


Review Article

Eco-friendly Biosynthesis of Metallic Nanoparticles and Their Antibiofilm Activity against Bacterial Pathogens: A Review

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Article Info

Keywords: Plant extract, Nanoparticles, Antibacterial, Antibiofilm.**Received:** 28.10.2025**Accepted:** 21.11.2025**Published:** 27.11.2025 © 2025 by the author's. The terms and conditions of the Creative Commons Attribution (CC BY) license apply to this open access article.

Abstract

The increasing prevalence of antibiotic-resistant bacteria has created an urgent need for novel antimicrobial strategies. Metallic nanoparticles, especially those synthesized via green and eco-friendly methods, have emerged as promising alternatives due to their potent biological activities and biocompatibility. This review highlights recent advances in the eco-friendly biosynthesis of metallic nanoparticles using plant extracts, microorganisms, and other natural sources as reducing and stabilizing agents. The discussion focuses on their physicochemical properties, mechanisms of antibacterial and antibiofilm action, and their effectiveness against clinically important pathogens such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Escherichia coli*. Additionally, the review emphasizes how green-synthesized nanoparticles interfere with quorum sensing, inhibit biofilm formation, and enhance bacterial membrane disruption. Overall, eco-friendly metallic nanoparticles represent a sustainable and effective approach for combating multidrug-resistant bacteria and biofilm-associated infections, providing a promising direction for future biomedical applications.

1. Introduction

Antibiotic resistance is one of the most significant challenges facing the healthcare sector in the modern era, especially with the increasing incidence of infections that do not respond to traditional drug treatments [1]. Among these bacteria, *Pseudomonas aeruginosa* is considered one of the most dangerous pathogens, causing chronic and difficult-to-treat infections, especially in hospitals and among immunocompromised patients [2]. This is due to its high capacity to form biofilms, complex structures surrounded by a protective layer that renders it highly resistant to environmental factors and antibiotics, enhancing its ability to survive and multiply in harsh conditions [3].

In light of this challenge, nanotechnology has emerged as a promising solution to combat bacterial infections [4]. Silver nanoparticles (AgNPs) have gained widespread interest in scientific circles due to their unique physical and chemical properties, which give them a superior ability to kill bacteria through multiple mechanisms, such as free radical production, cell wall penetration, and interference with biological processes within the bacterial cell [5].

However, traditional chemical methods for manufacturing these particles may require the use of harmful and expensive chemicals [6]. Which prompted researchers to turn to green synthesis as an environmentally friendly and safer alternative [7].

This approach relies on using plant extracts or microorganisms to reduce metal ions and convert them into nanoparticles, without the need for harsh industrial conditions or chemical solvents [8]. Among these sources, *Allium sativum* (garlic) is a plant rich in active compounds such as allicin, which exhibits antibacterial and antifungal properties [9]. Garlic contains a group of bioactive compounds such as allicin and diallyl sulfides, which play a major role in reducing the growth of bacteria and destroying their cell walls [10]. In addition to its reducing properties, garlic makes it an ideal material for the green and safe synthesis of silver nanoparticles [11].

This study focuses on the use of *Allium sativum* extract in the synthesis of silver nanoparticles and the evaluation of their antimicrobial efficacy against the biofilm-forming bacterium *Pseudomonas aeruginosa*. The results of this study may open new horizons in the field of nanotherapy by providing natural alternatives that can be used in the pharmaceutical and medical industries to combat bacterial infections, especially those resistant to traditional antibiotics.

2. Green Synthesis of Silver Nanoparticles using *Allium sativum*

In recent years, nanotechnology has emerged as a rapidly growing field with broad applications in medicine, biotechnology, and environmental science. Among different metallic nanoparticles, silver nanoparticles (AgNPs) are the most widely studied due to their remarkable antibacterial, antifungal, and antiviral properties [12]. Traditional methods for synthesizing AgNPs often involve toxic chemicals and high-energy processes, which raise environmental and biological safety concerns. Therefore, green synthesis using biological materials has become a sustainable and eco-friendly alternative [13] and Figure 1.

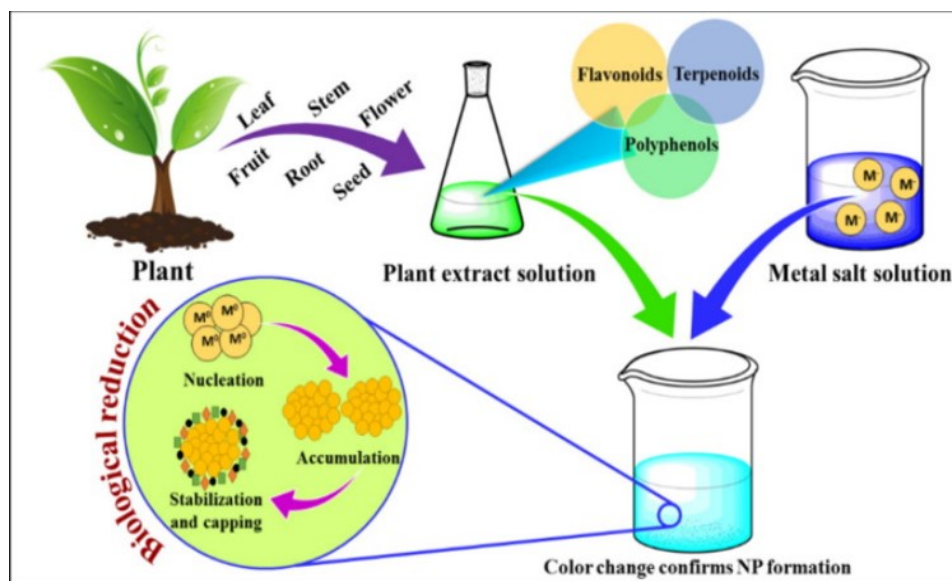


Figure 1: Green Synthesis of Silver Nanoparticles

One of the most effective natural sources for green synthesis is *Allium sativum* (garlic), a medicinal plant rich in bioactive compounds that can act as both reducing and stabilizing agents during nanoparticle formation [14]. *Allium sativum* contains several bioactive molecules such as allicin, S-allyl cysteine, flavonoids, phenolics, and sulfur-containing compounds [15].

These compounds play a crucial role in reducing silver ions (Ag^+) from silver nitrate solution to elemental silver (Ag^0), leading to the formation of nanoparticles.

At the same time, these biomolecules stabilize the nanoparticles by capping their surface, preventing aggregation and ensuring long-term stability [16].

The synthesis process begins when an aqueous extract of garlic is mixed with a silver nitrate ($AgNO_3$) solution. The bioactive compounds in garlic extract donate electrons to silver ions, reducing them to metallic silver. This reduction is visually indicated by a color change from colorless to brownish-yellow, due to surface plasmon resonance (SPR) - a characteristic optical property of silver nanoparticles [17].

Characterization of Synthesized AgNPs

The synthesized AgNPs are typically characterized using various analytical techniques:

- **UV-Visible Spectroscopy:** To confirm nanoparticle formation by detecting the SPR peak (usually between 400–450 nm).
- **FTIR (Fourier Transform Infrared Spectroscopy):** To identify functional groups responsible for reduction and stabilization.
- **SEM and TEM:** To determine the size, shape, and morphology of nanoparticles, which are usually spherical and range between 10–50 nm.
- **XRD (X-ray Diffraction):** To verify the crystalline nature of the nanoparticles [18].

Advantages of Using *Allium sativum* Extract

- **Eco-friendly and non-toxic:** avoids hazardous chemicals.
- **Cost-effective:** Uses easily available natural material.
- **Biocompatible:** Suitable for biomedical applications.
- **Dual functionality:** Garlic compounds reduce and stabilize nanoparticles simultaneously.
- **Enhanced biological activity:** Synergistic effects between silver and garlic's natural antimicrobial molecules [19].

Applications of *Allium sativum*-Mediated AgNPs

1. **Antimicrobial Activity:** Effective against a wide range of pathogenic bacteria including *Pseudomonas aeruginosa*, *E. coli*, and *Staphylococcus aureus*.
2. **Antibiofilm Properties:** Disrupt bacterial biofilms and inhibit quorum sensing.
3. **Wound Healing:** Promote faster recovery due to antimicrobial and anti-inflammatory effects.
4. **Medical Device Coatings:** Prevent microbial colonization on surfaces.
5. **Environmental Uses:** Useful in water purification and antimicrobial packaging materials [20].

Green synthesis of silver nanoparticles using *Allium sativum* represents a simple, sustainable, and biologically safe approach for nanoparticle production [21]. Garlic's natural phytochemicals enable effective reduction and stabilization of silver ions, resulting in stable, bioactive nanoparticles with strong antimicrobial and antibiofilm properties [22]. This eco-friendly method provides a promising platform for biomedical and environmental applications, supporting the transition toward green nanotechnology [10].

Antibacterial Activity of *Allium sativum* - Based AgNPs

Although the mechanism of AgNP synthesis using “bio-factories” has not been fully studied, there are currently many data on this subject. Most researchers note several areas through the antibacterial effect of AgNPs is realized, such as;

1. Cell membrane destruction and leakage of its cellular contents.
2. Binding to functional groups of proteins, causing protein denaturation and cell death.
3. Penetration inside the cell and damage of intracellular structures (vacuoles, ribosomes).
4. Inactivation of the respiratory chain.
5. Blocking DNA replication and/or turning it into a condensed form.
6. Alteration in the transcription processes.
7. Inhibition of protein biosynthesis (translation).
8. Denaturation of enzymes transporting nutrients through the bacterial cell membrane.
9. Generation of reactive oxygen species (ROS).
10. Modulation of signal transduction pathways. According to these ways, AgNPs are able to destroy a wide range of microorganisms, including both Gram-negative and Gram-positive, making them a wonderful remedy even against antibiotic-resistant strains of bacteria [23].

Another possible mechanism of antimicrobial activity is due to free radical formation when AgNPs interact with bacteria, resulting in the release of silver ions inside the cell, causing the cell contents to leak out and ultimately initiating protein denaturation [24]. ROS are by-products of normal oxygen metabolism, and their level is controlled by the antioxidant protection system, such as glutathione/glutathione disulfide (GSH/GSSG). ROS are formed by interactions with enzymes and/or biomolecules, causing cell damage. Excessive production of ROS, such as superoxide, hydrogen peroxide, and hydroxyl radicals, often promotes cellular oxidative stress in microorganisms. Therefore, the ability of AgNPs to generate ROS and free radicals and thus enhance oxidative stress in cells is often mediated by their antibacterial activity [25].

AgNPs synthesized using garlic extract exhibit strong antibacterial activity against both Gram-positive and Gram-negative bacteria. In the case of *Pseudomonas aeruginosa*, the nanoparticles disrupt the bacterial cell membrane, generate reactive oxygen species (ROS), and interfere with cellular respiration and DNA replication. The synergistic effect of silver ions with garlic's sulfur compounds enhances the antimicrobial potency, often exceeding that of chemically synthesized AgNPs [26].

Anti-Biofilm Activity against *Pseudomonas aeruginosa*

The main intracellular signaling pathway governing biofilm formation and maturation mechanisms is quorum sensing (QS) [27]. QS is a bacterial communication system which regulates gene expression in proportion to cell density. When the quorum is reached, small extracellular molecules called autoinducers (AIs) are released and bind to their receptors in a neighboring cell to activate the transcription of virulence genes. In *P. aeruginosa*, three QS circuits named las, rhl and pqs are described. The las and rhl systems can be found in several pathogens. They produce and recognize acyl-homoserine lactone-like AI: the N-(3-oxododecanoyl)-L-homoserine lactone (3-oxo-C12-HSL) odDHL and the N-butanoyl-L-homoserine lactone (C4-HSL) BHL, that activate their transcription factors LasR and RhlR, respectively. A third specific pqs system produces and detects the 2-heptyl-3-hydroxy-4(1H)-quinolone PQS (*Pseudomonas* quinolone signal) [28] and Figure 2.

The extracellular aqueous polymeric matrix is composed of proteins, rhamnolipids, extracellular DNA (eDNA) and exopolysaccharides named Pel, Psl and alginate [29]. Psl and Pel maintain structural integrity and are necessary for the cell adhesion to biotic or abiotic surfaces. Their production is stimulated by c-di-GMP. The alginate is essentially secreted by mucoid strains in the lungs of CF patients in response to stressful conditions (nutrients deprivation, antibiotics, slower cell growth, high osmotic pressure). In addition to preserving matrix structural cohesion, the alginate helps evade the immune system by resisting phagocytosis and macrophage-mediated free radical damage, but also diminishes antibiotic diffusion [30]. DNA, which is excreted following eukaryotic cell lysis induced by pyocyanin, is mainly a cell–cell interconnecting component. It contributes to the mushroom-like structure of the biofilm, can serve as a nutrient source and appears as a proinflammatory molecule by activating neutrophils. It can also reduce antibiotic penetration and promote horizontal gene transfer, and it takes part in the coordination of cell movements during twitching motility, thus contributing to biofilm expansion [31]. In the early stages of biofilm formation, soluble lectins A and B (LecA and LecB), aggregating proteins implemented at the outer membrane surface, strengthen the biofilm stability by binding to matrix and membrane saccharides from other cells [32]. At this stage, rhamnolipids stimulate the LPS composition adaptation and the swarming motility. After irreversible adhesion and production of the extracellular matrix, bacteria grow in microcolonies and proliferate in the biofilm.

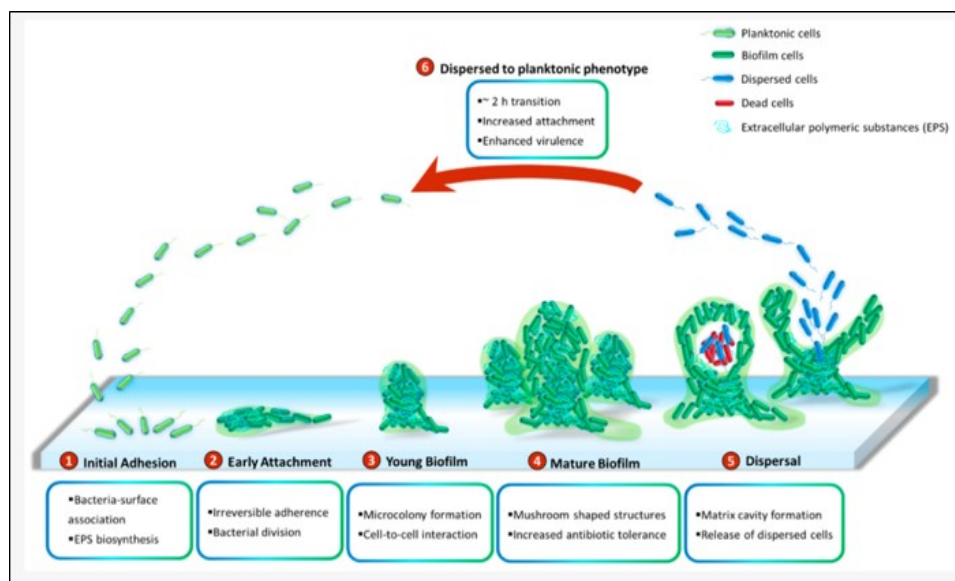


Figure 2: Stages of biofilm

Pseudomonas aeruginosa forms complex biofilms that protect the bacteria from antibiotics and host defenses. Studies show that *A. sativum*-derived AgNPs significantly inhibit biofilm formation and reduce the metabolic activity of sessile cells [33].

Mechanistically, AgNPs interfere with quorum sensing pathways by down regulating genes such as *lasI*, *lasR*, *rhlI*, and *rhlR*, which are essential for biofilm formation and virulence factor production [34]. Additionally, the nanoparticles can penetrate the extracellular polymeric substance (EPS) matrix and cause structural disintegration of mature biofilms [35].

Cytotoxicity and Environmental Considerations

The exploitation of AgNPs is in the infancy stage and the overall evaluation of their health hazards is so far not promising, although a number of products are already on the market. In the immediate future, we expect that the use of AgNPs in human health will increase significantly because of their utility, principally in relation to pandemic control. The lethal outcomes of AgNPs in organisms depends on several factors, such as the route of exposure (penetration, concentration and duration), factors associated with vulnerable organisms and factors associated with AgNPs' intrinsic toxicity, bioavailability and accumulation in organisms [36].

Three possible methods of consuming AgNPs are: inhalation, parenteral/dermal and oral. The cytotoxic effects of AgNPs depend on their size, tissue allocation, infiltration competence and cellular absorption [37].

Although *A. sativum*-based AgNPs are generally considered less toxic compared to chemically synthesized ones, their biosafety still depends on concentration and exposure duration. Low doses are typically safe for mammalian cells, but further *in vivo* studies are needed to confirm long-term biocompatibility and environmental safety [38].

The cytotoxic efficacy toward L929 mouse fibroblast cells, by means of the Cell-Counting-Kit-8 (CCK-8) method, were conducted as follows. First, 6×10^3 cells per well were placed into 96-well plates and incubated for 24 h at 37°C under 5% carbon dioxide. The cells were then treated with different nanomaterial concentrations for 24 h. The culture medium containing 10% CCK-8 was added into each well and cultured at 37°C under 5% carbon dioxide for 2 h. The viability was detected at 450 nm using the ELISA spectrophotometer [39].

3. Conclusion and Future Perspectives

Green-synthesized silver nanoparticles using *Allium sativum* offer a promising and eco-friendly strategy for combating antibiotic-resistant pathogens like *Pseudomonas aeruginosa*. Their dual antibacterial and anti-biofilm activities highlight their potential for applications in medical devices, wound dressings, and infection control. Future research should focus on optimizing synthesis parameters, understanding molecular mechanisms of action, and evaluating clinical applicability through *in vivo* models.

Article Information

Disclaimer (Artificial Intelligence): The author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.), and text-to-image generators have been used during writing or editing of manuscripts.

Competing Interests: Authors have declared that no competing interests exist.

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