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Green-Synthesized Silver Nanoparticles and Their Potential for Antibacterial Applications

Zdenka Bedlovičová and Aneta Salayová

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.72138

Abstract

The prevalence of infectious diseases is becoming a worldwide problem, and antimicrobial drugs have long been used for prophylactic and therapeutic purposes, but bacterial resistance has creating serious treatment problems. The development of antibiotic resistance makes scientists around the world to seek new drugs that would be more effective. The use and search for drugs obtained from plants and other natural products have increased in recent years. It is well known that silver and its compounds have strong antibacterial activity. Silver, compared to the other metals, shows higher toxicity to microorganisms, while it exhibits lower toxicity to mammalian cells. The progress in the field of nanotechnology has helped scientists to look for new ways in the development of antibacterial drugs. Silver nanoparticles (AgNPs) are interesting for their wide range of applications, e.g. in pharmaceutical sciences, which include treatment of skin diseases (e.g. acne and dermatitis) and other infectious diseases (e.g. post-surgical infections). Various antibacterial aids, such as antiseptic sprays, have also been developed from AgNPs. In this chapter, we have focused on various synthesis methodologies of AgNPs, antibacterial properties, and the mechanism of action.

Keywords: silver nanoparticles, plant extracts, antibacterial activity, green synthesis, biosynthesis

1. Introduction

Frequent use of antibiotics results in resistance of pathogens against them. This is the health and the life-threatening reality. It is therefore necessary to look for new sources of effective potent drugs. Nature is an inexhaustible source of health-promoting substances. Combination of knowledge in natural medicine with modern technology leads to the discovery of new drugs. One of the most promising sources in recent years has been shown to be plant extracts,
which are rich in antioxidant and antimicrobial compounds that have been used as a nanoparticle synthesis agent [1–3]. Nanotechnology and nanoscience have been established recently as an interdisciplinary subject dealing with biology, chemistry, physics, and engineering. The term “nano” is derived from the Greek word dwarf in the meaning of extremely small. The size of nanoparticle is between 1 and 100 nm [4, 5]. Unique biological, chemical, and physical properties of silver nanoparticles (AgNPs) lead to the wide range of applications in spectroscopy, sensors, electronics, catalysis, and pharmaceutical purposes [6]. It is well known that silver has an inhibitory effect toward many bacterial strains and microorganisms commonly presented in medical and industrial properties [7], e.g. in the medical industry including creams containing silver to prevent local infections of burns or open wound, dental work, catheters, plastics, soaps, pastes, and textiles [8–11]. Many authors confirmed that AgNPs show efficient antimicrobial properties and kill bacteria at low concentrations (mg L⁻¹) [12] without toxic effect on human cells [13, 14].

The presented review deals with the possibilities of the synthesis of AgNPs with respect to green synthesis as well as their antimicrobial activity and its determination. Finally, we focused on the mechanism of action theories of silver nanoparticles.

2. Synthesis of silver nanoparticles

Production of nanoparticles can be achieved through different methods. Generally, we can divide these methods into physical, chemical, and biological. Some of these methods are simple and make good control of nanoparticle size by affecting the reaction process. On the other side, there are still problems with stabilization of the product and in obtaining monodisperse nanosize using achieved method [15]. In Table 1, we show list of some synthesis techniques of AgNPs and some of them are further briefly described.

2.1. Physical and chemical methods

For a physical approach, the nanoparticles are prepared using evaporation-condensation, which could be carried out in a tube furnace at atmospheric pressure [16]. This method has

<table>
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<tr>
<th>Silver nanoparticles synthesis</th>
<th>Chemical methods</th>
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<td>Physical methods</td>
<td>Reduction, Sonochemical, Photochemical, Electrochemical, Microwave</td>
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<tr>
<td>Arc discharge</td>
<td></td>
<td>Using essential oils</td>
</tr>
</tbody>
</table>

Table 1. Selected techniques for the preparation of AgNPs.
some disadvantages due to large space of tube furnace, high energy consumption, and long time for achieving thermal stability. For these reasons, various methods of synthesis of AgNPs by physical methods were developed, for example, a thermal decomposition for the preparation of nanoparticles in powder form [17]. Conventional physical methods, such as pyrolysis, were used by Pluym et al. [18]. The advantages of physical methods are speed and no use of toxic chemicals, but there are also some disadvantages, such as low yields and high energy consumption [19].

Chemical methods provide an easy way to prepare AgNPs in solution (water or organic solvent could be used). The most common method of synthesis of silver nanoparticles is reduced by organic and inorganic reducing agents. Generally, various reducing compounds, such as sodium ascorbate, sodium borohydride, hydrogen, Tollens reagent, and $N,N$-dimethylformamide, could be used for Ag$^+$ ion reduction. The reduction leads to the formation of metallic silver Ag$^0$, which is followed by agglomeration into oligomeric clusters. These clusters eventually lead to the formation of metallic colloidal silver particles [20]. It is essential to use stabilizing agents during the preparation of AgNPs to avoid their agglomeration [21]. It is necessary to note that polymeric compounds such as poly (vinyl alcohol), poly (vinyl pyrrolidone), and polyethylene glycol are effective protecting agents for the stabilization of nanoparticles [22, 23].

### 2.2. Green synthesis

As we described, there are various chemical and physical methods of synthesis of silver nanoparticles. These methods are cost and toxic for the environment [24]. These facts lead to the look for new, simple, and eco-friendly alternatives that would not harm human and animal health. The revolution in the world of synthesis of silver nanoparticles has brought the development of the green synthesis techniques. The biologically provided synthesis of nanoparticles has been shown to be simple, low cost, and environmentally friendly. In the green synthesis, the reduction procedure is performed by a natural-based material including bacteria, fungi, yeast, plants and plant extracts, or small biomolecules (e.g. vitamins, amino acids, or polysaccharides) [25–27]. With the development of reliable methodology to produce nanoparticles, several attempts of in vivo and in vitro synthesis of AgNPs have been realized. In this chapter, we divided the green synthesizing methods into in vivo synthesis, where for the biogenic production of silver nanoparticles, whole cells were used. In this approach, silver reduction can happen intracellularly or extracellularly with the formation of silver nanoparticles on the cell walls. Another case of synthesis of AgNPs is in vitro method. These procedures perform outside of a living organism with a cell-free extract, since the nanoparticles do not need whole cells for their synthesis. Generally, the process of biosynthesis involves three steps. The first step is, the phase of reduction of Ag$^+$ ions; the second is growth step, where larger aggregates are observed; and finally, the third, in which the stabilization of nanoparticles with capping agents is proceeded [28]. In Table 2, we reported the most recently published green synthesis of silver nanoparticles.

#### 2.2.1. In vitro synthesis of AgNPs

In the so-called green approach, the reduction procedure is performed by a natural-based material, most commonly a plant extract containing substances with the antioxidant and
<table>
<thead>
<tr>
<th>Species</th>
<th>Reducing/capping agent</th>
<th>Precursor Charac. techn.</th>
<th>Tested bacteria</th>
<th>Method</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pelargonium endlicherianum</em></td>
<td>Root extract</td>
<td>5 mM AgNO₃</td>
<td>SEM UV/Vis DLS Zeta pot.</td>
<td>G⁻: E. coli, <em>Pseudomonas aeruginosa</em> G⁺: <em>Staphylococcus epidermidis</em></td>
<td>[66]</td>
</tr>
<tr>
<td><em>Lantana camara</em></td>
<td>Terpenes-rich extract of leaf</td>
<td>1 mM AgNO₃</td>
<td>XRD UV/Vis FTIR SEM Zeta pot.</td>
<td>G⁻: E. coli, <em>Pseudomonas aeruginosa</em> G⁺: <em>Staphylococcus aureus</em></td>
<td>[63]</td>
</tr>
<tr>
<td><em>Diospyros sylvestrica</em></td>
<td>Root extract 10 mM silver acetate</td>
<td>UV/Vis SEM TEM XRD</td>
<td>G⁻: E. coli, <em>Klebsiella pneumoniae</em>, <em>Pseudomonas aeruginosa</em>, <em>Proteus vulgaris</em> G⁺: <em>Bacillus subtilis</em>, <em>Bacillus pumilis</em>, <em>Streptococcus pyogenes</em>, <em>Staphylococcus aureus</em></td>
<td>Macrodiffusion broth method, MIC</td>
<td>[67]</td>
</tr>
<tr>
<td><em>Adhatoda vasica</em></td>
<td>Leaf extract 50 mM AgNO₃</td>
<td>UV/Vis XRD SEM TEM</td>
<td>G⁻: <em>Salmonella typhimurium</em> G⁺: <em>Bacillus subtilis</em></td>
<td>Agar diffusion method</td>
<td>[68]</td>
</tr>
<tr>
<td><em>Carica papaya</em></td>
<td>Peel extract 0.25–1.25 mM AgNO₃</td>
<td>UV/Vis FTIR XRD DLS AFM Zeta pot.</td>
<td>G⁻: E. coli, <em>Klebsiella pneumoniae</em> G⁺: <em>Staphylococcus aureus</em>, <em>Bacillus subtilis</em></td>
<td>Disc-diffusion method</td>
<td>[61]</td>
</tr>
<tr>
<td><em>Alstonia scholaris</em></td>
<td>Leaf extract 1 mM AgNO₃</td>
<td>UV/Vis XRD EDS SEM AFM FTIR</td>
<td>G⁻: E. coli, <em>Pseudomonas aeruginosa</em> G⁺: <em>Klebsiella sp.</em>, <em>Bacillus sp.</em></td>
<td>Agar well-diffusion method</td>
<td>[69]</td>
</tr>
<tr>
<td><em>Ocimum tenuiflorum,</em> <em>Solanum tricocatum,</em> <em>Syzygium cumini,</em> <em>Centella asiatica,</em> <em>Citrus sinensis</em></td>
<td>Leaves/peel extract 1 mM AgNO₃</td>
<td>UV/Vis XRD AFM SEM</td>
<td>G⁻: E. coli, <em>Pseudomonas aeruginosa</em>, <em>Klebsiella pneumoniae</em> G⁺: <em>Staphylococcus aureus</em></td>
<td>Agar well-diffusion method</td>
<td>[70]</td>
</tr>
<tr>
<td>Species</td>
<td>Reducing/capping agent</td>
<td>Precursor</td>
<td>Charac. techn.</td>
<td>Tested bacteria</td>
<td>Method</td>
</tr>
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</tr>
</tbody>
</table>
| *Ficus religiosa*    | Leaf extract           | 1 mM AgNO$_3$ | TEM UV/Vis EDX FTIR DLS DSC Zeta pot. | G-: *E. coli*, *Pseudomonas fluorescens*, *Salmonella typhi*  
G+: *Bacillus subtilis* | Disc-diffusion method | [71] |
| *Tamarindus indica*  | Fruit extract          | 5 mM AgNO$_3$ | UV/Vis XRD EDX SEM TEM FTIR | G-: *Pseudomonas aeruginosa*, *Salmonella typhi*, *E. coli*, *Klebsiella pneumonia*  
G+: *Bacillus cereus*, *Staphylococcus aureus*, *Microcococcus luteus*, *Bacillus subtilis* | Agar diffusion method | [72] |
| Alfalfa              | *In vivo seeds*        | AgNO$_3$   | XRD TEM         | —                                                                                | —                   | [52] |
| **Mushrooms**        |                        |           |                |                                                                                  |                     |      |
| *Pleurotus ostreatus*| Water extract          | 1 mM AgNO$_3$ | UV/Vis SEM TEM EDX FTIR | G-: *E. coli*, *Pseudomonas aeruginosa*  
G+: *Bacillus subtilis*, *Bacillus cereus*, *S. aureus* | Disc-diffusion method, MIC | [34] |
| *Ganoderma neo-japonicum* (Imazeki) | Water extract          | 1 mM AgNO$_3$ | UV/Vis XRD TEM | —                                                                                | —                   | [35] |
| *Pleurotus florida*  | Water extract          | 1 mM AgNO$_3$ | UV/Vis XRD FTIR TEM AFM | G-: *Salmonella typhi*, *Proteus mirabilis*, *Providencia alcalifaciens*  
G+: *S. aureus* | MIC                         | [36] |
| **Larvae**           |                        |           |                |                                                                                  |                     |      |
| *Oecophylla smaragdina* | Tissue extract        | 1 M AgNO$_3$ | UV/Vis TEM EDX | G-: *E. coli*  
G+: *S. aureus* | Disc-diffusion method, MIC MBC | [73] |
<p>| <strong>Algae</strong>            |                        |           |                |                                                                                  |                     |      |
| <em>Gracilaria corticata</em> | Water extract          | 1 M AgNO$_3$ | UV/Vis XRD FTIR TEM EDS | —                                                                                | —                   | [74] |</p>
<table>
<thead>
<tr>
<th>Species</th>
<th>Reducing/capping agent</th>
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<th>Charac. techn.</th>
<th>Tested bacteria</th>
<th>Method</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pterocladiella capillacea</em></td>
<td>Water extract</td>
<td>1 mM AgNO$_3$</td>
<td>UV/Vis FTIR TEM EDX</td>
<td>$G^-$: E. coli, <em>Pseudomonas aeruginosa</em> $G^+$: S. aureus, B. subtilis</td>
<td>Agar well-diffusion technique</td>
<td>[64]</td>
</tr>
<tr>
<td><em>Sargassum longifolium</em></td>
<td>Water extract</td>
<td>1 mM AgNO$_3$</td>
<td>UV/Vis XRD FTIR TEM</td>
<td>—</td>
<td>—</td>
<td>[51]</td>
</tr>
<tr>
<td><strong>Bacteria</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Acinetobacter spp.</em></td>
<td>Cell-free extract</td>
<td>0.5–5 mM AgNO$_3$</td>
<td>UV/Vis XRD TEM EDX</td>
<td>$G^-$: E. coli, <em>Pseudomonas aeruginosa</em>, <em>Enterobacter aerogenes</em>, <em>Shigella sonnie</em>, <em>Salmonella typhi</em> $G^+$: S. aureus, <em>Streptococcus mutans</em></td>
<td>Disc-diffusion method, MIC</td>
<td>[62]</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>Culture supernat</td>
<td>1 mM AgNO$_3$</td>
<td>UV/Vis TEM</td>
<td>—</td>
<td>—</td>
<td>[40]</td>
</tr>
<tr>
<td><em>Klebsiella pneumonia</em>, <em>Escherichia coli</em>, <em>Enterobacter cloacae</em></td>
<td>Culture supernat</td>
<td>1 mM AgNO$_3$</td>
<td>UV/Vis TEM EDS</td>
<td>—</td>
<td>—</td>
<td>[41]</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>Culture supernat</td>
<td>1 mM AgNO$_3$</td>
<td>UV/Vis TEM FTIR DLS</td>
<td>—</td>
<td>—</td>
<td>[25]</td>
</tr>
<tr>
<td><em>Pseudomonas stutzeri</em></td>
<td>In vivo</td>
<td>50 mM AgNO$_3$</td>
<td>TEM EDX</td>
<td>—</td>
<td>—</td>
<td>[53]</td>
</tr>
<tr>
<td><em>Bacillus cereus</em></td>
<td>Biomass</td>
<td>1 mM AgNO$_3$</td>
<td>UV/Vis TEM EDX</td>
<td>$G^-$: E. coli, <em>Pseudomonas aeruginosa</em>, <em>Klebsiella pneumoniae</em>, <em>Salmonella typhi</em> $G^+$: S. aureus</td>
<td>Agar well-diffusion technique</td>
<td>[55]</td>
</tr>
<tr>
<td><strong>Fungi</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Verticillium</em></td>
<td>Fungal biomass</td>
<td>0.2 mM AgNO$_3$</td>
<td>UV/Vis TEM XRD SEM EDX</td>
<td>—</td>
<td>—</td>
<td>[57]</td>
</tr>
</tbody>
</table>
reducing properties, e.g. aldehydes, ketones, terpenoids, flavones, or carboxylic acids [29].
In the field of plant-mediated nanotechnology, various plant extracts of specific parts such as root, bark, stem, leaves, seed, fruit, peel, and flower have been used for the synthesis of silver nanoparticles [30–32]. In general, silver nitrate aqueous solution is used for the reaction with the plant extract leading to rapid formation of stable nanoparticles. The plant extract is usually prepared by suspending dried powdered parts of plant in distilled or deionized water or organic solvent, most often ethanol and methanol. Extraction is carried out in various ways (different temperature, time, extract concentration, and pH). After extraction, the solid residues are removed, and the filtrate is used for the synthesis of silver nanoparticles. It is investigated that green synthesis using plant extracts is faster than microorganisms, such as bacteria or fungi [30]. The biosynthesis of silver nanoparticles from different parts of plants is schematically described in Figure 1.

<table>
<thead>
<tr>
<th>Species</th>
<th>Reducing/capping agent</th>
<th>Precursor</th>
<th>Charact. techn.</th>
<th>Tested bacteria</th>
<th>Method</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillium brevicompactum</td>
<td>Water extract</td>
<td>1 mM AgNO₃</td>
<td>UV/Vis, TEM, FTIR, XRD</td>
<td>—</td>
<td>—</td>
<td>[39]</td>
</tr>
<tr>
<td>Biomolecules</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-Glucan</td>
<td>Solution</td>
<td>0.5–1 mM AgNO₃</td>
<td>UV/Vis, EDX, TEM, DLS</td>
<td>G−: E. coli, Methyllobacterium spp., Sphingomonas spp</td>
<td>Disc-diffusion method</td>
<td>[75]</td>
</tr>
<tr>
<td>Pectin</td>
<td>Water solution</td>
<td>1–10 mM AgNO₃</td>
<td>TEM, XRD, DSC, TGA, DLS, FTIR, Zeta pot.</td>
<td>G−: E. coli</td>
<td>MIC</td>
<td>[26]</td>
</tr>
<tr>
<td>Glutathione</td>
<td>1 mM water solution</td>
<td>1 mM AgNO₃</td>
<td>FTIR, AFM, SEM</td>
<td>G−: E. coli</td>
<td>Optical density</td>
<td>[46]</td>
</tr>
</tbody>
</table>


Table 2. Green synthesized AgNPs and their antibacterial activity, if determined.
It is well known that edible mushrooms are rich in proteins, saccharides, vitamins, amino acids and other compounds, which can be used as reducing agents in biosynthesis of silver nanoparticles. The aqueous extract of edible mushroom *Volvariella volvacea* was used for the synthesis of AgNPs. Author thought that as mushrooms are rich in proteins, there is increased productivity of nanoparticles compared to other biosynthesis routes already reported [33]. In recent time, there has been published another papers that described green synthesis from mushroom extracts, for example, *Pleurotus ostreatus* [34], *Ganoderma neo-japonicum* [35], and *Pleurotus florida* [36].

Interestingly, the use of microorganism extract has resulted in an easy method to synthesize nanoparticles with characteristic shapes, size, and morphology. Extracts from microorganisms (fungi, bacteria, yeasts, actinomycetes) may act as reducing and capping agents for the synthesis of AgNPs. The reduction of Ag⁺ ions is proceeded by biomolecules found in extract (enzymes, proteins, amino acids, polysaccharides or vitamins). In case of microorganisms, the extracts can be made by two methods. The first is by washing the biomass and dissolving the cells in water or a buffer [37], and the second is by used medium in which biomass has grown [38]. The aqueous extract from a fungus *Penicillium brevicompactum* was attempted [39]. Of course, specific bacteria can be used for the synthesis of nanoparticles. An interesting approach of green biosynthesis of AgNPs using supernatant of *Bacillus subtilis* and microwave irradiation in water solution has been studied [40]. Authors reported extracellular biosynthesis of silver nanoparticles and avoid the aggregation of microwave radiation they used. The rapid biosynthesis of AgNPs using the bioreduction of aqueous Ag⁺ ion by the culture supernatants of *Klebsiella pneumonia*, *Escherichia coli*, and *Enterobacter cloacae* was reported [41]. An extensive volume of literature reported successful biosynthesis of AgNPs using microorganisms including bacteria, fungi, yeasts, and actinomycetes.
Reduction of Ag⁺ ions is achieved using biomolecules that also serve as a capping agent. Reaction is mostly performed in water solution, which is considered as environmentally friendly solvent system. The extraction and purification of biomolecules require one or more steps in production process, and for this reason, it could be needed more time and would be not economic. The solution of this problem is using simple molecules, such as saccharides and polysaccharides as reducing agents. A simple method for silver nanoparticles was described by Raveendran et al., who used α-D-glucose as reducing agent in gently heated system [42]. In another work, the AgNPs were synthesized using pectin from citrus [26]. Authors found optimal conditions and some advantages, such as short reaction time, almost 100% conversion of Ag⁺ ion to Ag₀ and very good reproducibility and stability of the product. There were used many approaches using polysaccharides for synthesis of AgNPs, e.g. dextrin [43], cellulose [44], or polysaccharides isolated from marine macro algae [45]. Preparation of silver nanoparticles using other isolated or purified biomolecules has also been studied. For example, as reducing and capping agents were used glutathione [46], tryptophan residues of oligopeptides [47], natural biosurfactants [48], oleic acid [49], etc.

Essential oils could be one of the alternative methods for biosynthesis of AgNPs. With respect to chemical composition of essential oils (phenols, flavonoids, terpenes), essential oils have been successfully used for the preparation of silver nanoparticles. Usually, reduction is used for aqueous solution of silver nitrate, and essential oils serve as reducing and capping agents [50].

Algae extracts have great efficiency in green synthesis of nanoparticles. There have been a few articles published reporting this method. Rajeshkumar and his co-workers published an algae-mediated preparation of AgNPs using purified brown algae *Sargassum longifolium* water extract. The extract was mixed with silver nitrate water solution and kept at the room temperature [51]. Bioreduction of Ag⁺ ions by algae extracts is similarly proceeded due to content of phytochemicals (carbohydrates, alkaloids, polyphenols, etc.).

2.2.2. *In vivo* synthesis of AgNPs

Under the term *in vivo*, we understand the biosynthesis of nanoparticles in living organisms, either extracts or isolated biomolecules. Gardea-Torresdey published the very first article discussing about synthesis of silver nanoparticles using living plant Alfalfa (*Medicago sativa*). They found that Alfalfa root is able to absorb silver in neutral form (Ag⁰) from agar medium and transport it into the shoots of plant where Ag⁰ atoms arrange themselves to produce AgNPs [52]. Marchiol et al. [28] reported the *in vivo* formation of silver nanoparticles in plants *Brassica juncea*, *Festuca rubra*, and *Medicago sativa*. The rapid bioreduction was performed within 24 h of exposure to AgNO₃ solution. TEM analyses indicated the *in vivo* formation of AgNPs in the roots, stems, and leaves of the plants, which had a similar distribution but different sizes and shapes. The contents of reducing sugars and antioxidant compounds were proposed to be involved in the biosynthesis of AgNPs.

Some microorganisms resistant to metal can survive and grow in the presence of metal ions. The first evidence of bacteria synthesizing silver nanoparticles was observed using
*Pseudomonas stutzeri* AG259 strain [53]. Since the first evidence of bacteria producing AgNPs in 1999, different bacteria were used, e.g. *Lactobacillus* strains [54], *E. coli* [25], or *Bacillus cereus* [55]. Fungi have been observed as good producers of silver nanoparticles due to their tolerance and capability of bioaccumulation of metals. When fungus is exposed to the Ag⁺ ions, it produces enzymes and metabolites, which protect it from undesirable foreign matters resulting in production of AgNPs [56]. Many reports dealing with biosynthesis of silver nanoparticles using fungi or yeasts have been published. For example, fungus-mediated synthesis of silver nanoparticles was described by Mukherjee [57]. They isolated the fungus *Verticillium* from the *Taxus* plant, and after mycelia growth and separation, they suspended dry mycelia in distilled water and added the Ag⁺ ions to prepare silver nanoparticles. They found that the AgNPs were formed below the cell wall surface due to reduction of silver ions (Ag⁺) by enzymes presented in the cell wall membrane. Extracellular production of silver nanoparticles was described, for example, by Sadowski, who prepared nanoparticles from *Penicillium* fungi isolated from the soil [58].

### 3. Antibacterial activity

The discovery of the first antibiotics has dramatically changed the quality of human life, but the development of the natural mechanism of bacterial resistance has been forced scientists to develop more effective antimicrobial drugs. The interest about the use of nanoparticles as antibacterial agents has seen a dramatic increase in the last few decades. The unique properties of silver nanoparticles have allowed exploiting in medicinal field. The most studies have been attended to their antimicrobial nature. Since silver nanoparticles show promising antimicrobial activity, researchers use several techniques to determine and quantify their activity on various Gram-positive and Gram-negative bacteria.

#### 3.1. Methods of evaluation of antibacterial activity

To evaluate the antimicrobial activity different methods are currently used, the results of which are given in different ways. Commonly used techniques to determine the antimicrobial activity of biogenic silver are the minimal inhibitory concentration (MIC), the minimal bactericidal concentration (MBC), time-kill, the half effective concentration (EC₅₀), well-diffusion method, and disc-diffusion method. The most commonly used is disc-diffusion method developed in 1940. These well-known procedures are comprised of preparation of agar plates incubated with a standardized inoculum of test microorganism. Then, the sterile discs (about 6 mm in diameter) impregnated with AgNPs at a desired concentration are placed on the agar surface. According to agar well-diffusion method, the tested concentration of AgNPs is introduced into the well with a diameter of 6–8 mm punched into agar. Cultured agar plates are incubated under conditions suitable for tested bacteria, and the sensitivity of the tested organisms to the AgNPs is determined by measuring the diameter of the zone of inhibition around the disc or well. This method is contributed and beneficial for its simplicity and low cost and is commonly used in antibacterial activity of Ag nanoparticles evaluation [59].
The antibacterial properties of silver nanoparticles are often studied by employing dilution methods, quantitative assays, the most appropriate ones for the determination of MIC values. Minimal inhibitory concentration (MIC) is usually expressed in mg mL$^{-1}$ or mg L$^{-1}$ and represents the lowest concentration of the AgNPs, which inhibits the visible growth of the tested microorganism. Either broth or agar dilution method may be used for quantitative measurement, the \textit{in vitro} antimicrobial activity against bacteria. The minimum bactericidal concentration (MBC) is less common compared to MIC determination and is defined as the lowest concentration of antimicrobial agent killing 99.9% of the final inoculum after incubation for 24 h. The most appropriate method for determining the bactericidal effect is the time-kill test and can be also used to determine synergism for combination of two or more antimicrobial agents. These tests provide information about the dynamic interaction between different strains of microorganism and antimicrobial agents. The time-kill test reveals a time-dependent or a concentration-dependent antimicrobial effect. The varied time intervals of incubation are used (usually 0, 4, 6, 8, 10, 12, and 24 h), and the resulting data for the test are typically presented graphically [59].

3.2. Antibacterial activity of AgNPs

Antimicrobial activity of silver is well known. Silver has been used for treatment of several diseases since from ancient time [60]. The AgNPs synthesized by different methods were widely tested against number of pathogenic bacteria with evidence of strong antimicrobial activity against a broad-spectrum bacteria including both Gram-negative and Gram-positive. Some researchers have been reported that the AgNPs are more effective against Gram-negative bacteria [61–63], while opposite results have also been found [64]. The difference in sensitivity of Gram-positive and Gram-negative bacteria against AgNPs may result from the variation in the thickness and molecular composition of the membranes. Gram-positive bacteria cell wall composed of peptidoglycan is comparatively much thicker than that of Gram-negative bacteria [2, 65].

The importance of antibacterial activity study on different bacterial strains becomes from the importance of understanding the mechanism, resistance and future application. The latest studies on antimicrobial properties are summarized in Table 2.

Although the antibacterial effect of silver nanoparticles has been widely studied, there are some factors affecting the antimicrobial properties of AgNPs, such as shape, size, and concentration of nanoparticles and capping agents [30]. Nakkala et al. [71] analyzed AgNPs with the average size of 21 nm, and the size distribution was found to be 1–69 nm prepared by medicinal plant \textit{Ficus religiosa}. These nanoparticles showed excellent antibacterial activity in \textit{P. fluorescens}, \textit{S. typhi}, \textit{B. subtilis}, and \text{E. coli}. Bacterial cells exposed to lower concentration of AgNPs exhibited delays of growth which may be due to the bacteriostatic effect, while at the higher concentration (of 60 and 100 μg), the AgNPs were found to exhibit bactericidal effect as no growth was observed.

The smaller particles with a larger surface-to-volume ratio were able to reach bacterial proximity most easily and display the highest microbicidal effects than larger particles [19, 69, 76]. Normally, a high concentration leads to more effective antimicrobial activity, but particles of small sizes can kill bacteria at a lower concentration. Furthermore, apart from size and concentration, shape
also influences the interaction with the Gram-negative organism *E. coli* [19, 77]. Pal et al. [78] discussed about depending of nanoparticles’ shape and size on antibacterial activity against Gram-negative bacteria *E. coli*. They found that observed interaction between nanoparticles of silver with various shapes and *E. coli* was similar, and the inhibition results were variable. They speculated about the fact that AgNPs with the same surface areas, but different shapes, may have unequal effective surface areas in terms of active facets [78]. Sadeghi et al. [79] found different antimicrobial effects of nanosilver shapes (nanoparticles, nanorods, and nanoplates) for *S. aureus* and *E. coli*. SEM analysis indicated that both strains were damaged and extensively inhibited by Ag-nanoplates due to the increasing surface area in AgNPs.

### 3.3. Mechanism of action

In the past decade, silver nanoparticles as antimicrobial agents have attracted much attention in the scientific field. Although several reviews have described the AgNPs’ mechanism in detail, the exact mechanism of the antibacterial effect of silver and AgNPs remains to be not fully elucidated. Most studies considered multiple mechanisms of action but simplified the main tree of different mechanisms determine the antimicrobial activity of silver nanoparticles: (1) irreversible damage of bacterial cell membrane through direct contact; (2) generation of reactive oxygen species (ROS); and (3) interaction with DNA and proteins [80–83]. The damage of cell membranes by AgNPs causing structural changes renders bacteria more permeable and disturbs respiration function [84]. Interestingly, Morones et al. [84] demonstrated the existence of silver in the membranes of treated bacteria as well as in the interior of it by transmission electron microscopy (TEM) analysis. Another aspect of mechanism is the role of Ag⁺ ions release. Research has shown that the Ag⁺ ions at a lower concentration than that of AgNPs can exert the same level of toxicity [60]. Several evidences suggest that the silver ions are important in the antimicrobial activity of silver nanoparticles [81, 85]. Durán et al. [81] discussed that silver ions react with the thiol groups of proteins, producing bacterial inactivation, and inhibit the multiplication of microorganisms. Ag⁺ in μmol L⁻¹ levels had weakened DNA replication due to uncoupling of respiratory electron transport from oxidative phosphorylation, which inhibits respiratory chain enzymes and/or interferes with membrane permeability. On the other side, silver ion can interact with the thiol groups of many vital enzymes and inactivate them and generate reactive oxygen species (ROS) [29]. The AgNPs can act as a reservoir for the monovalent silver species released in the presence of an oxidizer. [85] Ag⁺ release was found to correlate with AgNP size, the silver nanoparticles antibacterial activity below 10 nm is mainly caused by the nanoparticle itself, while at larger sizes, the predominant mechanism occurs through the silver ions [81]. Lee et al. [86] studied the mechanism of antibacterial action on *Escherichia coli*. A novel mechanism for the antibacterial effect of silver nanoparticles, namely the induction of a bacterial apoptosis-like response, was described. They observed accumulation of reactive oxygen species (ROS), increased intracellular calcium levels, phosphatidylserine exposure in the outer membrane which indicate early apoptosis, disruption of the membrane potential, activation of a bacterial caspase-like protein and DNA degradation which is the sign of late apoptosis in bacterial cells treated with silver nanoparticles (Figure 2).

Antimicrobial activity of silver nanoparticles combined with various antibiotics is currently being studied, and the synergistic antibacterial effect has been found. Singh et al. [62] studied
individual and combined effects of AgNPs with 14 antibiotics. They found that synergistic action of AgNPs and antibiotics resulted in enhanced antibacterial effect. Exposure of bacteria in combination of AgNPs and antibiotics reduced the MICs significantly, and the bacteria were found to be susceptible to all of the tested antibiotics, except cephalosporins, where no change was observed. The significant reduction of required antibiotic dose up to 1000-fold in combination with small amount of AgNPs could achieve the same effect. The study on bacterial strains resistant to one or more antibiotics belonging to the β-lactam class indicated that the addition of AgNPs decreased MIC on the susceptibility range, therefore, addition of AgNPs not only reduced MIC, but also caused bacteria sensitivity to antibiotic. Briefly, simultaneous action of AgNPs with antibiotics could prevent the development of bacterial resistance. These results are in accordance with findings reported by Gurunathan [76], who observed synergistic effects of silver nanoparticles in the presence of conventional antibiotics on Gram-negative bacteria E. coli and K. pneumoniae. The viability of bacteria was significantly reduced by more than 75% in combination of sublethal dose of meropenem and AgNPs. Evidence of a synergistic effect resulting from the combination of silver nanoparticles with five different antibiotics was declared by reducing MIC against multiresistant, β-lactamase, and carbapenemase producing Enterobacteriaceae [87]. The resistance on antibiotic treatment of S. aureus is fast growing global problem due to slow development of new effective antimicrobial agents. Akram et al. [88] investigated synergic effect of five various antibiotics and AgNPs applied in combination with blue light against methicillin-resistant S. aureus (MRSA). These triple combinations of blue light, AgNPs, and the antibiotic considerably enhanced the antimicrobial activity against MRSA, in comparison with treatments involving one or two agents.

The biofilm formation is adjunctive problem of resistance on antimicrobial agents and chronic bacterial infections. It was proposed that Ag-NPs can impede biofilm formation [89]. Hwang et al. [90] found that combination of AgNPs with ampicillin, chloramphenicol, and kanamycin against various pathogenic bacteria inhibits the formation of biofilm. Deng et al. [91] examined the synergistic antibacterial mechanism of four different classes of conventional antibiotics in combination with AgNPs against the multidrug-resistant bacterium Salmonella typhimurium. The antibiotics enoxacin, kanamycin, neomycin, and tetracycline interact with AgNPs strongly and forming antibiotic-AgNPs complex, while no such effects were observed for ampicillin and penicillin. This complex with AgNPs interacts more strongly with the Salmonella cells and causes more Ag⁺ release, thus creating a temporal high concentration of Ag⁺ near the bacterial cell wall that ultimately causes cell death.
4. Conclusion

The use of silver nanoparticles provides an opportunity to solve a global problem of bacterial resistance toward antibiotics. The possibilities of silver nanoparticles synthesis are very broad. In the last decade, there has been dramatically grown scientific interest in nanoparticles biosynthesis by various reducing and capping agents presented in biological sources including plants, plant extracts, microorganism, or larvae. The natural green synthesis approach is an eco-friendly and cost-effective due to the fact that no toxic and dangerous chemicals are used.

One of the key aspect in the design of more potent antibacterial system is the understanding its mode of action. Generally, nanoparticles are well established as promising alternative to antibiotic therapy or combinational therapy because they possess unbelievable potential for solving the problem with the development of pathogens resistance. Finally, from this point of view, silver nanoparticles represent product with potential application in medicine and hygiene, and the green synthesis of AgNPs can pave a way for the same.

Acknowledgements

The authors would like to acknowledge financial support of IGA UVLF 13/2016: “Antioxidant and antibacterial activity of silver nanoparticles prepared using plant extracts.”

Conflict of interest

The authors declare no conflict of interest.

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