Mechanistic aspects of plant-based silver nanoparticles against multi-drug resistant bacteria

Shahid Wahab, Tariq Khan, Muhammad Adil, Ajmal Khan

ARTICLE INFO

Keywords:
Biogenic silver nanoparticles
Multi-drug resistant bacteria
Natural extracts

ABSTRACT

Resistance among pathogenic bacteria to the existing antibiotics is one of the most alarming problems of the modern world. Along with reducing the use of antibiotics, and antibiotic stewardship, an alternative to antibiotics is much needed in the current scenario to combat infectious diseases. One alternative is to produce nanomaterials, especially, silver nanoparticles (AgNPs) against antibiotic-resistant bacteria. AgNPs are the most vital and fascinating nanoparticles because of their unique structural and functional properties and application against pathogenic bacteria. However, the synthesis of AgNPs remains a problem because of the chemicals and energy requirements and the byproducts of the reactions. Concerns have been raised about using chemically and physically synthesized nanoparticles because of their potential risks to the human body, animals, and environment. Green synthesis of these nanoparticles is a better alternative to physical and chemical approaches. Plant-based synthesis in turn is a method which can provide AgNPs that are cost-effective and eco-friendly as well as biocompatible. The specific features of size, morphology and shape of plant-based AgNPs give them the potency to fight multi-drug resistant bacteria. A detailed look into mechanistic aspects of the action of AgNPs against resistant bacteria with a focus on characteristic properties of AgNPs is required. This review discusses in detail these aspects and the potential of plant-based AgNPs as a solution to antibiotic resistance.

1. Introduction

The world health organization has declared antimicrobial resistance (AMR) among the top 10 global public health threats to human life (WHO, 2016). According to the WHO, a growing list of infections – including throat infections, foodborne diseases, tuberculosis, gonorrhea, and pneumonia are offering harder fronts to antibiotics during the fight of these antibiotics against the causative agents of these infections. According to the United States Pharmacopeia, approximately 700,000 deaths per year occurs due to antimicrobial resistance which is likely to grow to 10 million by the year 2050 (Betts et al., 2018). Apart from deaths, antibiotic resistance is related to the burden on hospitals and thus the ultimate economic burden. Antibiotic resistant infections have been reported to cause 2.5 million hospital days in European union and 3.2 million hospital days in Thailand (ECDC, 2009).

Therefore, there comes a much-needed call for alternatives to antibiotics. Scientists have aimed at developing alternate techniques to address this challenge (Singh et al., 2020). Among the many advances made during this century, nanomaterial have found to be a better option in fighting against the multi-drug resistant bacteria (Kim et al., 2007) because of their noticeable properties like their large surface area to volume ratio (Khalil et al., 2013). The property of having large surface area to volume ratio enhances their affinity to membrane. Furthermore, the affinity towards the negatively charged membrane increases because of the presence of positive charge on the nanoparticles. Bacteria cannot easily become resistant to nanoparticles as nanoparticles at the same time target numerous routes for killing bacteria (Singh et al., 2020). Metallic nanoparticles including silver nanoparticles (AgNPs) are the best studied nanoparticles having the potential to address the problem of antibiotic resistance (Gemmell et al., 2006). Silver nanoparticles (AgNPs) are known to have key applications in the research area of multi-deciplines such as gene delivery in molecular biology, drug delivery in pharmaceutical industry, use in biosensors mainly in biochemistry, environmental sciences for handling the problems of pollution and agricultural sciences. AgNPs are now used in novel ways in the form of nanomedicine due to the fact that AgNPs posses unique physical and chemical
2. Antibiotic resistant bacteria; a simple introduction

Different classes of antibiotics are used to fight against infectious microbes. Antibiotics eliminate such microbes through various antimicrobial mechanisms such as interference in transcription and translation, inhibition of enzymes, and alteration of membrane structure (Kohanski et al., 2010). However, some microbes have developed antibiotic resistance (Padiyara et al., 2018). Antibiotic resistance is one of the major cause of lack of efficiency of antimicrobial agents. This leads to consumption of higher drug doses, longer stays in hospitals, higher toxicity, and increased mortality (Pelgrift and Friedman, 2013). Various factors including over-prescription of antibiotics, misuse of antibiotics, overuse of antibiotics, their widespread agricultural use, and availability of fewer new antibiotics contribute towards antibiotic resistance (Ventola and therapeutics, 2015). Are Among the recently known microorganisms that have developed resistance are Vancomycin resistant *Staphylococcus aureus*, *Enterococcus faecium*, and *Enterococcus faecalis* (Cetinkaya et al., 2000), carbapenem-resistant *Enterobacteriaceae*, penicillin-resistant Streptococcus pneumoniae, and multidrug-resistant Acinetobacter baumannii, *Mycobacterium tuberculosis*, *Pseudomonas aeruginosa*, *Salmonella enterica* and *Vibrio cholera* (Betts et al., 2018). Some of the possible known mechanisms used by bacteria to develop antibiotic resistance include decreased uptake and increased efflux pumps, acquiring and expression of drug resistant genes, modification of antimicrobial target, alteration of antimicrobial drugs through the development of drug degrading enzymes, production of competitive inhibitors, emergence of persistier cells, biofilm formation, and swarming (Figure 1) (Blecher et al., 2011). These mechanisms ultimately cause lesser accumulation of antibiotics in bacterial cells which in turn lower the therapeutic level of the drug (Huh and Kwon, 2011). Thus, a higher and repeated dose of drugs will be required which leads to harmful effects both on humans and animals.

3. Solutions to antibiotic resistance

In order to tackle these problems, new drugs need to be developed. Scientists are now interested in rapid diagnosis and targeted therapy. This could be achieved by either modifying or completely avoiding the utilization of conventional antibiotics. Metal nanoparticles such as copper, silver, titanium, and zinc could be the possible alternative. These nanoparticles are antimicrobial in nature. Metals use different mechanisms i.e. damage to DNA and protein, membrane degradation, and generation of reactive oxygen species to kill microorganisms (Abn et al., 2018). Metal nanoparticles possess unique tunable physical properties such as size and shape. Bulk metals can be transformed to metal nanoparticles (Singh et al., 2017b). This transformation not only reduces the size but also results in the formation of different shape nanoparticles such as spherical, octahedral, rod, and triangular shaped (Singh et al., 2015). These geometrical variations are very useful for antimicrobial applications as the antimicrobial activity of nanoparticles is dependent on the surface area available for biological components interactions. Therefore, metallic nanoparticles, especially, AgNPs have become one of the highly promising alternative to combat antibiotic resistance and fight resistant microbes (Huh and Kwon, 2011). Different studies have reported the antimicrobial applications of both biologically and chemically synthesized metallic nanoparticles. The results have shown that biologically synthesized nanoparticles possess higher antimicrobial effect as compared to chemically synthesized nanoparticles (Singh et al., 2016).

4. Why green synthesis of nanoparticles?

Metallic nanoparticles are more promising antimicrobial agents due to their large surface area to volume ratio (Gong et al., 2007). Among the different types of metal nanoparticles, magnesium, gold (Gu et al., 2003), copper, titanium, zinc (Schabes-Retchkiman et al., 2006), alginate (Ahmad et al., 2006), and silver nanoparticles (Rai et al., 2009) have been shown to possess strong antibacterial activities. Chemically synthesized nanoparticles use chemicals as a reducing agent, hence converting Ag ion to AgNPs. But because of the harmful effects and the low biocompatibility of the chemically synthesized nanoparticles, green synthesis of the nanoparticles is favored. Green synthesized nanoparticles are eco-friendly reducing agents (Park, 2014a). There is an ongoing debate about the toxic effects of AgNPs. The debate focus on whether chemically or physically synthesizing these nanoparticles especially AgNPs is safe or not. To address this issue, biologically synthesis of AgNPs is considered.

In the biogenic synthesis of AgNPs, plant parts are used to prepare an extract and the process is performed in a hot or cold solvent. Compounds found in plants such as ascorbic acid, reducing sugars, citric acid amino acids, alkaloids, terpenoids, and flavonoids present in different parts of plants acts as reducing agent, quickly converting Ag + to AgNPs (Barros et al., 2018). Carson et al. experimented with the biogenic synthesis of AgNPs from a medicinal plant. They prepared an aqueous extract from *Dulcis terv* (Verbenaceae) to synthesize AgNPs using microwave irradiation and its formation was confirmed by ultraviolet-visible spectroscopy. Other characterization techniques included X-ray photoelectron spectroscopy, Transmission electron microscopy and X-ray diffraction (Carson et al., 2020).
Table 1. AgNPs synthesis in different plants, their parts used and the morphological characteristics of biosynthesized AgNPs.

| Plants                  | Part            | Shape       | Size         | Reference                          |
|-------------------------|-----------------|-------------|--------------|------------------------------------|
| Mentha pulegium         | Leaves          | Anisotropic | 5-50 nm      | (Abd Kelkawi et al., 2016)         |
| Syrius benoin           | Plant extract   | Spherical   | 12-38 nm     | (Du et al., 2016)                 |
| Acorus Calamus          | Rhizome         | Spherical   | 59 nm        | (Nayak et al., 2015)              |
| Ficus religiosa         | Leaves          | Spherical   | 21 nm        | (Nakkala et al., 2017)            |
| Iresine herbstii        | Leaves          | Cubic       | 44-64 nm     | (Dipankar and Murugan, 2012)      |
| Prunus persica          | Plant extract   | Spherical   | 40-98 nm     | (Kumar et al., 2017)              |
| Aloe vera               | Leaves          | Spherical   | 15.2 nm      | (Chandran et al., 2006)           |
| Carica papaya           | Fruit           | Cubic       | 15 nm        | (Jain et al., 2009)               |
| Memecylon edula         | Leaves          | Triangular  | 50-90 nm     | (Elavazhagan and Arunachalam, 2011)|
| Handelia trichophylla   | Plant extract   | Spherical   | 20-50 nm     | (Yazdi et al., 2019)              |
| Anacardium occidentale  | Leaves          | Spherical   | 40-60 nm     | (Balavigneswaran et al., 2014)    |
| Synsepalum Dulcisicum   | Plant extract   | Spherical   | 4-26 nm      | (Lateef et al., 2016)             |
| Alphonsea sclerocarpa   | Plant extract   | Irregular   | 33 nm        | (Doddapaneni et al., 2018)        |
| Solemnium lycopersicum  | Plant extract   | Cubic       | 21.11 nm     | (Baran and Hilal, 2019)           |
| Salvia officinalis      | Leaves          | Spherical   | 41 nm        | (Okaiyeno et al., 2020)           |

AgNPs based on living extracts or biosynthesized AgNPs have an advantage of being eco-friendly, clean and no use of toxic chemicals in the production process. Other advantages are this method being simple enough and the one-pot reaction, amenability to scale up, elimination of the toxic chemicals, increased biocompatibility, enhanced colloidal stability and the cost-effectiveness. Thus, biosynthesized AgNPs are preferred over the chemical and physical methods of AgNPs production. In addition, they do not involve the use of use of abiotic factors such as maintaining high temperature, pressure maintenance, and use of energy. Bacteria, fungi, plants, plant-based compounds, algae, carbohydrates, and microorganisms are among the main source for the synthesis of green nanoparticles (Park, 2014b). The extracts of these organisms contain protein, amino acid, enzymes, carbohydrates, vitamins, and secondary metabolites which take part in AgNPs synthesis as both reducing and capping agents.

Plants are, in turn, better synthesizers among the other biological approaches owing to the amount of plant resources available. Plants also provide a superior platform for nanoparticle manufacturing. They contain harmless compounds and act as natural capping agents. Furthermore, the use of plant extracts lowers the cost of microbe isolation and culture medium, improving the cost-competitive viability of microorganism-based nanoparticle production (Sharma et al., 2009). The benefit of employing plants for nanoparticle production is that they are readily available, safe to handle, and contain a wide range of secondary metabolites. Several plants are now being researched for their function in nanoparticle production.

5. Silver nanoparticles (AgNPs) as a solution to antimicrobial resistance

AgNPs is very a viable alternative solution to antimicrobial resistance. A plethora of studies have confirmed that AgNPs possess enormous potential against pathogenic bacteria. For instance, in a study by Jia et al. (2007), AgNPs were mixed with Poly (Vinyl Alcohol) (PVA) nanofibers and the antibacterial properties were assessed against Staphylococcus aureus and Escherichia coli. A significant reduction in bacterial activity confirmed that the nanoparticles coated fibers are efficient antibacterial agents. Similarly, in another study, AgNPs were chemically synthesized using silver nitrate and sodium borohydride and its antimicrobial activity was evaluated against S. aureus and E. coli wherein they observed marked activity of these nanoparticles especially against pathogenic E. coli (Kim et al., 2007). AgNPs have also been shown to be potent against biofilm formation in bacteria. Biofilms are structure developed when bacterial cells adhere to surfaces and make a conjugate. Palanisamy et al. (2014) investigated the antibacterial properties of chemically synthesized AgNPs in the formation of biofilms in P. aeruginosa. Results from the resistant strain indicated that 20 μg/mL of AgNPs inhibited growth with an inhibition rate of 56% in biofilm forming P. aeruginosa. This suggests that AgNPs can be used as an alternative to combat P. aeruginosa infections. Despite the properties discussed above, chemically synthesized AgNPs, however, possess problems of the use of potentially toxic environmentally unsafe chemicals. We will now highlight the importance of plants in conferring antibacterial properties to AgNPs.

5.1. Contribution of plants to the properties of antibacterial AgNPs

Medicinally important biomolecules such as amino acids, proteins, vitamins, alkaloids, phenolics, saponins, tannins and terpenoids present in the plant extracts are found to be involved in the reduction as well as stabilization of silver ions (Kulkarni and Muddapur, 2014). Variety of plants and plant extracts have been reported to facilitate the synthesis of AgNPs (Table 1). We will discuss different plants used and the influence they put on the different properties of these AgNPs (Figure 2).

5.1.1. Characterization of antibiotic silver nanoparticles

For characterization of AgNPs different types of analytical techniques are used including UV-vis spectroscopy, X-ray diffraction, Fourier transform infrared (FTIR) spectroscopy, scanning electron microscopy (SEM), and transmission electron microscopy (TEM). UV-vis spectroscopy is usually used to monitor the synthesis of AgNPs through the combined oscillation of electrons on their surface in resonance with incident light. This in turn gives surface plasmon resonance (SPR)
Table 2. Table summarizing different studies reporting antimicrobial activity of plant-based silver nanoparticles (AgNPs).

| Plants name                | Plants parts | Size (nm) | Shapes                  | Bacterial strain                | Antibacterial potency                                           | References                                      |
|----------------------------|--------------|-----------|-------------------------|--------------------------------|------------------------------------------------------------------|-------------------------------------------------|
| Justicia Adhatoda L.       | Leaves       | 5-50 nm   | Spherical               | P. aeruginosa                   | Inhibition the growth of bacteria                               | Bose and Chatterjee, 2015                      |
| Carica papaya L.           | Fruit and leaves | 25-50 nm | Cubic                   | E. coli and P. aeruginosa       | Inhibition the growth of bacteria and disrupting the membrane   | Jain et al., 2009                               |
| Artemisia nilagirica       | Leaves       | 70-90 nm  | Square/spherical/hexagonal | S. aureus, B. subtilis, E. coli | Inhibition of the growth of bacteria and degradation of the membrane | Vijayakumar et al., 2013                       |
| Trianthema decandra        | Roots        | 15 nm     | Cubic                   | P. aeruginosa and E. coli       | Clear inhibition zone and membrane disrupting                  | Geethalakshmi and Sarada, 2010                 |
| Emblica officinalis        | Fruit        | 15 nm     | Spherical               | Staphylococcus, B. subtilis, E. coli, K. pneumonia | Disrupting cell membrane, permeability, and respiration function of the cell. Also, penetrate the bacteria and cause cell death | Shakesh et al., 2015                           |
| Cranegus douglasi           | Fruit        | 29.28 nm  | Spherical               | S. aureus and E. coli           | Produce a clear zone of inhibition in bacteria                  | Ghaffari-Moghadam and Hadi-Dabanlou, 2014     |
| Cleome viscosa             | Fruit        | 20-50 nm  | Spherical and irregular | S. aureus, B. subtilis, E. coli, K. pneumonia | Produce a clear zone of inhibition in bacteria                  | Lakshmanan et al., 2018                        |
| Prosopis farcta            | Leaves       | 10.8 nm   | Spherical               | S. aureus, B. subtilis, E. coli, P. aeruginosa | Produce a clear zone of inhibition in bacteria                  | Miri et al., 2015                              |
| Petroselinum crispum       | Leaves       | 25-30 nm  | Spherical               | S. aureus, E. coli, K. pneumonia | Prevent the growth of bacteria                                 | Roy et al., 2015                               |
| Psidium guajava            | Leaves       | 25-35 nm  | Spherical               | A. falcatus and E. coli         | Inhibit the growth of bacteria                                  | Wang et al., 2018                              |
| Moringa oleifera           | Leaves       | 9-11 nm   | Spherical               | E. falcatus, E. coli, S. aureus, P. aeruginosa, and K. pneumoniae. | Reduce the growth of bacteria                                 | Moodley et al., 2018                           |
| Cerapogia thwaitesi        | Leaves       | 100 nm    | Spherical               | S. typhii and B. subtilis       | Inhibit the growth of bacteria                                  | Mathukrishnan et al., 2015                     |
| Holoptelea integrifolia    | Leaves       | 32-38 nm  | Cubic                   | E. coli and S. typhimurium      | Inhibit the growth of bacteria                                  | Kumar et al., 2019                             |
| Caricum longa L.           | Leaves       | 15-40 nm  | Spherical               | P. aeruginosa, E. coli, and S. aureus | Reduce the growth and also kill the bacteria                   | Maghima and Alharbi, 2020                     |
| Aegle marmelos             | Fruit        | 159-181 nm| Cubic                   | P. aeruginosa, B. cereus, and S. dysenteries | Inhibit the growth of bacteria                                 | Devi et al., 2020                              |
| Terminalia bellerica       | Fruit        | 10 nm     | Spherical               | K. pneumoniae and P. aeruginosa | Inhibit the growth of bacteria                                  | Andra et al., 2019                             |
| Artemisia marschalliana    | Stem, fruit, leaves | 5-50 nm | Spherical               | B. cereus, A. baumannii, P. aeruginosa and S. aureus | Inhibit the growth of bacteria                                 | Salehi et al., 2016                            |
| Borago officinalis         | Leaves       | 30-80 nm  | Spherical               | S. aureus, v. parahaemolyticus, P. aeruginosa, and E. coli | A clear zone of inhibition and led bacteria to death            | Singh et al., 2017a                            |
| Solanum trilobatum         | Fruit        | 12.50-41.90 nm | Spherical               | E. falcatus, K. pneumoniae, S. mutans, and E. coli | Inhibit the growth of bacteria                                  | Raman et al., 2015                             |
| Ocimum basilicum           | Leaves       | 3-25 nm   | Spherical               | S. aureus, P. aeruginosa, and E. coli. | Produce a clear zone of inhibition against bacteria             | Malapermal et al., 2017                        |
| Pimpinella anisum          | Seed         | 3-16 nm   | Spherical               | S. pyogenes, A. baumannii, K. pneumoniae, S. typhii, and P. aeruginosa | Inhibit the growth of bacteria and disrupting their membrane   | AliSalhi et al., 2016                           |
| Handelidin trichophylla    | Aqueous extract | 20-50 nm | Spherical               | E. coli, P. aeruginosa, S. aureus and B. subtilis. | Prevent the growth of bacteria                                 | Yazdi et al., 2019                             |
| Terminalia arjuna          | Bark extract | 30-50 nm | Spherical               | E. coli                         | Produce a clear zone of inhibition against bacteria             | Ahmed et al., 2017                             |
| Morinda citrifolia         | Leaves       | 10-60 nm  | Cubic                   | E. aerogenes, E. coli, B. subtilis, K. pneumoniae, B. cereus and P. aeruginosa | Inhibitory action against bacteria                        | Sathishkumar et al., 2012                      |

(continued on next page)
Table 2 (continued)

| Plant name                  | Part used     | AgNPs size (nm) | Shape   | Reference                                                                 |
|-----------------------------|---------------|-----------------|---------|---------------------------------------------------------------------------|
| *Salvia splendens*          | Aqueous extract | 15–20            | Cubic   | (Raj et al., 2018)                                                        |
| *P. vulgaris*, *B. subtills*, and *S. aureus* | (continued) | 23 | Spherical          | (Gomaa, 2017a)                                                           |
| *Grewia aviscences*         | Leaves        | 50–70            | Spherical | (Sana et al., 2015)                                                        |
| *Nigella sativa*            | Seed          | 34               | Cubic   | (Vijayakumar et al., 2020)                                               |
| *Tribulus terrestris*       | Fruit         | 16–28            | Spherical | (Gopinath et al., 2012a)                                                  |

5.1.2. The role of AgNPs in antibiotic resistance is a function of their important properties

The antimicrobial activity of silver nanoparticles depends upon several factors including the type of microorganism, pH, temperature (Marambio-Jones and Hoek, 2010), different size, shapes (Zhou et al., 2012), and the concentration of AgNPs and zeta potential (Li et al., 2013; Pal et al., 2007). A general look at the above studies initiate the debate that size and shape among other properties play an important role in the antibacterial activities of the plant-based AgNPs (Table 2). Size and shape tends to be among the most important features of AgNPs which confer upon them their activity against pathogenic bacteria (Figure 3).

5.1.3. Importance of the size of plant-based AgNPs

Smaller nanoparticle having large interactive surface area is proved to be good bacticidal agents than large ones (Kvitak et al., 2008; Morones et al., 2005). The smaller nanoparticles have a greater surface area and is the most important property that affects antimicrobial activity, due to this it can provide high interaction area and give bacticidal effect more than larger particles (Gurunathan et al., 2014; Raza et al., 2016). For example, Li et al. (2013) reported that the antibacterial activity decreases with an increase in the size of the of the nanoparticles. Similarly, Yacamán et al. (2001) observed that the nanoparticles size less than 50 nm have an effective antimicrobial activity. It has been observed that nanoparticles with diameter between 5 to 15 nm have superior antimicrobial activity. According to Sondi and Salopek-Sondi (2004), greater antibacterial activity has been shown for smallest nanoparticles (5 nm).
They easily get attached to the cell membrane, damage the cell, and increase the permeability of the membrane, and finally resulting in cell death. Similarly, Morones et al. (2005) reported that nanoparticles having diameter around 10 nm have potent activity against bacteria.

In a study, aqueous extract of Alternanthera dentate was used to synthesize spherical shaped AgNPs with diameter of 50–100 nm. These AgNPs showed significant antibacterial activity against E. coli, E. faecalis, Klebsiella pneumonia and P. aeruginosa (Palanisamy et al., 2014). The extracts of Cocos nucifera was used to synthesize AgNPs of diameter 22 nm. These nanoparticles exhibited significant antibacterial activity against Bacillus subtilis, P. aeruginosa, K. pneumoniae, and Salmonella paratyphi (Mariselvam et al., 2014). Euphorbia hirta leaf extract was used to prepare 40–50 nm spherical shaped silver nanoparticles. These NPs were supposed to have strong antibacterial property against S. aureus and B. cereus. The Silver nanoparticles with size of 20–30 nm can be synthesized from the extract of Acalypha indica leaves. The nanoparticles produced had strong activity against V. cholera and E. coli (Krishnaraj et al., 2010). Leaf extract of Moringa oleifera had been used to prepare 57 nm sized silver nanoparticles with high antimicrobial potential against many types of pathogens i.e. C. kruisi, S. aureus, K. pneumonia and C. tropicalis (Prasad and Elumalai, 2011). Leaf extract of Garcinia mangostana having reducing property as well as antibacterial property could be helpful in the biosynthesis of 35 nm sized Silver nanoparticles (Veerasamy et al., 2011). Extract of Cacumen platycladi has great antibacterial activity and can be used in biosynthesis of silver NPs. To synthesize hexagonal and cubic shaped silver nanocrystals with the size of 31–40 nm, bark of Cinnamomum zeylanicum could be used (Huang et al., 2011).

5.1.4. Importance of the shape of plant-based AgNPs

Furthermore, different shapes of AgNPs exhibit several types of interaction with the cell membrane and show their different type of antimicrobial activity by damaging the membrane. It has been reported that when the shape of AgNPs is truncated triangular, hexagonal, and octahedral the antimicrobial activity will be highest against gram-negative bacteria. E. coli (Alshareef et al., 2017; Chen and Carroll, 2002). Similarly, El-Zahry et al. (2015) showed that AgNPs in the shape of hexagonal exhibit the highest antibacterial activity while triangular AgNPs display no activity against E. coli. Similarly the antibacterial activity conducted against B. subtilis, K. planticola, K. pneumoniae, S. nematodiphila, and E. coli by spherical shape AgNPs synthesized from Planomicrobium sp. That show decrease in growth rate with the increased in concentration of AgNPs, which suggested that biologically AgNPs can be an effective bactericidal food covering material (Rajeshkumar and Malarkodi, 2014). Similarly biological synthesis of AgNPs from Bacillus brevis and check their activity against pathogens such as Staphylococcus Aureus and Salmonella Typhi which show significant result in the from of zone of inhibition in the diameter of 7 nm–19 nm (Saravanan et al., 2018). Similarly, plant extract of Boerhaavia diffusa was used to synthesize face centered cubic AgNPs with an average size of 25 nm. The antibacterial potential of these nanoparticles was evaluated against Aeromonas hydrophila, Pseudomonas fluorescens, and Flavobacterium branchiophilum. These AgNPs showed highest sensitivity to P. branchiophilum (Kumar et al., 2014). Spherical shaped AgNPs, were synthesized using the fruit body extract of Tribulus terrestris. The antibacterial activity of these AgNPs was observed against Bacillus subtilis, E. coli, P. aeruginosa, Streptococcus pyogens, and S. aureus (Gopinath et al., 2012b). Similarly, Das et al. (2013), synthesized spherical shaped AgNPs using the leaf extract of Seshania grandiflora. These AgNPs showed significant antibacterial activity against human pathogens; S. aureus and S. enterica. A critical analysis of these examples shows that shape triggered by plant-based synthesis of AgNPs confers them potent antibacterial activity.

5.1.5. Concentration of AgNPs is important for combating antibiotic resistance

The concentration of nanoparticles is another important factor to be considered during the activity against antibiotic resistant bacteria. For instance, Kim et al. (2007) report that at low concentration of AgNPs, the growth of E. coli was inhibited while the inhibitory effect on similar concentration was poor against S. aureus. In a report by Pazos-Ortiz et al. (2017), AgNPs fixed in poly-epsilon-caprolactone were tested against gram-positive and gram-negative bacteria which showed major antibacterial effects against these bacteria when applied in 12 mM concentrations. However, higher concentrations of AgNPs that are chemically synthesized is not safe for the environment or other body functions. Biologically synthesized AgNPs even if applied in higher concentrations could be more safer to use. Ahmed et al. (2017) investigated the antibacterial efficiency of Terminalia arjuna based AgNPs (TA-AgNPs) against E. coli. Different concentrations (50, 100, 150, and 200 μg/mL) were used to assess the antibacterial effectiveness of TA-AgNPs. At higher concentrations of the AgNPs solution (200 μg/mL), the development of the pathogens was highly repressed, indicating the role of concentration of AgNPs in effective antibacterial action of the TA-AgNPs. Besides, it was found that the inhibition zone increased with the increase in the concentration of TA-AgNPs. Similarly, Dinesh et al. (2015) synthesized AgNPs from Aloe vera extracts and used against the bacteria B. subtilis, K. pneumoniae, and S. typhi using the disk diffusion method. AgNPs showed inhibitory action at a maximum concentration of 150 mg/L against all three bacteria. The zone of inhibition observed was 80 mm for B. subtilis.
B. subtilis were treated with chitosan AgNPs which indicate significant antibacterial activity against bacteria. Another study reported the antibacterial activity of plant based AgNPs and chemically synthesized AgNPs. The results revealed that both type of AgNPs showed antimicrobial potential against S. aureus and E. coli. However, the maximum zone of inhibition in case of plant based AgNPs was higher as compared to chemically synthesized AgNPs i.e. 4mm and 1mm respectively. The maximum inhibitory concentration of plant based AgNPs in S. aureus and E. coli was observed to be 10.24 mg/ml and 5.12 mg/ml respectively. For chemically synthesized AgNPs the MIC was found to be 10.24 mg/ml in both S. aureus and E. coli (Mousavi-Khattat et al., 2018). It has also been reported that plant based AgNPs produced maximum inhibition zones when compared with the standard antibiotic i.e. streptomycin. In case of streptomycin the inhibition zones were 9.41 mm and 10.12 mm for S. aureus and E. coli respectively. While for plant based AgNPs the inhibition zones were 12.47 mm and 16.27 mm in S. aureus and E. coli respectively (Senthil et al., 2017). Another study reported the antibacterial activity of sodium borohydride based AgNPs revealing the MIC values to be 100ppm for both S. aureus and E. coli. But in case of biosynthesized AgNPs the MIC values has been reported to be 31.25 and 62.5 ppm respectively for S. aureus and E. coli (Ocsoy et al., 2017).

5.1.6. Zeta potential of AgNPs as an important features of plant-based AgNPs

Zeta potential is also an important property that can affect antimicrobial activities. The interface between cell membranes and nanoparticles is based on electrostatic adhesion. The activity of AgNPs against bacteria is based on the electrostatic attraction in which bacterial component has a strong negative charge and AgNPs are less charged and their activity decrease the zeta potential on the surface of both bacteria (Abbassazdegan et al., 2015). For example, according to El Badawy et al. (2011) positively charge AgNPs are more toxic toward Bacillus species than negatively charged AgNPs. This is because the cell surface of Bacillus species is negatively charged, and it causes repulsion of negatively charged AgNPs. In another study by Ahmad et al. (2017), E. coli and B. subtilis were treated with chitosan AgNPs which indicate significant alteration of the membranes of bacteria because of the positive charge during interaction with the cell surface (zeta potential -11.6 and -7.5 mV). A biologically synthesized AgNPs from Mikia scabrella leaf extract was tested against multidrug resistance gram negative bacteria such as the nosocomial pathogens of Pseudomonas aeruginosa, Acinetobacter sp., and Klebsiella pneumoniae. In the size range from 18-12 nm with spherical shape and -21.7mV zeta potential for stable AgNPs, that exhibited significant antimicrobial activity for therapeutic application in nanomedicine (Prabakar et al., 2013).

6. Mechanism of antibacterial activity of AgNPs

There are three general pathways on which they perform their antimicrobial activity: (1) Degradation of the cell membrane and cell wall, (2) entry into the cell and disruption, and (3) Oxidative stress (Dukal et al., 2016; Slavin et al., 2017).

6.1. Degradation of the cell membrane and cell wall

Bacterial cell wall and membranes perform mainly the function of protecting them against harmful microbes and different types of environmental stress. These membranes also facilitate the transport of different types of useful nutrients (Madigan et al., 2014; Silhavy et al., 2010). It is suggested that AgNPs bind to the bacterial cell wall by ionic bond and generate a high proton motive force strength to disrupt the action of the enzymes containing thiol groups (Sereemaspun et al., 2008). For example, the bactericidal effects of the Avicennia marina based AgNPs against the E. coli were observed to be due to the possible dissipation of the proton motive force (Gnanadesigan et al., 2012). The action of AgNPs depends on the composition of the cell membrane and cell. In a study by Singh et al. (2010), Argemone mexicana leaf extract was used to synthesize green nanoparticles and the antibacterial assays were done against E. coli and P. aeruginosa. The study suggested two-pronged antibacterial activity i.e., the bactericidal effects of the AgNPs and the membrane disrupting capability of the polymer subunit. The gram-positive bacteria have thicker cell wall because of the presence of lipopolysaccharides in lesser quantity in their wall and they provide a tough barrier to AgNPs as compared to gram-negative. The higher amount of lipopolysaccharides and less peptidoglycan in cell walls of gram-negative bacteria makes them thinner in composition along with the composition of the cell membrane. Their sustenance, composition and negative charge on them makes them adhesive to AgNPs (Aziz et al., 2015; Gopinath et al., 2017; Pal et al., 2007; Rai et al., 2012; Sowmya et al., 2018). As mentioned earlier, the activity of AgNPs against bacteria is based on the electrostatic attraction between bacterial surfaces and AgNPs (Abbassazdegan et al., 2015). Due to this attraction and activity, the inside environment of the cell and their membrane polarization changes leading to cell death (Abalkhil et al., 2017;
Gomaa, 2017b). The bacteria which are not treated with AgNPs show no physiological and morphological changes while other bacteria that are treated with AgNPs show changes in their morphology, and their integrity is disturbed. Due to the disruption of the cell wall and permeability of the membrane, the components of the bacterial cell such as nucleic acid, proteins, enzyme, metabolites, and source of energy are released outside (Gomaa, 2017b; Li et al., 2013; Ravichandran et al., 2018; Yuan et al., 2017). Attachment of AgNPs to the cell wall of bacteria and its degradation is the basis of such mechanism of antimicrobial activity (Ansari and Alzohairiy, 2018; McQuillan et al., 2012).

6.2. Intracellular penetration and damage caused by AgNPs

When the AgNPs enter a bacterial cell, it binds to the proteins and DNA and causes conformational changes thus converting it into other less stable states affecting their function (Bondarenko et al., 2013; Gogoi et al., 2006; Hsueh et al., 2015). Studies reported that the presence of sulfur and other amino groups on the membrane surface facilitates the entry of AgNPs to the cell which leads to the destruction of DNA. AgNPs interact with biomolecules such as DNA, protein, and lipids due to the interaction of AgNPs with these biomolecules that have adverse effects on bacteria. For example, Gomathi et al. (2017) reported that Datura stramonium based AgNPs have antibacterial activity against E. coli. In this study it has been concluded that Ag ions from AgNPs penetrate inside into bacterial cell and cause severe damage to bacteria and led cell to death. Similarly, Jyoti et al. (2016) reported that AgNPs with large surface area, synthesized from Urtica dioica interact with bacterial cell and penetrate bacterial cell and release Ag + ions which interact with phosphorous and sulfur compound such as DNA and inhibit their replication and led the cell to death.

6.3. Oxidative stress in treated bacterial cells

Oxidative stress is usually produced by the introduction of antimicrobials to the bacterial cell. One way that inhibit the growth of resistant microbes is the induction of reactive oxidative species (ROS) in these microbes. Normally antimicrobials are used to elevate the levels of ROS. But new research shows that the introduction of AgNPs to resistant microbes raises the levels of ROS which leads to these resistant species. Research conducted by Khan and Ali (2020) shows that the introduction of AgNPs results in exceeding the level of ROS in the resistant microbial species such as Xanthomonas citri, S. aureus, and Erwinia carotovora. The results showed that AgNPs applied to bacterial suspension increased the quantity of ROS which subsequently resulted in the inhibition of bacteria. According to Das et al. (2017) AgNPs mediated the increase in ROS when subjected to resistant strains E. coli and S. aureus. The study showed that when these resistant strains are treated with AgNPs they raise the levels of ROS in these microbes which cause cellular inhibition of these microbes. Similarly, according to Kim et al. (2011) AgNPs formed ROS which damage cell membrane, protein, and DNA of both bacteria E. coli and S. aureus.

7. Combined synthesis of AgNPs with antibiotics to combat antibiotic resistance

AgNPs possess another importance function against resistance in the form conjugates with existing antibiotics (Figure 4). When antibiotics are attached with AgNPs, their stability, selectivity and functionality become enhanced and they target the drug very specifically (Kingsley et al., 2006). AgNPs conjugated with antibiotics (such as erythromycin, vancomycin, amoxicillin, clindamycin) showed enhanced antimicrobial activity against S. aureus and E. coli (Shahverdi et al., 2007). These antibiotic showed clear inhibition zones against both strains but erythromycin conjugated with AgNPs showed the highest activity against S. aureus with a clear zone of inhibition of 14 mm. In another set of experiments AgNPs conjugated with antibiotics (including gentamycin, tetracycline, ciprofloxacin, chloramphenicol) effectively targeted multidrug resistant bacteria (such as Micrococcus luteus, Staphylococcus epidermidis, and K. pneumoniae). The results showed that AgNPs-Antibiotics showed clear zones of inhibition (6–10 mm) when applied against these bacteria as compared to antibiotics alone (Tyagi, 2016).

Plant-based AgNPs have also found an important role in creating conjugates with antibiotics. According to Bonde et al. (2012), AgNPs synthesized via extracts of Murray koenigi were applied as combination with antibiotics such as ampicillin, streptomycin, gentamycin, and tetracycline and produced a synergistic effect against resistant pathogens such as E. coli and S. aureus. Gentamycin when combined with AgNPs showed the highest antibacterial activity against E. coli (4.06 fold increase) while tetracycline showed the highest activity against S. aureus (2.16 fold increase). In a study by Anjum et al. (2018) gentamycin was conjugated with AgNPs synthesized from Azadirachta indica which showed significant antibacterial activity against S. pneumoniae and S. aureus by interacting with the cell wall and inhibit the growth, and cause cell wall lysis, and inhibit bacterial replication. According to Halawani et al. (2020) biosynthesis of AgNPs from Rosa damascena conjugated with cefotaxime showed maximum antibacterial activity against E. coli (zone of inhibition = 23–37 mm). Bio-fabricated AgNPs conjugated with antibiotics to which resistance has been developed can prove a way forward towards fighting multidrug resistance. In one of our studies, we observed that combination of AgNPs with a broad-spectrum Ciprofloxacin showed better antibacterial activity as compared to AgNPs alone and ciprofloxacin separately. The zones of inhibition zone of S. somne, S. typhi C. amalonaticus and E. coli in response to Cipro-AgNPs and ciprofloxacin were 33 mm, 35.5 mm, 35.5 mm and 38.5 mm, respectively. Therefore, it can be suggested that the AgNPs along with Ciprofloxacin might have worked in interaction and resulted in better antibacterial activity against pathogens (Adil et al., 2019). Several studies have reported that AgNPs functionalized with biomolecules possess more antibacterial potential as compared to AgNPs used alone (Some et al., 2020).

8. Conclusion

AgNPs have act against pathogenic bacteria because of their unique properties like small size, specific shape which provide a larger surface area for interaction with pathogenic bacteria to destroy them easily. AgNPs synthesized through green methods having many advantages like the cost-effectiveness, safety, and less toxicity to non-targeted living organisms. Plant-based AgNPs have distinctive antimicrobial properties that deem them fit for use as an alternative to antibiotics. They have been shown to have antimicrobial activity against both gram-positive and gram-negative bacteria which depends on size, shape, concentration, and zeta potential. Antimicrobial activity of AgNPs alone or in combination with some antibiotic drugs is also promising. Green AgNPs can be conjugated with many antibiotics that increase their stability and functionality and show enhanced antimicrobial activity against many bacteria. However, future studies must focus on the toxicological and toxicokinetic actions of nanoparticles as antibacterial agents.

Declarations

Author contribution statement

All authors listed have significantly contributed to the development and the writing of this article.

Funding statement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.
Padiyara, P., Inoue, H., Sprenger, M., 2018. Global governance mechanisms to address antimicrobial resistance. J. Res. Treat. 11, 1178633718760887.

Pal, S., Tak, Y.K., Song, J., 2007. Does the antibacterial activity of silver nanoparticles depend on the shape of the nanoparticle A study of the gram negative bacterium Escherichia coli. Appl. Environ. Microbiol. 73, 1712–1720.

Palanisamy, N.K., Ferina, N., Amirulhanni, A.N., Mohd-Zain, Z., Hussaini, J., Ping, L.J., Durairaj, R., 2014. Effect of properties of chemically synthesized silver nanoparticles found against Pseudomonas aeruginosa. J. Nanobiotechnol. 12, 2.

Park, Y., 2014a. A new paradigm shift for the green synthesis of antibacterial silver nanoparticles utilizing plant extracts. Toxicol. Res. 30, 169–178.

Park, Y., 2014b. New paradigm shift for the green synthesis of antibacterial silver nanoparticles utilizing plant extracts. Toxicol. Res. 30, 169–178.

Pazos-Ortiz, E., Roque-Ruiz, J.H., Hinojos-Madarro, L., 2014. Synthesis of silver nanoparticles for the use in biomedical applications. In: Kaur, S., Roy, K., Sarkar, C., Ghosh, C. (Eds.), Phytosynthesis of silver nanoparticles using fucoidan isolated from Spatoglossum Schult. plant extract. Int. J. K. J. Sci. Res. 11, 180–182.

Prajapati, P., 2007. Effect of silver nanoparticles on Bacillus subtilis (NCIM 2533) and their antibacterial activity against pathogenic bacteria. Microb. Pathog. 116, 221–226.

Sahithikumar, G., Golshari, A., Saragam, K., Prakash, S., Sivaramakrishnan, S., 2012. Phyto synthesis of silver nanoscale particles using Morinda citrifolia L and its inhibitory activity against human pathogens. Colloids Surf. B: Biointerfaces 99, 288–295.

Sachdeva, S., Aslam, A., Shukla, S., 2014. Antibacterial activity of green synthesized silver nanoparticles against Pseudomonas aeruginosa, Escherichia coli, and Staphylococcus aureus. Int. J. Microbiol. 2014, 967201.

Sakhuja, G., Gobinath, C., Karpagam, K., Hemamalini, V., Premkumar, K., 2018. Biofabrication of Ag nanoparticles using Moringa oleifera leaves extract: antibiotic and antibacterial activities. RSC Adv. 8, 10117–10122.

Sahputra, M., Purnomo, B., Nam, S.-H., Seo, J.M., 2014. Silver nanoparticles as a new generation of antimicrobial agents. J. Environ. Sci. 26, 754–760.

Sardar, S., Dhandapani, M., Parida, R., 2019. Antimicrobial activities of green synthesized Ag nanoparticles against S. aureus and E. coli. J. Photochem. Photobiol. B: Biol. 198, 103536.

Saravanan, M., Barik, S.K., MubarakAli, D., Prakash, P., Pugazhendhi, A., 2018. Synthesis methodology and antimicrobial activity of silver nanoparticles synthesized by green method using Moringa oleifera leaf extract. Nano Res. Lett. 13, 401.

Sathishkumar, G., Gobinath, C., Karpagam, K., Hemamalini, V., Premkumar, K., 2011. Biofabrication of Ag nanoparticles using Moringa oleifera leaves extract: antibiotic and antibacterial activities. Biotechnol. Adv. 29, 95–109.

Sharma, V.K., Yang, R.A., Liu, Y., 2009. Silver nanoparticles: green synthesis and their antimicrobial activities. Adv. Colloid Interface Sci. 145, 83–96.

Si, Z., 2017. Silver nanoparticles as a new generation of antimicrobial agents. J. Environ. Sci. 53, 4–10.

Singh, A., Gautam, P.K., Verma, A., Singh, V., Shivapriya, P.M., Shivalkar, S., Sahoo, A.K., Samanta, S.K., 2020. Green synthesis of metallic nanoparticles as effective alternatives to treat resistant bacterial infections. Review 25, e004277.

Singh, A., Jain, D., Upadhyay, M., Khandeval, N., Verma, H., 2010. Green synthesis of silver nanoparticles using Argemone mexicana leaf extract and evaluation of their antimicrobial activities. Dig. J. Nanomater. Bios. 5, 483–489.

Singh, H., Do, J., Yi, T.-H., 2017a. Green and rapid synthesis of silver nanoparticles using Borago officinalis leaf extract: antifungal and antibacterial activities. Artificial Cell Nanobiotechnol. Biotechnol. 45, 1310–1316.

Singh, P., Kim, Y.-J., Zhang, D., Yang, D.-C., 2016. Biological synthesis of nanoparticles from plants and microorganisms. Trends Biotechnol. 34, 588–599.

Singh, P., Kim, Y.-J., Singh, H., Mathiyalagan, R., Wang, C., Yang, D.-C., 2015. Biosynthesis of anisotropic silver nanoparticles by Bhavagreas indica and their synergistic effect with antibiotics against pathogenic microorganisms. J. Nanomater. 2015, 1–7.

Singh, P., Singh, H., Ahn, S., Castro-Aceituno, V., Jimenez, Z., Simu, S.Y., Kim, Y.J., Yang, D.C., 2017b. Pharmacological importance, characterization and applications of gold and silver nanoparticles synthesized by Panax ginseng fresh leaves. Artificial Cell Nanomater. Biotechnol. 45, 1415–1424.

Slavin, V.N., Anis, J., Hafei, U.O., Bach, H., 2017. Metal nanoparticles: understanding the mechanisms behind antibacterial activity. J. Nanobiotechnol. 15, 1–20.

Some, S., Sarkar, B., Biswas, K., Jana, T.K., Bhattacharyya, D., Dam, P., Mondal, R., Kumar, A., Deb, A.K., Sadat, A., 2020. Bio-molecule functionalized rapid one-pot green synthesis of silver nanoparticles and their efficacy towards the multidrug resistant (MDR) gut bacteria of silkworms (Bombyx mori). RSC Adv. 10, 22742–22757.

Soni, I., Salopek-Sondi, B., 2004. Silver nanoparticles as antimicrobial agent: a case study on E. coli as a model for Gram negative bacteria. J. Colloid Interface Sci. 275, 177–182.

Sowmya, C., Lavakumar, V., Venkateshan, N., RaviChandran, D., Vaigopal, D., 2018. Exploration of Phyllanthus acidus mediated silver nanoparticles and its activity against infectious bacterial pathogen. Chem. Cent. J. 12, 1–8.

Sorena, R., Anita, B., Ramakrishnan, S., Gunasekaran, K., Nakerkan, S., 2020. Synthesis and characterization of AgNPs using plant extracts. Int. J. Curr. Microbiol. Appl. Sci. 9, 1939–1947.

Tayy, P.K., 2016. Use of biofabricated silver nanoparticles conjugated with antibiotic against multidrug resistant pathogenic bacteria. Biol. Insights 1, 1–2.

Veerasamy, R., Xin, T.Z., Gunasegaran, S., Xiang, T.F.W., Yang, E.F.C., Jeyakumar, N., Dhinaraj, S.A.J., 2011. Biosynthesis of silver nanoparticles using mangosteen leaf extract and evaluation of their antimicrobial activities. J. Saudi Chem. Soc. 15, 113–120.

Ventola, C.L., 2015. The antibiotic resistance crisis: part 1: causes and current status. J. Pharm. Ther. 40, 277–280.

Vijayakumari, M., Priya, K., Nancy, F., Nooridah, A., Ahmed, A., 2013. Biosynthesis, characterisation and anti-bacterial effect of plant mediated silver nanoparticles using Artemisia nilagirica. Ind. Crop. Prod. 41, 235–240.

Vijayakumari, S., Divya, M., Chen, J., Brumtha, M., Silva, L.P., Duran-Lara, E.F., Shreema, K., Ramji, S., Dasgupta, N., 2020. Biological compounding of cative silver nanoparticles with the seed extracts of blackcumin (Nigella sativa): a potential antibacterial, antidiabetic, anti-inflammatory, and antioxidant. J. Inorg. Organomet. Polym. Mater. 1–12.

Wang, L., Wu, Y., Xie, J., Wu, S., Wu, Z., 2018. Characterization, antioxidant and antimicrobial activities of green synthesized silver nanoparticles from Pinus guajia leaf aqueous extracts. Mater. Sci. Eng. C 86, 1–8.

WHO, 2016. Antibiotic Resistance. World Health Organization.

Yacamán, M.J., Ascencio, J., Liu, H., Garde-Torressey, J., 2001. Structure shape and stability of nanometric sized particles. J. Vac. Sci. Technol. B: Microelectron. Nanometer Struct. Proc. Measure. Pheno. 19, 1091–1103.

Yazdi, M.E.T., Amiri, M.S., Hosseinzadeh, H.A., Oskue, R.K., Mosavee, H., Pakravan, K., Darroudi, M., 2019. Plant-based synthesis of silver nanoparticles in Handelia triphylla and their biological activities. Bull. Mater. Sci. 42, 155.

Yuan, Y.-G., Peng, Q.-L., Gurunathan, S., 2017. Effects of silver nanoparticles on multiple drug-resistant strains of Staphylococcus aureus and Pseudomonas aeruginosa from mastitis-infected goats: an alternative approach for antimicrobial therapy. Int. J. Mol. Sci. 18, 569.

Zhou, Y., Kong, Y., Kundu, S., Cirillo, J.D., Liang, H., 2012. Antibacterial activities of gold and silver nanoparticles against Escherichia coli and bacillus Calmette-Guinier. J. Nanobiotechnol. 10, 19.