



## Biological Activities of Silver Nanoparticles Against: Gram-positive Bacteria and Gram-negative

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### Abstract:

In the past two decades, nanotechnology and secondary particle research have become one of the most important areas in modern materials science research, attracting great interest in the fields of electronics, biology, solar energy conversion. Catalysis medicine and water treatment due to the distinctive properties of secondary particles. Among the noble metals, we find that silver (Ag) is the preferred metal in the field of biological systems, living organisms, and medicine. Humans have known its medicinal properties for two thousand years 11-13, and it has been used as antibacterial agents since the nineteenth century, and its uses have now diversified to include many New physical, chemical and biological specializations. Ag-NPs are one of the most widespread silver nanoparticles, with about 500 tons of annual global production. The demand for secondary story particles (Ag - NPS) is increasing in abundance to be used in many applications Fields such as medicine, pharmacy, companies, healthcare, food, cosmetics, etc.

Considering their various properties (physical, chemical, and biological), they can be exploited for different purposes. There are many ways to prepare Ag-NPs, including physical, chemical, and biological. Although there are many types of nanomaterials, Ag-NPs have proven to be the most effective for their resistance to microbes, bacteria, fungi, viruses, and other microorganisms. On the other hand, we find that the biosynthesis of secondary silver particles, has become an emerging branch of nanotechnology, as microorganisms and plant extracts have recently been used in the synthesis of nanoparticles because they are rich in biologically active compounds.

**Keywords:** Silver Nanoparticles, Gram-positive, Bacteria, Gram-negative

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# Introduction

Nanoparticles are solid particles with a size within the range of 10-1000 nanometers (1). Nanotechnology refers to any technology carried out at the nanoscale level where matter is restructured at the atomic and molecular levels with a size within the range of 1-100 nanometers (2). This technology is involved in multidisciplinary fields, covering a wide and diverse range of sciences from engineering, biology, physics and chemistry (3).

Nanotechnology has made tremendous progress over the past decades, especially between 2005 and 2010, where its development has clearly and exponentially increased over previous years, in the number of products that contain or require nanoparticles for their production. This development has been supported by their unique general properties, in particular particle size, large surface area, high chemical stability, surface reactivity, charge, and shape relative to their regular-sized counterparts (4,5).

Nanotechnology has been developed as a new strategy in the field of antimicrobials to put an end to resistant microbes. Metal nanoparticles such as platinum, copper, silver and gold contain antimicrobials against bacteria that cause fungi and various diseases. In particular, silver nanoparticles have promising applications in nanotechnology and medicine, and their bactericidal activity against bacterial infections has been approved, as well as their anti-viral and anti-fungal effects that cause various diseases (6).

Among nanometals, silver nanoparticles have been used since the 1880s. Because silver has broad-spectrum antimicrobial activity against a wide range of microorganisms. Silver nanoparticles have received unlimited attention due to their exceptional properties such as chemical stability, catalytic activity, excellent conductivity, and most importantly as an antimicrobial and antifungal action against fungal species such as *Aspergillus fumigatus*. Silver is also known to be non-toxic and harmless to the human body in low concentrations, unlike other metal nanoparticles. (7).

The use of silver nanoparticles (AgNPs) has been increasing on a global scale particularly in the fields of textiles, technology, health, and food because of their remarkable features. (8,9) Among these, the health sector has been predicted to show a significant consumption of AgNPs. AgNPs were demonstrated to show antibacterial properties against various bacteria and also to be able to show antiviral properties, especially against the HIV-1 virus. [10] In addition, AgNPs have anti-inflammatory activity, and have been tested on animals and humans.[11]

These properties qualify silver nanoparticles as one of the most promising materials in catalysis. They are also used in sensing and imaging applications, including DNA detection (12). Aim of the study: Through this study, we decided to identify the secondary compounds and shed light on silver compounds. Learn about the methods of preparing Ag-NPs, with all their physical and chemical types, especially the biological method. Identify the effect of silver nanoparticles on Gram-positive and Gram-negative bacteria. Recognition Biological applications of silver nanoparticles

## Silver nanoparticles

Mineral secondary particles, especially particulate matter, have been known to have strong toxic effects on a wide range of microorganisms. Thanks to these broad-spectrum antimicrobial properties, silver has been widely used for biomedical applications and other environmental disinfection processes for centuries.[13]

Silver secondary particles have strong interaction with microorganisms due to their high surface area. Thus, these nanoparticles attack the bacterial surface, by penetrating these organisms and lead to the disruption of vital functions of the organism due to a change in membrane fluidity, thus increasing cell permeability.[14]

Bacterial membrane proteins contain sulfur and phosphorus in their structure. Both silver nanoparticles, such as silver ions, can interact with these proteins and inhibit DNA functions by interacting with these chemicals. Silver nanoparticles or silver ions can also attack the respiratory chain in bacterial mitochondria and lead to cell death.[15]

## Synthesis of silver nanoparticles

In general, AgNPs can be synthesized using several methods, including physical, chemical, and biological. It is unfortunate that chemical or physical synthesis has the potential to be dangerous to the environment. Thus, new studies have been appearing on the synthesis of AgNPs using biological means (green synthesis) by employing natural ingredients. Basically, the synthesis of AgNPs needs three essential ingredients: a precursor (Ag source, usually silver nitrate [AgNO<sub>3</sub>]), a reducing agent (to reduce Ag<sup>+</sup> ions to Ag), and a stabilizing agent (to avoid agglomeration). [16] For biological synthesis, it is necessary to employ natural materials such as bacteria, fungi, algae, plants, or yeast. This method of synthesis offers many advantages such as low cost since the reducing and stabilizing agents can be obtained freely. Also, biological synthesis can be carried out at room temperature and quickly. [17]

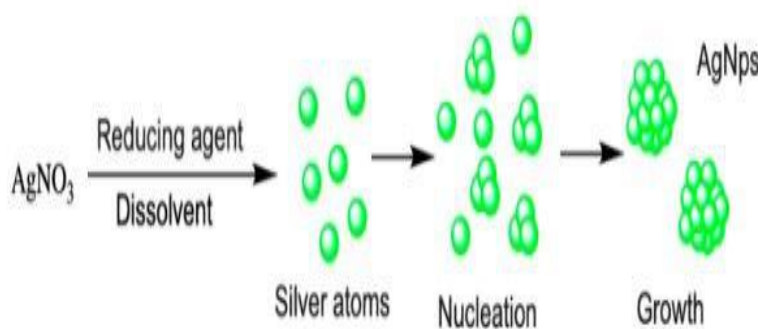
A common precursor used to synthesize AgNPs is AgNO<sub>3</sub>. When dissolved in water, AgNO<sub>3</sub> forms ions of Ag and NO<sub>3</sub>. Furthermore, the role of a plant extract as a reducing and stabilizing agent begins when the plant extract is mixed with AgNO<sub>3</sub> solution. The reducing agent is responsible for reducing Ag<sup>+</sup> ions from AgNO<sub>3</sub> to Ag particles. In addition, the stabilizing agent is employed to stabilize the produced AgNPs to avoid agglomeration. In general, plants containing flavonoids and terpenoids can be used for this synthesis. For instance, the main terpenoid, eugenol, present in *Cinnamomum zeylanicum* extracts was found to have the capability to act as a bio-reduction agent of gold(III) chloride trihydrate (HAuCl<sub>4</sub>) and AgNO<sub>3</sub>, forming nanoparticles. The flavonoid transforms from the enol form to the keto form, which can release a reactive hydrogen atom that is able to reduce metal ions to form nanoparticles, [18]

Synthesis procedures for producing AgNPs using roots, seeds, fruits, and leaves are well established. However, there is very little information available in the literature regarding the exploration of the capability of weed extracts for synthesizing AgNPs. In addition, the large use of weeds may also support the environment, particularly in agriculture, since they can be linked with the negative impact by monopolizing resources [19].

### Conventional Chemistry

Compared to other methods, chemical synthesis of AgNPs is relatively cheap and easy to implement at a large scale while maintaining a monodispersed size distribution. [20]

Among the variety of chemical methods available for the production, chemical reduction is the most widely used for this type of nanosystems. This process employs the use of three main components, (i) a metal precursor, (ii) reducing agents, and (iii) stabilizing agents [21]. Basically, two stages of nucleation and growth are involved (Figure 1). In this synthesis, the stabilizing agent can have a dual function, i.e., also acting as a reducing agent in the same reaction [22].



**Figure 1. Process for the synthesis of AgNPs.**

Appropriate average size, polydispersity, and shape of AgNPs can be achieved by controlling the nucleation stage, i.e., by monitoring the experimental parameters, such as the precursor used in the reaction, reducing agents, reagent concentration, pH, and temperature [23-24].

A critical step in the synthesis of AgNPs is their stabilization, especially in order to prevent agglomeration and oxidation processes. Therefore, one of the most common strategies is the use of stabilizing agents that are capable of protecting AgNPs. For the stabilization, chitosan, amine derivatives, thiols, or gluconic acid can be used. In addition,

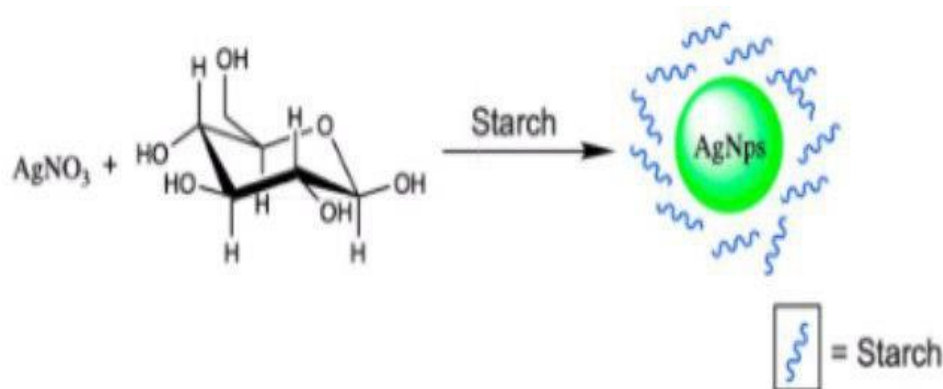
it has also been proven that the use of polymeric compounds, such as polyvinylpyrrolidone (PVP), polyacrylates, polyvinyl alcohol (PVA), polyacrylonitrile, polyacrylamide, or polyethylene glycol (PEG), is also useful. Finally, stabilization can be achieved by electrostatic repulsion by incorporating a negative charge on the surface of these NPs mainly through citrate groups [25].

Among all the chemical methods to obtain AgNPs, the Creighton method is the most widely used because it allows to produce monodispersed and small size (around 10 nm) nanoparticles [26]. In this method, the precursor agent is AgNO<sub>3</sub> and the reducing agent is NaBH<sub>4</sub>. The reaction that is carried out is as follows:

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### Green Chemistry

Although chemical synthesis has the advantages of being of low cost and of high performance, the use of reducing agents is harmful. Therefore, methods that use environmentally friendly reagents have been developed. Special interest is the method using β-D-glucose as a reducing agent to cause the chemical reduction of AgNO<sub>3</sub> salt. This method employs starch as a stabilizing agent (Figure2). These green synthesis is able to obtain AgNPs under 10 nm of mean size [28].



**Figure2. Chemical reduction of AgNO<sub>3</sub> salt from β-D-glucose.**

Ho et al. described a synthesis method using ascorbic acid as a reducing agent for the reduction of AgNO<sub>3</sub>. They were able to obtain hybrid AgNPs inside a polylysine shell modified by different fatty acids. This green synthesis method was able to develop AgNPs of mean size between 2 and 5 nm [29].

Nowadays eco-friendly methods of biosynthesis are being gradually replacing the traditional chemical synthesis with increasing publications about this topic in the last years. The biosynthesis of AgNPs uses the bases of chemical synthesis, but instead of a chemical entity, this takes advantage of the reductive properties of biological entities.[30]

### Physical Methods

There is a wide variety of physical methods for the synthesis of AgNPs, but evaporation/condensation is one of the simplest and best controlled. Moreover, laser ablation is also a method that allows to obtain a large number of nanoparticles in a short time. They are also the most widely used physical techniques. However, these methods are of high cost among other disadvantages, as the need to use a tubular furnace (which takes up a lot of space and consumes a large amount of energy) and the increasing temperature during the process requiring a long time to achieve thermal stability [31].

### Synthesis of silver nanoparticles by microorganisms:

#### Bacteria:

Recently, bacteria have shown their ability to biosynthesize Ag-NP. Nanoparticle synthesis can be extracellular or intracellular depending on the bacterial species.

There is a limit to the accumulation of nanoparticles that bacteria can survive. After that, nanoparticle accumulation can be toxic to bacteria because some microorganisms can tolerate low concentrations of metal particles and also grow under these conditions, due to their protection from this metal.[32] The first evidence of the production of secondary particles was isolated from a silver mine. When silver nitrate bacteria were placed in a solution of *Pseudomonas stutzeri* AG259, they produced secondary silver particles in their periplasmic space, which were pyramidal and hexagonal in shape and a size of up to 200 nanometers. In addition, A few bacterial strains (*B. amyloliquefaciens* (A. calcoaceticus, Gram-negative or Gram-positive), specifically) and *B. flexus* have been implicated in both Ag-NP biosynthesis.[33]

## **Fungi**

Many fungi have been widely used in the biosynthesis of secondary silver, and when compared to bacteria, they produce a large amount of nanoparticles, secrete larger amounts of proteins,[34] Hence higher yield of nanoparticles in biosynthetic approach.

Synthetic mechanisms depend on returning silver nitrate to secondary cation particles in the presence of enzymes present in the fungal structure, where silver ions are first absorbed on the fungal surface through interactions with chemical functional groups such as carboxyl anions and carboxyl and peptide bonds of proteins; Then the reduction process is performed by reducing the sugar from the sugars found on the fungi. One of the negative aspects of using this method in manufacturing AgNP is its slowness compared to plant extracts.[35]

## **Algae**

Algae have been exploited in the food, feed, cosmetics, fertilizer and pharmaceutical industries. Recently, research has focused on the biosynthesis of secondary particles by algae because they are easy to cultivate, are scalable, have rapid growth, and are an excellent source of secondary metabolites.[36] Therefore, interest in the biosynthesis of secondary particles by algae is rapidly increasing (112), as the use of algae is mainly due to their high ability to absorb metals and metal ion reduction, relatively low production costs, and most importantly their ability to produce secondary particles on a large scale 13-14). Another advantage is their ability to withstand extreme weather conditions more effectively than other microorganisms [37]. Live dry biomass can be used Another added advantage of using algae is the time required to synthesize secondary silver particles. Synthesis by algae requires less time compared to other microbes.

The time required by *E. coli* to produce... While *Caulerpa racemose* produces secondary particles only 3 h [38]. Silver nanoparticles made by algae contain a hydrophilic surface group such as carboxyl sulfate and hydroxyl sulfate, which gives them unique applicability. They can be used in medical treatment because the algae themselves do not create any toxic or harmful substance. Algae strains such as *Tetraselmis* 1161 have been approved for about 60 hours Ag-NPs [38] for the biosynthesis of new mineral *Scenedesmus*, *Desmodesmus*, *kochinensis* nanoparticles. Algae has the ability to convert minerals into new forms. And due to these characteristics, it has been variable Using algae to develop a range of nanomaterials.[39]

## **History of the effectiveness of nanosilver as an antibacterial:**

Silver has been used since ancient times to combat bacteria due to its ability to kill microorganisms and algae.

In the modern era after the advent of nanoscience, nanostructured materials have received intense focus from researchers - especially in recent decades - because their unique physical and chemical properties depend on their size. [40] Nano-silver technology is considered a new medical breakthrough, as silver manufactured at this very small size can eliminate more than 630 types of bacteria by destroying bacterial cell walls, and many fungi and viruses.

It has been widely used in the treatment of clinical diseases and burn wounds. Topical. This substance is also used to sterilize surgical instruments. There is a scientific study published in the American Journal of Nanotechnology in 2005, conducted by researchers from the Universities of Texas and Mexico, confirming that nanosilver at a scale of 1-

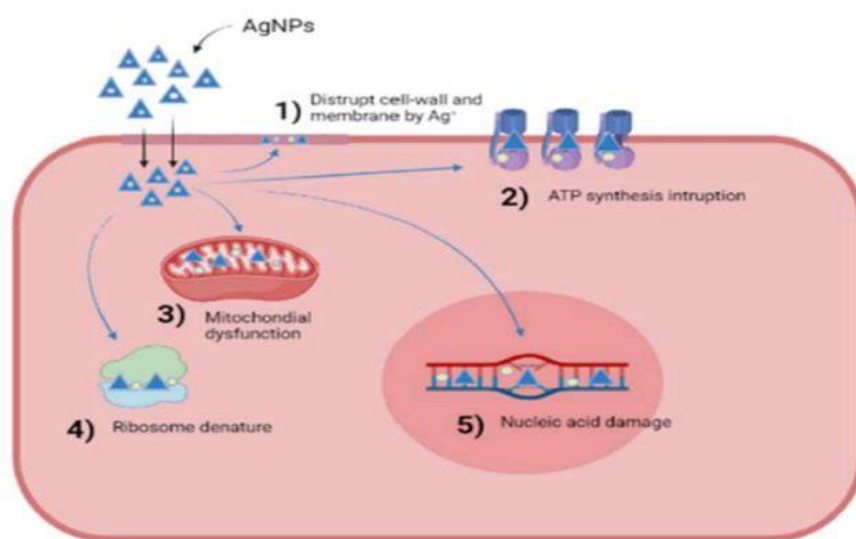


10 nanometers is capable of eliminating one of the most dangerous viruses to human health, which is the AIDS virus (HIV-1). ).[41]

### Antibacterial Activity of AgNPs

AgNPs seem to be alternative antibacterial agents to antibiotics and have the ability to overcome the bacterial resistance against antibiotics. Therefore, it is necessary to develop AgNPs as antibacterial agents. Among the several promising nanomaterials, AgNPs seem to be potential antibacterial agents due to their large surface-to-volume ratios and crystallographic surface structure. AgNPs were synthesized by four different types of saccharides with an average size of 25 nm, showing high antimicrobial and bactericidal activity against Gram-positive and Gram-negative bacteria, including highly multi-resistant strains such as methicillin-resistant *Staphylococcus aureus*. [41,42,44]

AgNPs synthesized by *Cryphonectria* sp. showed antibacterial activity against various human pathogenic bacteria, including *S. aureus*, *E. coli*, *Salmonella typhi*, and *Candida albicans*. Interestingly, these particular AgNPs exhibited higher antibacterial activity against both *S. aureus* and *E. coli* than against *S. typhi* and *C. albicans*. [Figure 3](#) shows the effectiveness of dose-dependent antibacterial activity of biologically synthesized AgNPs in *E. coli*. [45]



### Mechanism of the effectiveness of nanosilver as an antibacterial:

After binding Ag-NPs to the cell surface, they accumulate in the pits of the cell wall, disrupting the cell membrane [46]. Likewise, Ag-NPs also disrupt bacterial signal transduction via tyrosine residues. The dephosphorylate effect depends on peptide substrates, leading to termination of cell proliferation and apoptosis [47]

The antibacterial activity of Ag-NPs also depends on the dissolution status of Ag-NPs in the exposure medium of synthetic agents Processing, such as intrinsic Ag-NP properties (shape, size, capping agent) and surrounding media (organic and inorganic components), directly affects the effectiveness of Ag-NPs. The thick cell wall of Gram-positive bacteria may reduce the penetration of Ag-NPs into their cells; this is why In that Gram-negative bacteria are more susceptible to Ag-NPs. Biofilm formation in the oral environment protects bacteria from Ag and Ag-NPs by impeding their transport. The bioavailability and mobility of Ag-NPs in the biofilm are determined by the diffusion coefficients of Ag-NPs Ivan Sondhi (2004) also studied the biocidal activities of Ag-NPs against *E. coli* and confirmed the “pit” formation in the cell wall of this model of Gram-negative bacteria. A significant increase in permeability occurred due to the accumulation of Ag-NPs in the bacterial membrane, resulting in Cell death [48].

### Biological applications of silver nanoparticles

Due to their unique properties, AgNPs have been used extensively in house-hold utensils, the health care industry, and in food storage, environmental, and biomedical applications. Several reviews and book chapters have been dedicated in various areas of the application of AgNPs. Herein, we are interested in emphasizing the applications of AgNPs in

various biological and biomedical applications, such as antibacterial, antifungal, antiviral, anti-inflammatory, anti-cancer, and anti-angiogenic.[49]

## Discussion

The plant-based Ag-NP synthesis is highly preferred, in contrast to other technologies, because it contains active materials that contribute significantly to the Ag-NPs synthesis process, such as fluoids, alkaloids, and sugars, by returning Ag ions. This is evident from the color change of the mixture. The synthesis is also distinguished. Green quickly and can be completed in the collection of mineral salt extract temperature, pH and contact time.

Whereas when using living organisms in the biosynthesis process, it first requires collecting samples and then cultivating them in order for the biosynthesis process to take place after that.

Study of antibacterial activity: Ag-NPs prepared using biological methods were used to evaluate the antibacterial activity using several methods, including the disc spreading method and the growth inhibition method against several strains of Gram-negative and Gram-positive bacteria, and it gave good results with significant diameters of inhibition. Accordingly, most studies showed the possession of Ag-NPs antibacterial activity.

## Conclusion

Our interest in this research is based on identifying secondary compounds in all aspects, and focusing on nanosilver in terms of synthesis, applications, and estimation of antibacterial effectiveness. The number of scientific publications in this field on various methods of biosynthesis has increased, and a large number of them were based on the evaluation of secondary silver compounds obtained from plant extracts and showed that their importance lies in their containment of active compounds. Through our theoretical study, you find that nanoscience is a broad science through which secondary silver compounds have been able to break into all fields, and future studies indicate that it can play a prominent role in pushing countless microorganisms and plants that have been explored so far to achieve many field applications. Finally, a better understanding of the synthesis mechanism and properties of compounds could push the boundaries of this technology and expand its horizons beyond laboratory use.

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