of AgNPs. Producing nanoparticles from biological sources is far safer than using chemical or physical methods (Srivastava et al., 2021).

The impact of phytochemicals on the synthesis of silver nanoparticles (AgNPs) varies greatly depending on the plant species. More research is needed to elucidate the specific roles that phytochemicals play in AgNPs biosynthesis, such as reduction, immobilization, and dispersion, by quantitatively examining how the levels of specific functional phytochemicals vary across different plant tissues or species (Liu et al., 2023). Table 1 summarizes previous research on common qualitative phytochemical screening methodologies.

Table 1: *Common qualitative phytochemical screening tests for plant extracts*

Test	Phytochemical	Procedure	Observation	Role in AgNP Synthesis	Reference
Molisch test	Carbohydrates	1ml Molisch reagent +2ml aqueous extract and a few drops conc. Sulphuric acid.	A violet ring at the interface between two liquids	Weak reducing agent via aldehyde/hemiacetal groups; minor secondary role in capping	(Maheshwaran et al., 2024)
Froth (foam) test	Saponins	2–3 mL extract + water shake 30–60s.	Persistent froth (foam) layer	Surfactant-like stabilizer; reduces nanoparticle aggregation rather than driving reduction	(Mohlakoana, & Moteetee, 2021)
Dragendorff's test	Alkaloids	0.1ml extract and 2-3 drops of Dragendroff's reagent in a test tube.	An orange-red precipitate forms.	Nitrogen lone pairs coordinate Ag^{+} ions, assisting reduction and stabilization	(Shaikh & Patil, 2020)
Ferric chloride test	Tannin	2ml of extract, 1-2 drops of $FeCl_3$ solution.	Occurrence of blue-black or blue-green precipitate.	Strong reducing agent via phenolic hydroxyls; contributes to antioxidant-	(Khalid et al., 2023)

				antibacterial synergy	
Keller-Killiani test	Cardiac glycosides	5ml aqueous extract, 2ml glacial acetic acid,1 drop $FeCl_3$ and 1ml conc. H_2SO_4.	A brown ring forms at the interface.	Minor reducing contribution; limited direct evidence in the AgNP literature	(Maheshwaran et al., 2024)
Sodium Hydroxide test	Flavonoids	1 mL extract + 2 mL 10% NaOH. An intense yellow color is obtained; then add 1% HCl.	The yellow color becomes colorless.	Primary reducing agent via hydroxyl-to-quinone oxidation; also functions as a capping agent	(Salim et al., 2022)
Sulphuric acid test	Quinones	1ml plant extract and a few drops of concentrated H_2SO_4.	Red color forms.	Can act as electron shuttles, assisting Ag^+ reduction	(Ahmed & Majaz, 2022)
Lead tetraacetate test	Phenol	2 mL extract + 3 mL 10% lead acetate solution	A white precipitate forms.	Strong reducing and capping agent; associated with smaller, more stable nanoparticles	(Ahmed & Majaz, 2022)

Temperature, pH, reaction duration, metal salt volume, and plant extract concentration all have a complex relationship that influences particle size and shape (Ansari et al., 2023).

Because of its lower toxicity, many researchers have chosen to synthesize silver nanoparticles (AgNPs) using 1 mM silver nitrate salt (Asif et al., 2022). Some researchers used silver precursors in the 1-5 mM range and found that higher salt concentrations led to the formation of larger nanoparticles (Shaikh et al., 2021). Using a 5 mM salt concentration raised nanoparticle size from 12 ± 2 nm to 20 ± 3.3 nm. In addition, their studies used different doses of $AgNO_3$, ranging from 2 to 100 mM (Sikdar and Sikdar, 2023).

The pH level has a considerable impact on the surface charge of nanoparticles, affecting their colloidal stability and ability to assemble (Bélteky et al., 2021). At certain pH values, enhancing electrostatic repulsion among nanoparticles might prevent or reduce aggregation. This is critical for creating consistent and stable silver nanoparticle dispersions (Din et al., 2022).

Research indicates that the amount of extract directly correlates with the rise in the absorbance peak (Patel et al., 2024). Plant metabolites can affect the zeta potential of produced silver nanoparticles (AgNPs) (Yu et al., 2021). Zeta potential measurements were −20.7, −21.3, −12.0, and −11.3 mV for tea extract synthesis at 1%, 5%, 50%, and 100% concentrations, respectively. Furthermore, these findings indicate that the stability of silver nanoparticle (AgNP) suspensions improves with increasing extract concentrations (Sikdar, 2023).

Temperature is an important component in the green synthesis of silver nanoparticles. While the reaction normally happens at room temperature, resulting in extended synthesis periods, increasing the temperature can speed up the process. Higher temperatures reduce the concentration of Ag^{+} ions, resulting in a more uniform nucleation and smaller nanoparticles. Temperatures between 30 and 100°C are commonly used. The reaction rate often rises with temperature (Bélteky et al., 2021).

Green synthesis methods are increasingly replacing physical and chemical procedures due to concerns about the requirement for complex equipment and synthesis conditions. Aerosols, UV light, and thermal breakdown are all examples of traditional physical processes that frequently need high temperatures and pressures (Ying et al., 2022).
These systems have significant disadvantages, including dependency on hazardous materials, high prices with limited effectiveness, risky practices, and the production of harmful byproducts that harm the environment (Paiva et al., 2021). The complicated procedures related to the synthesis methods described, as well as their limitations, limit nanoparticle biocompatibility. As a result, there is an urgent need to develop a cost-effective, one-step, dependable, biocompatible, and non-toxic method for generating environmentally friendly nanoparticles (Nguyen et al., 2023).

These elements play critical roles in cell membranes, proteins, and DNA bases (Hashim et al., 2021). Silver nanoparticles are known to disrupt the integrity of bacterial cell walls and membranes, causing a variety of structural changes. Numerous studies have shown its efficacy against microorganisms. These studies show that smaller nanoparticles have a higher ability to penetrate bacterial cells (Yin et al., 2020).

AgNPs collect in the pit after attaching to the cell surface, causing the cell membrane to rupture. Their nanoscale dimensions allow them to pass through the cell wall and alter the structure of the cell membrane. Furthermore, denaturation of the cytoplasmic membrane can cause cell rupture and lysis (Anees et al., 2020). Another mechanism suggested functioning alongside the other two is the release of silver ions from the nanoparticles. Because of their size and charge, these ions can interact with biological components, potentially altering metabolic pathways, disrupting membranes, and even influencing genetic material (Bruna et al., 2021).

Silver ions also inhibit respiratory enzymes, causing an accumulation of reactive oxygen species (ROS) and impeding ATP synthesis (Morozova, 2021). Silver nanoparticles (AgNPs) also inhibit ribosome activity, which affects protein synthesis in bacterial cells. They also prevent bacterial signal transduction by eliminating phosphate groups from tyrosine residues on peptide surfaces, causing bacterial cell death and inhibiting microbial growth (Mikhailova, 2020).

Antibiotic resistance is a major public health concern in both developing and developed nations. The increasing prevalence of illnesses resistant to multiple medications is threatening the efficacy of antibiotic treatments (Oves et al., 2022).

Silver nanoparticles have significant antimicrobial properties. These nano-formulations have shown a strong ability to suppress the growth of a variety of microorganisms, including bacteria. Silver nanoparticles have been shown in studies to have strong antibacterial effects against both bacterial strains. They proposed that the variable sensitivity of the two bacterial species could be attributed to their distinct structural features, as well as the shape and size of the silver nanoparticles (Almatroudi, 2020). Table 2 highlights recent studies on the antibacterial activity of green-synthesized silver nanoparticles obtained from various plants against wound-associated infections.

Table 2: *Antibacterial activity of green-synthesized silver nanoparticles against wound-associated pathogens*

Plants used for AgNps	**Solvent/extract**	**Wound-associated Bacteria**	**Zone of inhibition**	**Reference**
Azadirachta indica leaves	Aqueous extract	*Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*	17.7mm, 18.7mm, 10.3mm,17.7mm	(Chinnasamy et al., 2021)
Olea europaea (olive fruit)	Aqueous extract	*Staphylococcus aureus, Salmonella typhi, Escherichia coli, Klebsiella oxytoca*	7.9mm, 23.6mm, 26.7mm, 24.1mm	(Ullah et al., 2023)
Moringa oleifera leaves	Distilled water	*Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae*	20.6mm, 21.0mm, 18.0mm	(Mohammed & Hawar, 2022)
Olea europaea leaves	Methanol	*Staphylococcus aureus, Salmonella typhi, Pseudomonas aeruginosa*	23mm, 24mm, 21mm	(Hashmi et al., 2024)

Ehretia cymosa leaves	Methanol	*Staphylococcus aureus, Escherichia coli*	17.8mm, 13.8mm	(Adeleye et al., 2023)
Calendula officinalis flowers	Ethanol	*Staphylococcus aureus, Escherichia coli*	22mm, 22mm	(Nanda et al., 2023)
Cucumis sativus fruit	extract	*Staphylococcus aureus*	2.5mm	(Iqbal et al., 2024)
Green tea leaves	Aqueous extract	*Klebsiella sp, Staphylococcus aureus*	10mm, 11mm	(Widatalla et al., 2022)
Hibiscus tiliaceus leaves	Deionized water	*Bacillus subtilis, Pseudomonas aeruginosa, Proteus vulgaris*	10.91mm, 10.47mm, 11.94mm	(Konduri et al., 2024)
Citrus sinensis peel	Aqueous extract	*Staphylococcus aureus, Bacillus subtilis, Escherichia coli, and Salmonella typhi*	25.2mm, 20.6mm,15.3mm, 10.8mm	(Gupta et al., 2025)

Health and medicine have experienced the most substantial research and development in nanoparticle-based antimicrobial technologies (Yaqoob et al., 2020). A recent investigation looked into the efficacy of a disinfectant with silver nanoparticles (AgNPs) as an active ingredient for cleaning surgical masks. The resultant formulation exhibited strong antibacterial capabilities, significantly inhibiting the growth of germs such as *E. coli*, *K. pneumoniae*, and *S. aureus* on treated masks (Valdez et al., 2021).

Catheters treated with silver nanoparticles (AgNPs) were tested for their ability to prevent infections and improve sterility. Catheters embedded with these nanoparticles have been shown in studies to suppress the growth and biofilm development of several pathogens, including E. coli, S. aureus, and C. albicans, for at least 72 hours. Furthermore, the addition of AgNPs greatly reduced the growth of Enterococcus and P. aeruginosa strains (Bruna et al., 2021). In addition to their antibacterial capabilities, research has investigated the healing benefits of silver and its nanoparticles, resulting in the development of several wound dressings with better healing and antibacterial effects (Rybka et al., 2022).

Their anti-inflammatory and anti-angiogenic properties make them useful in cancer therapy treatment (Simon et al., 2022). Silver nanoparticles can inhibit the activity of tyrosinase, reducing melanin synthesis and encouraging a lighter skin tone. Furthermore, their antioxidant properties aid in skin rejuvenation, reducing the visible signs of aging (George and Devi, 2024).

The effective clinical use of silver-based wound dressings provides an essential translational benchmark for green-synthesised silver nanoparticles (AgNPs). In the treatment of burns and chronic wounds, several FDA-approved commercial dressings, such as ionic silver hydrofiber dressings (Aquacel® Ag) and nanocrystalline silver dressings (ActicoatTM), have demonstrated good wound-healing outcomes and potent antibacterial activity. According to clinical data, these dressings minimize the need for dressing changes and enhance patient comfort while lowering bacterial burden, preserving a moist wound
environment, and promoting quicker wound healing (Shrestha et al., 2024). Despite variations in silver-release processes and dressing matrices, comparative investigations have demonstrated that several commercial silver dressings have similar antibacterial effectiveness. However, dressing composition, wound selection, and controlled and prolonged silver ion release all play a major role in their clinical performance. These results highlight the need for additional standardization of synthesis protocols, thorough safety evaluation, and well-designed clinical trials before green-synthesized AgNPs can achieve clinical translation comparable to commercially available silver dressings, despite their promising antibacterial properties.

Material and Methods

This review involves a search of peer-reviewed articles published between 2020 and 2025, with a particular emphasis on the green synthesis of silver nanoparticles (AgNPs) and their antimicrobial application in wound care. Databases such as ScienceDirect, SpringerLink, PubMed, Google Scholar, and Scopus produced studies using keywords like "green synthesis," "silver nanoparticles," "plant extracts," "antibacterial activity," and "wound infection." Only peer-reviewed works published in English were considered. Selected publications focused on plant-mediated synthesis methods, reaction conditions (such as pH, temperature, solvent type, and concentration), and antibacterial activity against wound-related pathogens such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*.

Characterization methods, including UV-Vis, FTIR, SEM, and XRD, were included in the extracted data. Studies that focused primarily on physical or chemical synthesis methods, cytotoxicity, or in vivo wound healing assessments were excluded to retain an emphasis on ecologically friendly synthesis procedures.

Instead of using a formal, systematic process (like PRISMA-guided), this review uses a narrative approach. Instead of using a pre-registered methodology with recorded screening and exclusion counts at every stage, researchers chose articles based on their relevance to plant-mediated AgNP synthesis and wound-related antibacterial activity, relevance, and methodological completeness of reporting. This method does not allow the quantitative meta-analytic pooling that a completely systematic review would offer, but it is suitable for synthesizing the wide range of synthesis pathways and antibacterial discoveries described in the literature. This distinction is clear, and urge future literature updates to use a PRISMA-based systematic methodology as studies on green-synthesized AgNPs increase.

Qualitative evaluation of the data helped identify phytochemical agents and important parameters that influence nanoparticle yield, size, and antibacterial activity. The studies that reported inhibition zones against wound-associated bacteria for green-synthesized AgNPs were compared.

Results and Discussion

Phytochemical-Mediated Synthesis of Silver Nanoparticles

Plant-derived phytochemicals play an important role in the green production of silver nanoparticles. Medicinal plant extracts contain bioactive components such as flavonoids, phenolics, terpenoids, and alkaloids, which act as natural reducing and capping agents. These biomolecules help reduce silver ions (Ag^+) to metallic silver under environmentally safe conditions.

The phytochemical profile changes depending on the plant species, extraction solvent polarity, plant part chosen, and extraction process, all of which have a direct impact on nanoparticle yield, size, and morphology. Optimizing these parameters improves the functional properties of AgNPs in biomedical applications (Dolai et al., 2021). Table 3 summarizes what each characterization technique confirms about green-synthesized AgNPs, the results typically expected, and how each property relates to antibacterial performance.

Table 3: *Characterization techniques for green-synthesized AgNPs and their relevance to antibacterial activity*

Technique	Confirms	Expected result	Link to antibacterial activity	References
UV-Vis spectroscopy	Formation of AgNPs via surface plasmon resonance (SPR)	Absorption peak typically at 400–450 nm; peak sharpness/position indicates size and monodispersity	Sharper, blue-shifted peaks indicate smaller, more uniform particles, associated with greater membrane penetration and potency	(Mandru et al., 2023)

FTIR	Functional groups (phenolic OH, C=O (amide), carboxyl) responsible for Ag^+ reduction and capping	Shifted/new bands (e.g., ~3400 cm^{-1} OH, ~1630–1650 cm^{-1} amide I) versus raw extract	Capping phytochemical identity governs surface charge/stability and thus sustained Ag^+ ion release	(Anastasiadis., 2025; Dai et al., 2023))
SEM/TEM	Particle morphology and size distribution	Predominantly spherical particles, tens of nanometers in diameter	Smaller size and higher surface-area-to-volume ratio increase bacterial membrane contact and efficiency	(Huq et al., 2026)
XRD	Crystallinity confirms face-centered cubic (FCC) metallic silver	Bragg reflections at 2θ = 38°, 44°, 64°, 77° corresponding to (111), (200), (220), (311) planes	Crystallinity affects particle stability and dissolution rate, indirectly influencing sustained ion release	(Mubeen et al., 2022)

These interpretations are consistent with established characterization practices in the plant-mediated AgNP literature.

Influence of Synthesis Parameters on AgNP Properties

Reaction conditions have a significant impact on AgNP synthesis, shape, and antibacterial activity. Alkaline pH (8-10) enhances silver ion reduction, resulting in smaller, uniform nanoparticles. The pH affects surface charge, influencing colloidal stability and preventing aggregation via electrostatic repulsion (Ansari et al., 2023). Temperature optimization is similarly crucial. Moderate heating (40-70°C) promotes phytochemical-silver ion interactions, resulting in improved particle dispersion and shorter synthesis times. However, high temperatures or extended reaction periods enhance particle agglomeration, which reduces antibacterial activity (Alharbi et al., 2022).

Silver Salt Concentration

Silver nitrate concentration has a considerable impact on nanoparticle size and distribution. Most research uses 1 mM $AgNO_3$ due to its low toxicity; however, doses ranging from 1-5 mM have been tested. Higher concentrations produce bigger nanoparticles, which may compromise stability due to increased aggregation potential (Bélteky et al., 2021).

Standardization and Reproducibility of Synthesis Parameters

Several research efforts have not thoroughly identified the synthesis variables causing batch-to-batch variability in green AgNP synthesis. Temperature, pH, shaking speed, incubation time, extract concentration, $AgNO_3$ concentration, and extract-to-$AgNO_3$ ratio were all tested simultaneously using a Plackett–Burman statistical screening design applied to *Citrus sinensis* peel-mediated synthesis. The study found that pH alone significantly impacted nanoparticle size and zeta potential (optimized zeta size 11.44 nm) (Mickky et al., 2024). Tight pH control should take precedence over other reaction conditions in standardization attempts, according to this factorial research.

To improve cross-study reproducibility, a minimum reporting checklist for future plant-mediated AgNP synthesis studies based on the parameters that are most frequently under-reported in the surveyed literature: (i) exact $AgNO_3$ molar concentration; (ii) reaction pH at the point of mixing; (iii) reaction temperature and heating method; (iv) plant extract-to-$AgNO_3$ volume ratio; (v) reaction/incubation time; and (vi) zeta potential and polydispersity index (PDI) reported alongside mean particle size. The quantitative cross-study comparisons that are missing from the current research would be possible with consistent reporting of these six factors.

Antibacterial Activity against Wound Pathogens

Mechanisms of Action

Green-synthesized AgNPs outperform crude plant extracts in terms of antibacterial activity due to their higher surface area-to-volume ratio, which allows for more effective interactions with bacterial membranes and intracellular targets.

Antibacterial mechanisms include disruption of bacterial cell wall integrity, membrane depolarization and increased permeability, production of reactive oxygen species (ROS), which causes oxidative stress, inactivation of respiratory enzymes and inhibition of ATP synthesis, disruption of protein synthesis via ribosomal dysfunction and DNA damage, and interference with replication. Phytochemical bio-capping layers improve nanoparticle stability, inhibit aggregation, and provide synergistic antibacterial effects via continuous silver ion release (Bruna et al., 2021).

Comparative Efficacy against Wound Pathogens

Recent research has reported that AgNPs derived from various plant extracts have substantial antibacterial properties against both Gram-positive and Gram-negative wound infections. AgNPs produced from *Citrus sinensis* peel, *Calendula officinalis* flowers, and *Moringa oleifera* leaves, for example, inhibit *Staphylococcus aureus* in zones ranging from 21 to 25 mm. Similarly, AgNPs derived from *Olea europaea* fruit, *Moringa oleifera* leaves, and *Calendula officinalis* flowers inhibit *Escherichia coli* in zones of 21-26 mm (Gupta et al., 2025; Mohammed & Hawar, 2022). These findings show that plant species, extraction solvents, and plant parts all have a major impact on antibacterial potency. Table 4 gathers physicochemical characterization data, particle size, shape, zeta potential, and synthesis conditions for representative plant sources previously discussed in Table 2. These studies provide this data directly from their original characterizations.

Table 4: *Physicochemical characteristics of green-synthesized AgNPs from selected plant sources*

Plant source	Particle size	Shape	Zeta potential	Antibacterial activity	Synthesis conditions	Reference
Azadirachta indica leaves	~33 nm (SEM/TEM)	Spherical	Not reported (related neem studies: −19.6 to −33.2 mV)	ZOI 10.3–18.7 mm vs. *B. cereus, E. coli, P. aeruginosa, S. aureus*	Aqueous leaf extract, room temperature	(Chinnasamy et al., 2021)
Olea europaea fruit	~77 nm (SEM)	Spherical, monodispersed	−40 mV	ZOI up to 27 mm vs. *E. coli* (highest); 7.9–24.1 mm range across tested strains	Aqueous fruit extract	(Ullah et al., 2023)
Citrus sinensis peel	30–40 nm (range 11–50 nm across studies)	Spherical	−14 to −23.7 mV (varies with pH/conditions)	ZOI 10.8–25.2 mm vs. *S. aureus, B. subtilis, E. coli, S. typhi*	Aqueous peel extract, 1 mM $AgNO_3$	(Gupta et al., 2025)
Green tea leaves	Not reported in original source	Spherical	Not reported	ZOI 10–11 mm vs. *Klebsiella sp., S. aureus*	Aqueous leaf extract	(Widatalla et al., 2022)

The inconsistent reporting of zeta potential and precise crystallite size for several other plant sources in Table 2 (*Moringa oleifera, Calendula officinalis, Ehretia cymosa, Cucumis sativus,* and *Hibiscus tiliaceus*-derived AgNPs) restricts a fully quantitative cross-study comparison. Rather than being a flaw in the current synthesis, this difference in reporting is itself a reproducibility gap that this study indicates as having to be addressed by future primary studies.

Factors Contributing to Enhanced Activity

Green-synthesized AgNPs outperform crude extracts in terms of membrane penetration due to nanoscale dimensions, sustained silver ion release from nanoparticle surfaces, synergistic effects between silver ions and phytochemical coatings, increased bioavailability and cellular uptake, and a multi-target mechanism addressing antibiotic resistance.

However, reported inhibition zones vary significantly (ranging from 2.5 mm to 26.7 mm), most likely due to variances in synthesis processes, bacterial strain sensitivity, testing methodology, and nanoparticle properties (size, shape, concentration).

Mechanism of Antibacterial Action of Green-Synthesized AgNPs

Instead of having a single mode of action, green-synthesized AgNPs use a series of physical and metabolic processes to produce their antibacterial effect. AgNPs weaken the integrity of the membrane after first adhering electrostatically to the bacterial cell wall, increasing permeability and allowing intracellular contents to seep out (Wang et al., 2024). Reactive oxygen species (ROS) result from the release of Ag^+ ions, which occurs from the nanoparticle surface and after intracellular uptake. Bacterial cell death results from the resulting oxidative stress, which destroys proteins, lipids, and DNA, slows important enzymes, and hinders DNA replication (Shabulal et al., 2024).

Figure 1 summarizes this pathway. The enhanced and more consistent antibacterial activity discussed throughout this review is directly caused by nanoparticle properties that support sustained, controlled ion release, such as smaller size, higher surface-area-to-volume ratio, and stable capping by phytochemicals, because Ag^+ ion release and ROS generation act synergistically rather than independently.

Limitations and Variability in Results

Analysis of the available literature finds significant variation in reported antibacterial efficacy. Inhibition zones range from 2.5 mm (*Cucumis sativus*) to 26.7 mm (*Olea europaea*), indicating variations in synthesis techniques, bacterial strain sensitivity, nanoparticle properties, and testing methods. This inconsistency highlights the necessity of uniform techniques. Furthermore, the precise phytochemical substances responsible for efficient nanoparticle production and increased antibacterial activity remain poorly characterized. Most papers lack comprehensive cytotoxicity and long-term stability data, which are crucial for clinical translation.

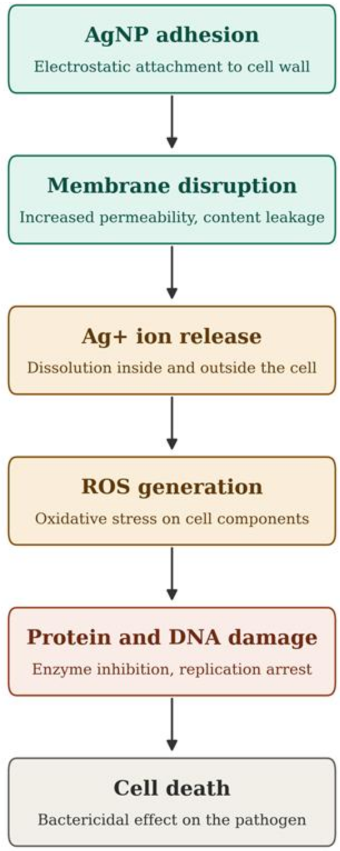

Fig. 1: *Proposed mechanism of antibacterial action of green-synthesized silver nanoparticles against bacterial cells, from initial adhesion through to cell death.*

Explaining Variability in Reported Inhibition Zones

Methodological heterogeneity and actual variations in nanoparticle potency explain the wide range of reported inhibition zones (2.5–27 mm) because studies differ in bacterial inoculum density, disk/well loading volume, incubation time, and whether zones were normalized to a fixed concentration of silver. Therefore, without such normalization, cross-study statistical comparison is not entirely valid, indicating a methodological gap in addition to the biological one. Notably, susceptibility does not consistently favor Gram-positive or Gram-negative organisms across studies; *Olea europaea*-derived AgNPs, for example, showed greater inhibition of Gram-negative *E. coli* (27 mm) than Gram-positive *S. aureus* (7.9 mm) (Ullah et al., 2023), contrary to the common assumption that Gram-positive bacteria are uniformly more susceptible to AgNPs.

Statistical Trend between Particle Size and Inhibition Zone

The analysis of particle size and zone-of-inhibition (ZOI) values against *E. coli* from six independently published green-synthesis studies, each using a different plant source and conducted by different research groups (Table 5), aimed to determine the correlation between the two variables.

Table 5: *Particle size and zone of inhibition (E. coli) compiled across independent green-synthesis studies*

Plant	Particle size (nm)	*E. coli* ZOI (mm)	Reference
Morinda citrifolia seed	3	20.45	(Morales-Lozoya et al., 2021)
Azadirachta indica leaf	33	18.7	(Chinnasamy et al., 2021)
Calluna vulgaris leaf	45	15.38	(Akalin, 2024)
Moringa oleifera gum	50	21.0	(Irfan et al., 2021)
Euphorbia serpens	55	20.0	(Ahmad et al., 2022)
Olea europaea fruit	77	27.0	(Ullah et al., 2023)

Between 2020 and 2025, six independent studies observed a positive but non-significant correlation between reported AgNP particle size and the zone of inhibition (ZOI) against Escherichia coli (Pearson $r = 0.49$, $p = 0.33$). The observed direction of the association contrasts with the commonly proposed mechanistic expectation that smaller nanoparticles, owing to their higher surface-area-to-volume ratio, exhibit greater antibacterial activity. However, the data did not show a statistically significant relationship, suggesting that particle size alone cannot reliably predict antibacterial potency.

This apparent inconsistency is likely attributable to substantial methodological heterogeneity among the included studies. Although all studies employed green-synthesized AgNPs, they differed in bacterial strain source, inoculum density, nanoparticle concentration, plant-derived capping phytochemistry, and antibacterial testing method (e.g., disk diffusion versus well diffusion). These factors may have influenced the observed antibacterial activity and could have obscured any underlying size–activity relationship.

The findings therefore illustrate the limitations of cross-study comparisons using at present available literature. Restricting analysis to recent (2020–2025) independently conducted studies does not allow us to confirm a consistent relationship between particle size and antibacterial activity without controlling for key experimental variables. Accordingly, these results support the need for standardization in reporting nanoparticle characterization and antibacterial testing parameters so that particle size can reliably predict AgNP antibacterial efficacy across different studies.

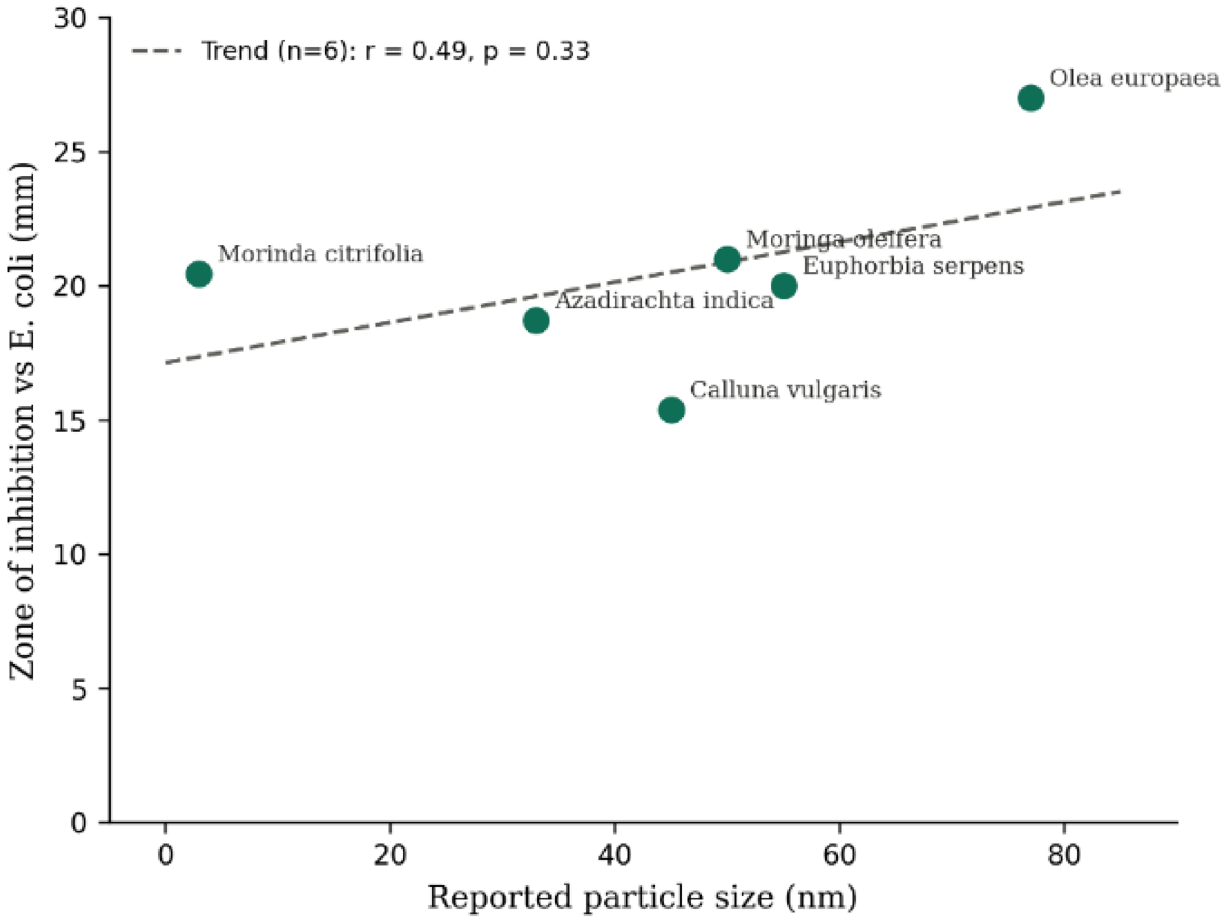

Fig. 2: *Relationship between reported AgNP particle size and zone of inhibition against E. coli across seven independent green-synthesis studies.*

Cytotoxicity toward fibroblasts, keratinocytes, and mammalian cells

Green-synthesized AgNPs are generally less cytotoxic than ionic silver at antibacterially relevant concentrations, according to in vitro studies using normal human dermal fibroblasts (NHDF) and keratinocytes (NHEK), despite the lack of in vivo and clinical data on cytotoxicity (Galandšová et al., 2016; Franková et al., 2016). Cytotoxicity is very size-dependent: smaller AgNPs (~5 nm) are significantly more hazardous to dermal fibroblasts than bigger particles (~40 nm). This effect can be applied to the generation of reactive oxygen species and oxidative stress, rather than to direct membrane rupture alone (Avalos et al., 2021). Cytotoxicity, like antibacterial efficacy, is not a fixed property of AgNPs and depends on synthesis route, capping phytochemical, and particle size, as evidenced by the wide variation in reported IC_{50} values for skin/mammalian cell lines by plant source, ranging from ~37 µg/mL (HEK293, Citrus unshiu peel AgNPs) to over 480 µg/mL (chemically synthesized AgNPs on gingival fibroblasts) (Mafhala et al., 2024; Al Sufyani NM et al., 2022).

Conclusion

Green synthesis of silver nanoparticles (AgNPs) offers a promising and environmentally sustainable approach for combating wound-associated infections. Phytochemicals from plants act as natural reducing and stabilizing agents, influencing nanoparticle size, shape, stability, and antibacterial activity. AgNPs synthesized from *Citrus sinensis*, *Calendula officinalis*, *Moringa oleifera*, and *Olea europaea* demonstrate greater antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* than their corresponding crude extracts. Although green-synthesized AgNPs generally exhibit lower cytotoxicity than ionic silver at antibacterial concentrations, the specific phytochemical constituents responsible for efficient nanoparticle formation and enhanced antimicrobial activity remain poorly characterized, and quantitative relationships between phytochemical composition and nanoparticle properties are still largely unexplored. In addition, considerable variation in synthesis procedures, nanoparticle characterization, and antibacterial testing methods limits reliable comparison among published studies and hinders clinical translation. Future research should focus on elucidating antibacterial mechanisms, optimizing synthesis parameters, adopting standardized synthesis and reporting protocols, evaluating therapeutic indices by integrating cytotoxicity and antibacterial efficacy, and conducting rigorous in vivo safety and efficacy studies. Quantitative phytochemical analysis using HPLC or equivalent techniques is also required to identify bioactive constituents and correlate their concentrations with nanoparticle characteristics and antibacterial performance (Al Mutairi et al., 2022). These advances will improve reproducibility and facilitate the translation of plant-mediated AgNPs into clinically relevant wound-care applications.

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