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Natural Sources and Antimicrobial Activity of Green Synthesized Silver Nanoparticles Against Human Pathogens: An Integrative Review

Review Article

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Abstract

Emergence of infectious diseases and drug resistant pathogenic microbes are global health and development threats. Despite enhanced knowledge of microbial pathogenesis and use of new therapeutics, mortality and morbidity rates remain alarmingly high. Hence, there is an urgent need to find new antimicrobial agents and discover innovative methods to produce new drugs. Since nanoparticles have well-defined optical, chemical, electrical and mechanical properties, their use in combating the infections and resistant microbes is gaining traction in the twenty-first century. Engineered nano-materials (ENMs) synthesized from natural sources such as plants are an emerging area of interest in nano-biotechnology. Currently, there are a number of applications of this technology such as bio-sensing, biological separation, molecular imaging, and anticancer therapy. Green synthesis of silver nanoparticles (AgNPs) is an important part of nanotechnology where NPs can be synthesized using biological materials such as plant extracts, plant biomass, and microorganisms. This method is nontoxic to the environment and the green synthesis of AgNPs exerts remarkable antimicrobial effects against human pathogen strains making them useful on the clinical platform against drug-resistant microbes. AgNPs have strong antibacterial activity, so they are used in the treatment of infections caused by multidrug resistant bacteria, due to its high surface-to-volume ratio and its chemical and physical properties. This integrative review focuses on summarizing the green sources of AgNPs possessing antimicrobial properties that have an advantage over physical and chemical methods.

Keywords: Nanotechnology; Antimicrobial Activity; Silver Nanoparticles; Biological Synthesis; Human Pathogens; Natural Sources

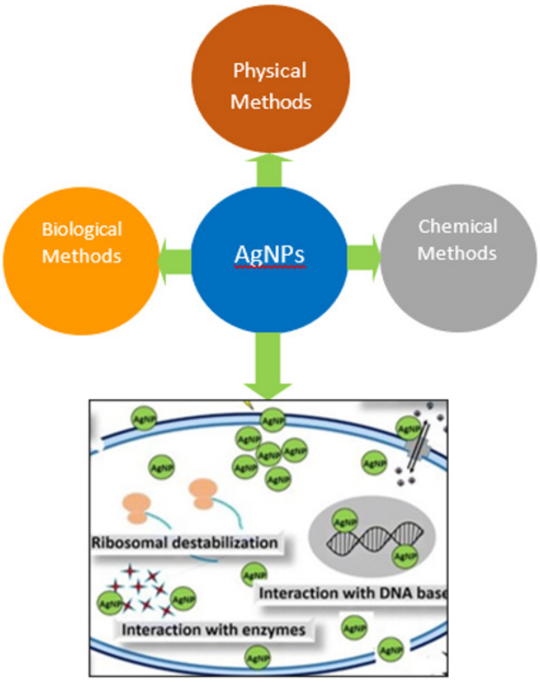
Abbreviations: ENMs: Engineered Nanomaterials; NPs: Nanoparticles; SERS: Surface-Enhanced Raman Scattering; PBS: Phosphate-Buffered Saline; DMF: Dimethyl Formamide

Introduction

Nanotechnology is a term that refers to manipulation and manufacturing of matter at a nanoscale [1]. The field of nanotechnology is a competitive area of research in material sciences and the interest in engineered nanomaterials (ENMs) via synthesis of nanoparticles (NPs) is rapidly increasing in the scientific community. NPs exhibit entirely new or improved properties because of specific characteristics, such as size (1-100nm), form, and structure [2-4]. NPs can be grouped as organic and inorganic NPs. Inorganic NPs, metallic NPs, and magnetic NPs, are semi-conductor NPs while organic NPs consist of carbon nanoparticles. NPs possess small area which shows notable catalytic reaction, bio-chemical activity, and atomic behavior as compared to the same chemical composition of larger particles [5]. Among nanoparticles, silver nanoparticles (AgNPs) have received great attention due to their unique chemical and physical properties, in addition to their use in many medical purposes. They have anti-fungal and antiviral activity, in addition to being anti-inflammatory and due to their potential use in catalysis, biological sensors, opto-electronics, DNA sequencing, antimicrobial characteristics, Surface-enhanced Raman Scattering (SERS), technology related to clean water, climate change and control, production of energy, information storage, and biomedical applications [6-19]. Because of their broad-spectrum antimicrobial activity, low toxicity, and low cost, AgNPs have become very popular medical EMNs in the last few years [20].

The present integrative review focuses on summarizing the green sources of AgNPs possessing antimicrobial properties that have an advantage over physical and chemical methods. There are many methods that are used to manufacture AgNPs, including chemical, biological, and physical methods as evaporation-condensation is a common procedure for the synthesis of NPs [Figure1]. In this method, component is placed in the center of furnace and vaporized into a gas carrier. Biological methods are one of the most important methods due to their low cost, no pollution to the environment, and no side effects. Biological methods depend on the use of living organisms such as plants or microorganisms such as bacteria, viruses, fungi [21].

Figure 1: Synthesis methods of AgNPs.



AgNPs, Fullerene, gold and Phosphate-Buffered Saline (PBS) are synthesized by evaporation-condensation procedure as well. However, this method has several draw backs as the tube furnace used in this technique occupies a lot of space and consumes a lot of energy while raising the temperature around source material. It also takes a lot of time to achieve thermal stability [22-23]. Furthermore, average tube furnace needs several minutes of preheating to reach a constant working temperature and power of several kilowatts. One of the disadvantages of this procedure is the existence of defects in the product surface structure while the physical characteristics of NPs depend on the product surface structure. Regardless of the process, physical procedure is used as a measuring tool and NPs synthesis is used for long term experiments for the study of inside toxicity [24]. AgNPs can also be produced by chemical reduction method, which is the commonly used method. Several reducing agents, inorganic and organic are used for Ag ions reduction, e.g, sodium citrate, N,N-dimethyl formamide (DMF), poly-ethylene glycol block copolymers, Ascorbate, Tollen's reagent, essential hydrogen, and sodium borohydride (NaBH4) [25-27]. Capping agents are used for size stabilization. The advantage of this technique is that in a short period, considerable number of NPs can be synthesized. The drawback of this method is that chemicals used for the synthesis are toxic and result in byproducts that are not eco-friendly. These issues led to the biosynthesis of NPs through green route which does not require the use of toxic chemicals, this is in response to a growing need to find aneco-friendly alternative. Production of green AgNPs is an important branch of nanotechnology used for the production of NPs by using the materials such as plant extracts, plant biomass, and microorganisms. Green production over physical and chemical procedure is eco-friendly, cost effective and easily scaled up for large scale production of NPs. Also, high pressure, temperature, energy and toxic chemicals are not used in the green synthesis of AgNPs, which gives this method an advantage over the chemical and physical methods [28,29]. To solve the issues presented by the conventional methods, the scientific community is taking the green route using microorganisms like yeast, fungi, bacteria and actinomycetes, plant extracts, and diatoms of viruses.

Synthesis of AgNPs from Bacteria

Bacteria can be used to prepare nanoparticles both inside and outside the cell. Many lactic acid bacteria (LAB) such as *Lactobacillus* spp., *Pediococcus pentosaceus* and *Enterococcus faecium* have the ability to reduce silver ions to silver nanoparticles. As LAB has the ability to produce exopolysaccharides containing different monomers (glucose, galactose, mannose and fructose) that are used in the redox reactions that take place during the manufacturing process of silver nano-composites [30].

The synthesis of inorganic materials from bacteria occurs eitherintra or extracellular. The inorganic materials are potent biofactories for the formation of metal nanoparticles like silver and gold. AgNPs are said to be biocompatible but some bacteria are resistant to silver and can accumulate silver on theircell walls, which may be used as part of a silver recovery process. AgNP sare usually made by the strain of

Pseudomonas stutzeri AG259 which shows resistance for silver. The cells show accumulation of AgNPs in large number up to 200nm by using supernatants culture of psychrophilic bacteria [30-33]. Strains of Licheniformis bacillus have been used for the synthesis of AgNPs. The silver nitrate aqueous solution is added to L. *bacillus*, a variation seen in color from white yellow to brown resulting in AgNPs formation with the size range of 50nm stabilized via enzyme nitrate [34]. Culture supernatants of *Staphylococcus aureushave* also been used to produce AgNPs. However, for fast production of AgNPs, culture supernatants of several bacteria from Enterobacteriaceae family have been used [35].

Synthesis of AgNPs from Fungi

The manufacture of silver nanoparticles using fungi has sparked a wide controversy in various fields of science. Fungi are microorganisms that have the ability to produce proteins and enzymes that have the ability to reduce and stabilize silver particles. One of the most important species used is Aspergillus sp., *Fusarium* sp. In some methods, Trametestrogii was used in the synthesis of silver nanoparticles, as it has the ability to produce enzymes that have the ability to decompose the lignin present in cellulose. *Macrophominaphaseolina* was also used in the synthesis of silver particles at a low cost [36].

Fungi are another source of production of AgNPs. They are easy to handle and have potential capacity of bioaccumulation of metal, high binding capacity, and tolerance and intra-cellular uptake. Fungi secrete many enzymes which are used in silver nitrate (AgNO3) reduction and can be used via several procedures for the production of nanoparticles [37,38]. Antibacterial activity on textile fabrics is investigated by using strains of *Fusarium oxysporum* [39]. AgNPs mono disperse can be produced by utilizing fungus strain *Aspergillus flavus* with the average size of nanoparticles in range of 8.92 ± 1.61nm measured by the transmission electron microscopy procedure [39].

Synthesis of AgNPs from Plants and Plant Extracts

In the biological method, medicinal plants or microorganisms are used to manufacture nanoparticles. The use of medicinal plants is of great benefit as nanoparticles gain its benefits. These plants contain phytochemical compounds that have antioxidant and antibacterial properties that make nanoparticles [40]. Since ancient times, silver compounds have an anti-bacterial effect, and they are used due to their efficiency and cheapness compared to gold compounds. Silver nanoparticles do not have a toxic effect on eukaryotic cells in humans, but they have a toxic effect on prokaryotic cells, as well as in fungi and bacteria. It is not preferred to synthesis silver nanoparticles using Microorganisms because most of them are pathogenic. The most important plants used to prepare silver nanocomposites are *Memecylonedule*, *Callicarpa maingayi*, *Terminalia chebula*, *Trachyspermum ammi*, *Papaver somniferum*, *Bauhinia variegate* L., *Hevea brasiliensis, Aloe vera*, *Cestrum nocturnum* and tea leaf [41].

The first plant that was used for the production of nanoparticles metallic is *Alfalfa sprouts*. The roots of this plant absorb silver from the agar medium and transport it to the shoots of plants in the oxidation state. These silver atoms array themselves in shoots to synthesize the AgNPs [42]. *Ananas comosusis* also used in the synthesis of AgNPs and acts as reducing and stabilizing agent. Production of nanoparticles occurs through ultraviolet visible spectrometer, electron microscopy with high transmission resolution, X-ray spectroscopy with dispersive energy, diffraction area selected, and micrographs transmission electron microscopy showing rounded nanoparticles of 12nm diameter [43]. Peanut shell extract is another material used in the synthesis of AgNPs with its antifungal properties. Compared to commercial AgNPs, both were found to be similar, spherical, and oval with an average diameter between 10 and 50 nm as confirmed with FTIR, XRD peaks, UV-Vis spectra [44]. AgNPs from *Malus domesticaextract* were also spherical and their diameter was 20nmconfirmed with FTIR, XRD peaks, UV-Vis spectra [42]. Some other extracts used for the production of AgNPs are leaf extract of *Polyalthia longifolia* with D-sorbitol, extract of papaya fruit, *Ocimum sanctumleaf* extract, and carbo leaf extract, Ficus carica leaf extract, *Olea europaea*, *Abutilon indica*, Mulberry leaves, Olive leaf extra, *Acacia leucophloeaextract*, *Chrysanthemum indicum*, *Rumex hymenosepalus*, *Premna herbacea* leaf extract, and *Pathenium hysterophorus* leaf extract [45-58].

Using the extracts of plants has beneficial effects because they are safe and easily accessible [59]. Main compounds of capping and reduction of nanoparticles are bio-molecules including proteins, alcoholic component, amino acids, enzymes, flavones, phenols, carbohydrates, terpenoids and alkaloids [60,61]. The parameters like temperature, pH, metal salt, extract concentration, and contact time also affect the plant extracts [62]. Several reducing agents that were found to be effective capping agents in formation of AgNPs are *Jatropha curcas* latex, *Cassia auriculata* leaf extract, Geranium leaf extract, *Ficus benghalensis* leaf extract, *Acorus calamus* extract, *Boerhaavia diffusa* extract, *Acalypha indica* leaf extract, *Chenopodium album* extract, and *Alternanthera denates* extract [63-71].

Anti-Microbial Properties of AgNPs Against Human Pathogens

Silver nanocomposites are manufactured using actinomycetes. In this method, enzymes secreted from the cell wall of actinomycetes reduce the concentration of silver and help the proteins to stabilize silver nanoparticles [72]. Several studies established the antimicrobial properties of the green approach to AgNPs and showed that this approach is ecofriendly, efficient, and cost effective. Fungus *Trichoderma viridewas* used for the synthesis of AgNPs and the characteristics of the particles were studied via ultra-violet visible spectroscopy, electron micro-scope transmission, and electron scanning micro-scope. Their size varied from 1-50nm and the shape was globular. These particles showed exhibition resistant potential for human pathogenic bacteria [73]. Extract of grapes and orange wastes were used and characteristics of AgNPs were determined through electron microscopy transmission, reflectance diffuse infrared spectroscopy, and diffraction of X-ray. Particle size was monitored by UV-Vis spectroscopy. The AgNPs were crystalline and spherical in shape with the average diameter of grape particles being 5-50nm and 3-14nm for orange wastes. They exhibited the dose dependent strong antimicrobial activity against Escherichia coli and *Listeria monocytogenes* [74]. Similarly, *Caulerpa racemosa*, a marine alga was used and the characteristics of AgNPs were determined by electron transmission microscopy, reflectance diffuse infrared spectroscopy and diffraction of X-ray. Particle size was monitored by ultraviolet visible spectroscopy. AgNPs particles were crystalline and face centered cubic geometry in shape. The average diameter of particles obtained was 5-25nm. They exhibited the dose dependent strong anti-microbial properties against *Staphylococcus aureus* and *Proteus mirabilis* [75]. The antimicrobial activity of AgNPs obtained from banana peel extract was determined against pathogenic bacteria and yeast. The production of nanoparticles showed synergistic effect with levofloxacin antibiotic and increase in antimicrobial properties by 1.16-1.32-fold [76]. The antimicrobial activity of AgNPs obtained from *Escherichia coli* was determined against pathogenic bacteria such as *Vibrio cholerae*, *Salmonella typhi*, *Bacillus subtilis* and *Klebsiella pneumoniae*. The particles showed the antibacterial effects [77]. The antimicrobial activity of AgNPs synthesized from the carnivorous plant Dionaea muscipula against pathogenic bacteria was determined, and particles produced the high antimicrobial activity [78]. The antimicrobial activity of AgNPs synthesized from *Aegle marmelos* methanol extract was also determined against pathogenic bacteria. Evaluation of antimicrobial effect of these green synthesized AgNPs recorded the highest inhibitory activity against *Bacillus cereus* followed by *Pseudomonasaeruginosa* and *Shigella dysenteriae* [79].

The antimicrobial activity of AgNPs using *Tribulus terrestris* fruit extract produced the highest antibacterial activity against *Pseudomonas aeruginosa, Streptococcus pyogenes, Escherichia coli, Staphylococcus aureus* and *Bacillus subtilis.* T. terrestris mediated production of AgNPs is less expensive and shows vast antibacterial applications in new medicine [80]. Synthesized AgNPs from *Cocous nucifera* ethyl acetate and methanol extract possess strong antimicrobial properties against *Bacillus subtilis*, *K. pneumoniae*, *Salmonella paratyphi*, and *Pseudomonas aeruginosa* [81]. The green synthesis of T. triangulare AgNPs exhibited significant potential against *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* [82].

Conclusion

Growing need for nanotechnology and green chemistry has pushed for the adoption of development of green routes to produce EMNs using microorganisms, plants and other natural resources. The interest of scientific community in green production of NPshas extensively increased in recent years. Because of their cost-effectiveness, easy availability, nontoxic path, eco-friendly design, number of studies have been conducted on the extraction of plant mediated NPs and their application in different fields. Plants also produce several specific compounds that aid in the synthesis process and speed up the rate of formation. Green synthesis of AgNPs is an exciting and evolving part of nanotechnology impacting the environment and contributing to the long-term sustainability and growth of nano-science. Although the applications of green synthesis of AgNPs are expected to grow rapidly, there are some concerns about their long-term effects on animals and humans, and their aggregation in the environment, which need further investigations. Notwithstanding, the green synthesized AgNPs already have a wide range of applications such as antimicrobial activity against human pathogens, anticancer therapy, wound repair and bone healing, vaccine adjuvant, gene therapy, anti-diabetic agents, drug delivery, biosensors, catalysis, SERS and magnetic resonance imaging, and catalysis. It appears that we have only seen the tip of the iceberg so far and there is much more to be seen and found. Silver Nano composites have anti-bacterial effect as they are non-toxic, cheap and environmentally friendly. Silver nanoparticles showed broad activity against Gram-negative bacteria, in addition to having effective activity in multidrug-resistant strains of bacteria. In addition to being used in many applications in medical devices.

Conflicts of Interest

The authors declare no conflict of interest.

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