Green synthesis of Silver Nanoparticles and their antimicrobial applications

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Abstract The exceptional antibacterial properties of silver nanoparticles (Ag NPs) and their prospective uses in different fields have attracted a lot of interest in contemporary times. The chemical agents used in the preparation of Ag NPs are hazardous to human health and the environment. In contrast to chemical approaches, green synthesis techniques involve the use of natural resources, which accomplish the principles of green chemistry and sustainable development goals (SDGs). In this regard, this review article delves into a comprehensive analysis of the green synthesis methods employed for the production of Ag NPs and their utilization as diverse antimicrobial agents. In addition to exploring the many antimicrobial uses of Ag NPs production, this article attempts to give a thorough examination of the processes behind the antibacterial activity of Ag NPs. This review provides in-depth mechanisms of antimicrobial action, including rupture of membranes, production of reactive oxygen species (ROS), and disruption of cellular functions. Thus, this article explores recent insights into green synthesis approaches for the preparation of Ag NPs which are effectively utilized as antimicrobial agents.

1 Introduction

The most active area of material science research is nanotechnology, and the production of NPs is expanding rapidly globally. Ag NPs, out of all the metallic nanoparticles, have drawn the most attention and are known for having unique qualities like conductivity, anti-fungal, antibacterial, catalysis, chemical stability, anti-viral, and anti-proactive potentials [1,2]. Microorganisms (algae, bacteria, fungi) and plant extracts perform green synthesis because of their reducing and antioxidant properties, which turn metallic compounds into their metallic nanoparticles. Using silver nanoparticles increases the surface area for exposed microorganisms, which dramatically amplifies the antimicrobial effectiveness of silver, even though silver is commonly employed in nitrate form to give antibacterial benefits. Since NPs of metal oxide and metal have such good qualities as a high surface to high dispersion in solution and volume ratio, among other advantages, they have been the subject of much scientific and technological investigation among all other types of NPs.
Consequently, the antibacterial properties of metal and metal oxide NPs are enhanced. The scientific community is becoming very interested in AgNPs because of their extensive applications in food technology, chemistry, microbiology, parasitology, pharmacology, and cell biology. AgNPs are created when various biomolecules, such as ketones, phenolic acids, plant extract proteins, carboxylic acids, tannins, aldehydes, and oxidize Ag\(^+\) to Ag\(\text{0}\)\([3–5]\). A variety of physical and chemical methods can be used to fabricate NPs. Since surface structure greatly influences a material's physical qualities, the nanoparticles produced using this method include surface defects that may result in significant limitations \([6]\). They also require a lot of energy, high temperatures and pressures, are expensive, and take a long time. Chemical routes entail dangerous and poisonous substances that provide biological threats to the environment and public health.

On the other hand, no chemicals are used in the green synthesis process. Additionally, this approach is very straightforward, eco-friendly, stable, and one step. Due to their natural availability, quick generation, efficiency, low cost, and environmentally friendly character, NPs are now synthesized from a variety of plant sources, including stems, leaves, flowers and roots. Phytochemicals, which serve as stabilizing, capping and reducing agents for the production of nanoparticles, are abundant in plants and include terpenoids, saponins, vitamins, alkaloids, phenolics, amino acids, flavonoids, polysaccharides and proteins.

## 2 Green Synthesis

Microorganisms (bacteria, algae, fungi) and plant extracts perform green synthesis because of their reducing and antioxidant properties, which turn metallic compounds into their metallic nanoparticles. The following categories apply to green synthesis: (a) using microorganisms such as actinomycetes (prokaryotes), bacteria, yeasts and fungi; (b) using membranes, virus DNA, and diatoms as templates; and (c) using plants and plant extracts. The following sections provide a description of the green synthesis using plants, fungi, bacteria, and plant extracts. Due to its eco-friendly, non-toxic, simple, and cost-effective nature, using plant extract to create nanoparticles has drawn particular attention from researchers. The phytochemicals (amino acids, proteins sterols, alkaloids, phenolics, terpinoids, flavonoids etc.) serve as the nanoparticles' stabilizing and reduction agents. The synthesis techniques fall into two main categories "Top-down" and "bottom-up" approaches. The top-down method involves mechanically reducing the bulk form of silver metal to the nanoscale through techniques like mechanical milling, laser ablation, lithography, etc. (Figure 1), In order to prevent agglomeration, the bottom-up approach, also known as self-assembly, dissolves silver in a solvent, uses a reducing agent to reduce silver ions to their element, and stabilizes resulting natural AgNPs. An illustration of the different top-down and bottom-up methods for creating AgNPs is shown in Figure1. Stabilizing agents, reducing agents, and metal precursors are the three main ingredients that are frequently utilized in the chemical synthesis of AgNPs. Silver salts including silver perchlorate (AgClO\(_4\)), silver nitrate (AgNO\(_3\)), and silver tetrafluoroborate (AgBF\(_4\)) are reduced to form AgNPs primarily. AgNPs of various sizes, shapes, stability, and antibacterial activity are being produced by plants and microbes, and recently, this has become the focus of research \([7–10]\).
2.1 Plant extract

Silver nanoparticles have been effectively synthesized from several plant components, like roots, leaves, flowers, rhizomes and fruits. To remove trash and other undesired contaminants, different plant parts are collected from different sources and thoroughly cleaned with ordinary and distilled water. The pieces are then dried, pulverized, and used either fresh or powdered to create the extract. The crushed powder of the plant components are combined with deionized water or alcohol to make the extract. After that, mixture is heated for a short while below 60°C because prolonged heating at high temperatures can cause the photochemicals in the biomass extract to break down. The process of synthesizing of AgNPs involves addition of plant extract at different pH levels with solutions containing various concentrations of Ag salt in them, acting as a metal precursor, which then heated at varying temperatures. The biomaterials in the extract function as both a reducing agent and a stabilizing agent during the synthesis of AgNPs, this method of synthesis avoids the need for chemical stabilizers. UV-Vis can be used to track the advancement of AgNP production, as can visible color changes. Spectroscopy, which shows a distinct sharp peak at about 430–450 nm that is caused by the AgNPs’ surface Plasmon resonance (SPR). After AgNPs are effectively synthesized, the mixture is dried in low-temperature oven, centrifuged at high rpm to separate the nanoparticles, and then properly cleaned with solvents. The various plant extracts that have been effectively employed in the environmentally friendly production of AgNPs.

2.2 From leaf
Many different leaf extracts have been used to date for the biosynthesis of AgNPs. The synthesis of spherical AgNPs measuring 0.27 nm was reported for Skimmia laureola, and the efficacy of the product was evaluated against *K. pneumoniae*, *S. aureus*, *P. aeruginosa*, *E. Coli*, and *P. vulgaris*. Miri *et al.* used Prosopis farcta extract to carry out the biosynthesis of silver nanoparticles with an average size of 10.8 nm at room temperature (RT) [11].

To test the antimicrobial activity for synthesis of AgNPs against both Gram-negative (*PTCC 1399*, *Escherichia coli*, *PTCC*, *Pseudomonas aeruginosa*), and Gram-positive (*Staphylococcus aureus*, *PTCC 1431*) bacteria, and to compare results with control material the disc diffusion method was utilized. Findings demonstrated that each pathogen under test had an enhanced inhibitory diameter, suggesting that artificially generated AgNPs cause bacterial cellular damage and can therefore be employed as nanoantibiotics. Spherical biogenic AgNPs are also produced by Eclipta alba, Aloe vera, Leptadenia reticulata, and Momordica charantia. Tea leaf extract was used to create AgNPs in a different investigation. The synthesized NPs' bactericidal activity was evaluated against *E. coli* and *S. aureus*, and results indicated that former's inhibition action was more successful (89% inhibition rate) than the latter's (75% inhibition rate). Furthermore, the inhibition of bacterial cell-cell adhesion occurs when the NPs are treated against the bacteria. In order to biosynthesis AgNPs with a size distribution of 58 nm, Mukia maderaspatana leaf extract was used. The created nanoparticle was coupled with the antibiotic cetiriaxone to examine its antimicrobial efficacy against human infections, including *K. pneumonia*, *B. subtilis*, *S. aureus*, and *S. typhi*. The effectiveness of the pathogen suppression was then compared between the free nanoparticle and the drug. Comparing the AgNPs conjugated with cetiriaxone to the others, the results showed that their inhibitory activity was the highest. In testes using standard antibiotics, Taraxacum officinale leaf extract mediated AgNPs showed a strong positive reaction against both plant pathogens, *X. axonopodis* and *P. syringae* {dora} (e.g. streptomycin, oxytetracycline, tetracycline, and ampicillin). Combining tetracycline with AgNPs increased ZOI by around 40% as compared to antibiotics alone, significantly inhibiting the growth of specific phytopathogens. Despite the fact that the exact process is yet unknown, the scientists believe that the synergistic effect of NPs and antibiotics will increase bacterial cell membrane penetration, which will ultimately cause the bacterium to die and numerous cell organelles to be destroyed.

### 2.3 From fruits

Field of green nanoparticle production has extensively studied plant fruit extracts. A plethora of research exists regarding the environmentally friendly synthesis of silver nanoparticles using fruit extracts. The biogenic synthesis of spherical silver nanoparticles was successfully achieved by using fruit extracts from guava, *Emblica officinalis*, *Solanum trilobatum*, carambola *Helicteres isora*, to investigate a variety of microbial pathogens, including *E. coli*, *S. aureus*, *B. subtilis*, *S. mutans*, *B. cereus*, *S. typhi* and *K. pneumonia*. It has been observed, as fruit extract content rises, AgNP size reduces and antibacterial activity rises in response. When comparing Gram-positive to Gram-negative bacteria bacteria, Emblica officinalis had superior antibacterial activity. Owing to their extremely small size, guava, Helicteres isora, and Emblica officinals mediated AgNPs demonstrated excellent ZOI against the pathogens in question. In addition, fruit extracts from Lemon, *Nothapodytes nimmoniana* and *Syzygium alternifolium* Walp were used in the green synthesis of silver nanoparticles. Shape instruction AgNPs were directly applied to different strains of bacteria and demonstrated outstanding efficacy against bacterial strains by using CTAB in conjunction with lemon extract to modify form of silver nanoparticles. On the other hand, fruit extracts from *Nothapodytes nimmoniana*
and Syzygium alternifolium Walp function as bio-reducing and shape-directing agents during silver nanoparticles production.

Lemon extract’s antibacterial properties against four pathogens were examined in different investigation including, E. coli, P. aeruginosa and S. aureus, and compared it to the lemon extract, controls, and amikacin. Another bioreducing agent used in environmentally friendly synthesis of silver nanoparticles was lemon extract. The outcomes demonstrated that the artificially produced NPs are very effective at breaking the cells of bacterial strain, that ZOI is nearly identical to prescription amikacin antibiotic. The bactericidal efficacy of spherical silver nanoparticles against Gram-negative and Gram-positive bacteria was also investigated after they were made using a variety of fruit extracts, including apple, Momordica charantia extract, and Adansonia digitata L. AgNPs and extract together are said to cause greater damage to bacterial cell than Silver nanoparticles along with extract alone because of synergistic effect that phytochemicals that are capped on the surface of silver nanoparticles provide. Also, the tamarind fruit extract was used in the microwave assisted synthesis of spherical silver nanoparticles. The resulting AgNPs are highly stable and exhibit good bactericidal action without oxidatively forming. Also, the synthesis of AgNPs is a straightforward, quick, and inexpensive method. Strawberries, ginseng berries, and Phoenix dactylifera also effectively used for green production of spherical silver nanoparticles having strong antibacterial activity were Chaenomeles sinensis and Kigelia Africana fruit extract. The ZOI of AgNPs derived from ginseng-berry fruit extract against S. aureus (12.3 mm) was higher than that of E. coli (11 mm). Utilizing AgNPs made from Phoenix dactylifera extract showed significant bactericidal action against Gram-positive bacteria.

3 Mechanism

The possible process through which AgNPs work as antibacterial agents. According to reports, the bactericidal activity of AgNPs increases with their size because they give the bacterial membrane a larger surface area. The positively charged Ag ion and the negatively charged cell membranes combined to alter morphological distortion in the cell, which in turn caused cell leakage and ultimately, cell death. Moreover, AgNPs have a great binding affinity for the phosphorus and sulfur found in both external and intracellular membrane proteins. This binding influences the processes of cell respiration, replication, and ultimately, cell lifespan. In addition, AgNPs have the ability to attach to membrane protein thiol and amino groups, thus resulting in the production of (ROS) reactive oxygen species which prevent cell respiration. Interaction between AgNPs and the bacterial strain's peptidoglycan cell wall and plasma membrane accounts for their high bactericidal activity. Additionally, it has been proposed that AgNPs' interaction with cell walls improves membrane permeability by producing pits or pores that kill bacteria.
Fig.2. Process by which AgNPs work as bactericidal agents.

4 Applications of AgNPs

4.1 Antimicrobial activity of AgNPs

AgNPs have been shown to have potent antibacterial qualities by numerous studies. AgNPs with antibacterial and antiproliferative qualities were created by utilizing Acer oblongifolium plant extract [219]. Khodadadi et al. produced AgNPs by using Vaccinium arctostaphylos extract, and evaluated their antibacterial properties against gram-negative and gram-positive bacteria, such as Salmonella enteritidis and E. coli, as well as S. aureus and B. subtilis[12]. AgNPs typically exert their antibacterial effect by inactivating thiol groups in enzymes, which renders bacterial enzymes inactive, and by releasing silver ions. The liberated silver ions cause harm to the cytoplasm of the cells, prevent the reproduction of bacterial DNA, lower ATP levels, and ultimately kill the bacterial cells. Synthesized AgNPs mediated by Carthamus tinctorius waste extract were evaluated against both Gram-negative and Gram-positive bacteria, like P. fluorescens and S. aureus cells.

Enzymatic activities are inhibited by Ag ion produced from silver nanoparticles through interactions with phosphorus in DNA and proteins containing sulfur. Bacteria die as a result of maximal permeability, which is caused by NPs interacts with membrane proteins more easily when they are smaller than 20 nm[13]. AgNPs created using Gymnema sylvestre leaf extract were tested for their antibacterial efficacy against Escherichia coli and S. aureus. The bacteria's cell wall allowed the artificial AgNPs to cling to it and penetrate the bacterium. They have a substantial surface area which is small (nm). As a result, it impairs the system's normal operation, messes with the bacteria's breathing mechanism, and ultimately causes
death[14]. Mudhafar et al. (2020) evaluated the antibacterial efficacy of synthesized AgNPs generated from Salvia spinosa extract against B. subtilis and E. coli.[15]. AgNPs' bactericidal activity is most likely brought about by their adhesion to the generation of free radicals and cell wall. Alsammarraie et al. conducted a study on synthesis of green silver nanoparticles utilizing Curcuma longa then investigated the antibacterial effects against E. coli and Listeria monocytogenes[16]. Jain et al. reported that AgNPs derived from Ocimum Sanctum leaf extract showed antibacterial effectiveness against E. coli. [17]. AgNPs derived from Crocus sativus leaf extract have antibacterial activity against E. coli, Shigella flexneri, K. pneumoniae, P. aeruginosa, and B. subtilis, according to Bagherzade et al. [18]. AgNPs antibacterial effectiveness against P. aeruginosa, Micrococcus flavus, and K. pneumoniae was reported by Anandalakshmi et al. (2016)[19]. Silver nanoparticles were generated by leaf extract Pedalium murex.

5 Future challenges

- Standardization of Synthesis Procedures: The efficiency, repeatability, and scalability of green synthesis techniques for Ag NPs vary greatly. Creating standardized procedures to guarantee uniform nanoparticle size, shape, and quality will be a difficulty in the future.

- Techniques for Characterization: Precisely determining the characteristics of green-synthesised silver nanoparticles is essential to comprehending their characteristics and maximizing their antibacterial efficacy. Subsequent endeavors might concentrate on promoting characterization methods like X-ray diffraction (XRD), transmission electron microscopy (TEM), and dynamic light scattering (DLS).

- Optimizing Antimicrobial Efficacy: Ag NPs have strong antibacterial qualities, but they still need to be made more efficient against range of pathogens, such as ones that are resistant to drugs. This can entail altering the surface of Ag NPs or using combination treatments with additional antibiotics.

- Ensuring the biocompatibility and safety of Ag NPs is of utmost importance, given their planned use in biomedical applications. The issues of cytotoxicity, genotoxicity, and long-term impacts on the environment and human health may be explored in future studies.

- Stability and Storage: Ag NPs that have been green-synthesised may encounter problems with agglomeration, oxidation, and degradation over time, among other problems. Creating plans to extend the stability and shelf life of Ag NPs will be crucial for their use in real-world situations.

- Regulatory and Ethical Issues: Concerns about Ag NPs' approval, labeling, disposal, and possible environmental effects are brought up by their usage in antimicrobial applications. In the future, stakeholders, regulatory bodies, and researchers may work together to overcome these obstacles.

References

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