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Green synthesis of silver nanoparticles: properties and action against bacterial biofilms

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Abstract

Silver nanoparticles have gained the spotlight for their application in diverse fields over the last decade. Their properties range from optical, conduction, electrochemical and electromagnetic to biological properties like antioxidant, antimicrobial, anti-inflammatory, anti-tumor, and so on. Many investigations on silver nanoparticles show that they have the potential for eradication of antimicrobial resistance caused due to bacterial biofilms, which has become a setback against fighting microbial infections. Silver nanoparticles act against biofilms in multiple ways including damage to DNA, denaturation of proteins of the organism, creating pores or interfering with plasma membrane, generating reactive oxygen species, and many more mechanisms that make it difficult for a bacteria to gain resistance against these silver nanoparticles. Owing to their effective antimicrobial properties, their synthesis and properties are being explored and investigated. The most rational and sustainable way for the synthesis of silver nanoparticles is from plant extracts and microbes rather than chemical or physical synthesis which are high energy, temperature, machinery, labor, and cost requiring. Therefore, this review highlights the process of green synthesis for silver nanoparticles production, hinting at some of their important properties that make it unique and effective for applications in diverse felids as well as their action against bacterial biofilms as an upsurge against treating antibiotic resistance.

Key words: antibiofilm activity, antimicrobial activity, biofilms, green synthesis, silver nanoparticles.

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1. Introduction

Particles of size ranging from 1 to 100 nanometer (nm) are considered as nanoparticles and particles of silver in this range which is smaller than the smallest bacteria are known as silver nanoparticles shortly termed as AgNPs. Metal nanoparticles consist of tens or hundreds of uncharged metal atoms aggregated together with a surface composed of metal cations. Therefore materials when in nanoscale manifest certain distinctive properties [1]. Nanoparticles tend to possess superiority over normal-sized or bulk materials owing to properties like better scattering and absorption of light due to Surface Plasmon Resonance (SPR) [1].

By tuning the scale of the AgNPs, they are often engineered to deliver drugs and medicines, catalyze many reactions effectively, improve the lifetime and efficiency of batteries, serve as effective antibacterial agents, etc. The dynamic properties of AgNPs also allow them to have diverse utilizations in fields like environment, food, healthcare, energy, space, cosmetics, and electronics industry [2].

Th synthesis for AgNPs may be done using different chemical, physical or biological methods. Two major ways i.e. top-down (bulk material is broken into smaller particles) or bottom-up (smaller particles joined together to form the desired particle) approaches are used. Most chemical and physical methods require high-end machinery, heat, energy, chemicals, and are also time-consuming and labor-intensive [3]. Therefore the biological methods are now being explored and optimized for eco-friendly, cost-effective, and comparatively easier methods of AgNP synthesis [4].

AgNPs are also generated from silverware, even when we drink water from silverware or use silver cutlery we consume AgNPs and people are using silver cutlery for over millennia [5]. Colloidal silver, suspensions of silver nanoparticles in liquid were used even before antibiotics. The antimicrobial properties of silver are considered an effective way against the eradication of bacterial biofilms, a group or consortium of microorganisms that are attached with each other and even attached to the surface they are in through the production of a slimy layer made up of exopolysaccharides. These biofilms are responsible for the antibiotic resistance of various bacterial species. The application of AgNPs against bacterial biofilms is effective due to multiple mechanisms that it uses like DNA damage, protein denaturation, membrane damage by the accumulation of AgNPs, generation of ROS, and many more [6]. That is the reason why in this review, the basic focus is on the different methods used for green synthesis of AgNPs, evaluation of their properties that make the particles have varied application in different fields, and most importantly the application of AgNPs as antimicrobial agents against bacterial biofilms.

2. Synthesis of AgNP

Synthesis of AgNPs or metallic nanoparticles, in general, is grouped into top-down and bottom-up approaches involving physical, chemical, or biological modes for extraction [7]. Ball milling, grinding, are top-down where sol-gel methods, gas condensation, laser ablation being some of the bottom-up approaches.

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Some physical techniques for AgNP synthesis include mechanical milling, laser ablation, and evaporation-condensation [8]. The chemical mode of synthesis for AgNPs mainly includes chemical reduction using certain reducing agents for example Sodium borohydride NaBH4, Ascorbic acid, ammonium formate, sodium citrate, hydrazine elemental hydrogen, polyol, poly(ethylene glycol)-block copolymers, process, N, N-dimethylformamide (DMF), and many more [9,10]. Microwave-assisted synthesis, Microemulsion techniques are also used [8, 11]. But these forms of synthesis require heavy machinery as in milling and laser ablation, high energy for both physical and chemical modes, high heat for evaporation, also these techniques require more labor and some are very time-consuming.

2.1 Green Synthesis:

The bio-synthesis of AgNPs using different plants or microbes is known as the green synthesis approach which has been gaining a lot of desirability over physical and chemical methods [12]. The usage of microorganisms like bacteria, algae, fungi, or yeast has become widely recognized due to enormous potential for economical, environmentally sustainable outcomes that are less toxic, and needless use of harsh chemicals [13]. Basically, the synthesis can be intracellular or extracellular and the latter is mostly preferred. Microorganisms pose various enzymes that can detoxify, or reduce metals and this aspect has been applied for the green synthesis of various nanoparticles like gold and silver [14].

For addressing the large-scale synthesis of nanoparticles plant extracts can be used [14]. Ag NPs have been derived from various plants and from different plant parts including leaves, roots, fruits, bark, seed, callus, or stems extracts [5]. The mechanism for AgNP synthesis from different plant extract points directly towards proteins in plants that tap the silver ions to their surface which is due to the electrostatic interaction, or reduction because of phytochemicals such as flavonoids, quinones, organic acids, etc. these reducing agents have also seen to act as capping agents or stabilizers and no external agents are required for these [15, 16].

The process of bio-synthesis using microorganisms and plants has been demonstrated in Figure-1. The plant extracts or microbial cultures are added with metal salts, AgNO3, AgNP synthesis. For further refinement of the process of AgNP using green synthesis, temperature, pH, reaction conditions, reagents, aeration, mixing ratio, as well as incubation period must be modified [14]. *Ficus carica* fruits [17], *Opuntia ficus*-indica [2], *Citrus limetta* peel, [18] *Parkia speciosa* leaf, [19], *Moringa oleifere* Leaf [20], Bamboo Leaf [20], *Azadirachta indica* (Neem) Leaf [20] , *Microbacterium* sp. NV4 [21], *Bacillus subtilis* [22], *Lactobacillus reuteri* [23], *Chroococcus minutus* (strain, CRLSUM10) [24], *Klebsiella pneumoniae* [25] and many more plants as well as microbes have been used for AgNP synthesis.

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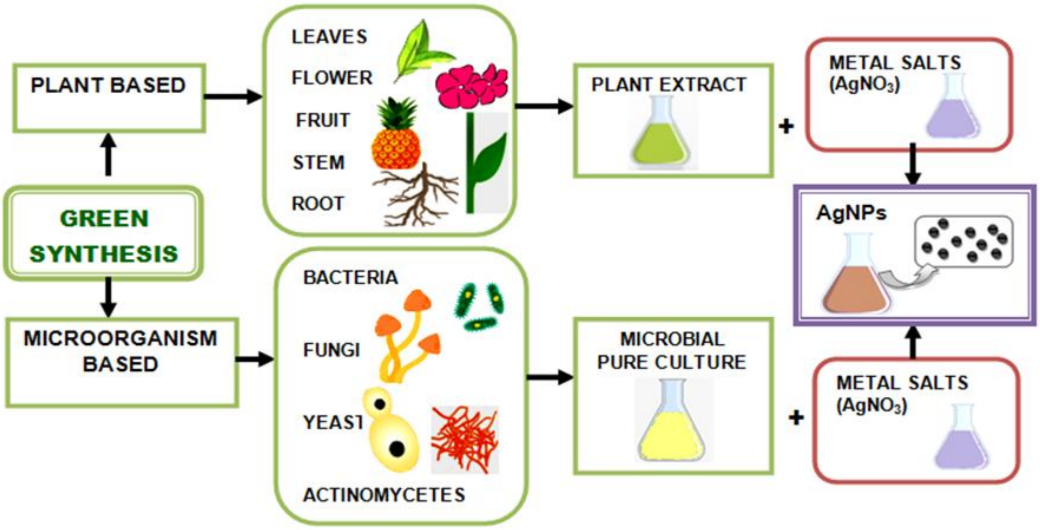

Figure1: Green synthesis of AgNPs from plants and microorganisms

3. Properties of AgNPs

AgNPs have many unique properties that tend to make them way more desirable than silver ions for different applications starting from conductors, photovoltaics, sensors to antimicrobial agents. These diverse applications are due to enhanced properties of AgNPs like excellent scattering and absorption of light, better conductance, better electromagnetic, optical, and electrochemical properties [26, 27].

When the nanoparticles are irradiated by light at a specific wavelength, the conduction free electrons present on the surface of the metal may undergo resonant oscillation and this phenomenon is called Surface Plasmon Resonance which aids the optical properties of AgNPs [26, 28]. The absorption, as well as the scattering of light via AgNP, can be modified or optimized by varying their refractive index, size, and shape. AgNPs synthesized by plant extracts show –ve zeta potential that can be advantageous as +ve zeta potential increases the cellular uptake as well as cytotoxicity[27, 29]. Silver commonly seen as shiny or metallic can turn blue, yellow, or maybe red due to its optical properties in nanoscale [26].

AgNPs have numerous biological properties that have provoked more research on them. Some of them include anti-cancer, anti-tumor, antioxidative, anti-inflammatory, antifungal, antibacterial, antiviral, and anti-angiogenic [30]. Ag ions are known to possess antimicrobial activity and Ag has been used as antimicrobial agents for a long time. Also, AgNPs, owing to their nano size and the large surface area tends to be better catalytic agents for chemical or biological processes [26].

It's seen that in the case of green synthesis certain compounds remain bound to the surface like flavonoids, lipids, tannins, polysaccharides, or proteins that tend to increase stability and adds to the antioxidant, antimicrobial activity, catalytic activity, and reduces the toxicity[27]. Some suggest that coating on AgNPS may cause a reduction in agglomeration rate and size

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also cause an adverse effect on cytotoxicity [29].

4. AgNPs against bacterial biofilms

Ag has been used for treating burns, wounds as well as used as antiseptics for a long time. AgNPs among metal nanoparticles have been proven to be better antimicrobial agents [6]. They have multiple modes of action against bacteria which makes them a suitable agent for combating antibiotic resistance due to the formation of bacterial biofilms. The activity of AgNPs on bacteria can be biocidal or inhibitory [5]. Factors like temperature, pH, species of bacteria, and concentration of AgNO3 decide the activity of the AgNPs [14, 5]. Some examples of green synthesized AgNPs effective against biofilms are mentioned in Table -1.

Many studies have been conducted to detect the efficacy of AgNPs against bacterial biofilms and antibiotic-resistant bacteria [31]. It's found that smaller-sized particles and stabilized AgNPs have better potential against biofilms. Inactivation of the biofilms can be influenced by stirring suggesting that AgNPs tend to inactivate biofilms by a process of biosorption. AgNPs can adequately strengthen the anti-bacterial, as well as anti-biofilm activity of many conventionally used antibiotics[6] like streptomycin, ampicillin, kanamycin, or vancomycin against Escherichia coli and Pseudomonas aeruginosa [6,32] Among new anti-biofilm approaches, include a coating of the medical devices with AgNPs may have the potential for combating the formation of biofilms [6].

The possible bacteriocidal mechanisms demonstrated till now (Figure-1) include- generation of free radicals like hydroxyl or superoxide anions that are considered as ROS (reactive oxygen species) [5], bonding of AgNPs to sulfhydryl groups leading to protein denaturation, entering of AgNPs into the bacterial cell wall which in turn may cause lethal damage to the microorganism leading to the cell death [33]. They also interact with the intracellular bacterial proteins, bacterial membrane proteins, phosphate residues of the bacterial DNA, and even interferes in the cell division of bacteria, all of which lead to bacterial apoptosis [5, 34].

Table 1. Anti-biofilm activity of certain biologically synthesized AgNPs

Agents used for AgNP synthesis	Size of AgNPs (nm)	Effective against biofilms formed by-	References
Clitorea ternatea (flower)	4-12	*Escherichia coli, Streptococcus pyogenes, Klebsiella pneumonia,* and *Staphylococcus aureus*	[35]
Thymus vulgaris (leaf)	75	Methicillin-resistant *Staphylococcus aureus* (MRSA) 090	[36]
Punica granatum (fruit)	68	*Pseudomonas aeruginosa*	[37]

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Mimusops elengi (fruit)	43	*Escherichia coli, Salmonella kentucky, Enterococcus durans, Listeria innocua, Staphylococcus epidermidis, Bacillus subtilis, Klebsiella pneumoniae,* and *Salmonella enteritidis.*	[38]
Citrus macroptera (fruit)	16	*Bacillus subtilis* and *Pseudomonas aeruginosa*	[39]
Carum copticum (seeds)	21.48 (average)	*Pseudomonas aeruginosa, Chromobacterium violaceum,* and *Serratia marcescens*	[40]
Olea europaea (fruit)	11.6 - 20.7	*Streptococcus mutans* and *Candida albicans*	[41]
Gelidium corneum	20-40	*Candida albicans*	[42]
Setosphaeria rostrata	2–20	*Pseudomonas aeruginosa*	[43]
Nocardiopsis sp. GRG1 (KT235640)	20-50	*Staphylococci* (MR-CoNS)	[44]
Spirulina platensis	29	*Pseudomonas aeruginosa* PA14	[45]

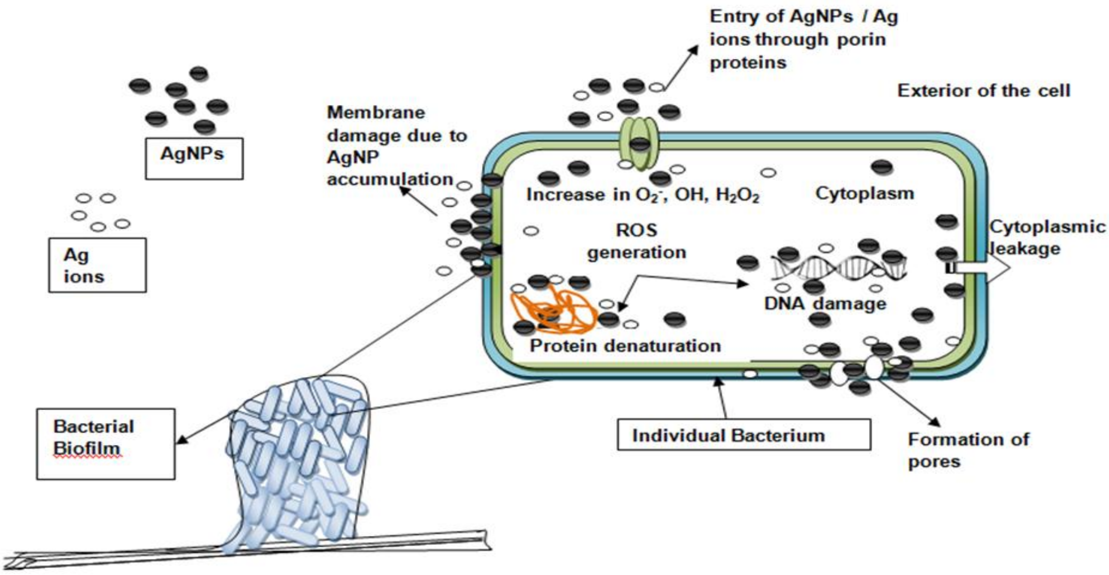

Figure 2: Multiple mechanisms of action of AgNPs against bacterial biofilms

Conclusion

Green syntheses of AgNPs have different merits over chemical or physical methods, because of which they are considered a potential and sustainable method. The unique properties posed by these make it a candidate apt for research on its synthesis, characterization, and antimicrobial activity. The AgNPs show ability to combat bacterial biofilms and this can be useful for upsurge for new antimicrobial agents due to growing cases of antimicrobial resistance. Therefore, this review addresses and emphasizes the plant or microbial-based green synthesis with properties of AgNP that can be useful in multidisciplinary applications mostly the biological field. Also, the mechanisms against bacterial biofilms have been put forth in hope of intriguing more research enthusiasm towards optimizing and upgrading these AgNPs as antibacterial agents.

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