Chapter

Green Synthesis of Metal Nanoparticles for Antimicrobial Activity

Jerushka S. Moodley, Suresh Babu Naidu Krishna, Karen Pillay and Patrick Govender

Abstract

The development and extensive spread of multi-drug resistant bacteria are considered as a major public health concern. Failures to control severe infections due to antibiotic resistance have augmented healthcare costs as well as patient morbidity and mortality. Presently, natural product-based therapeutics are gaining significant attention both for their antimicrobial effectiveness and for not persuading drug resistance. Furthermore, recent developments in nanoscience on new drug delivery systems built on nanostructured materials from plants and microbes have emerged which focus on targeted delivery and controlled release of therapeutic agents. This review examines the recent investigations on the biological activities of plant and bacterial biological material for silver nanoparticle (AgNP) synthesis. Also, the underlying mechanism of antimicrobial activities of silver nanoparticles against human pathogens will be discussed. A fact of the biological activities and/or chemical responses of plants is required, not only for the discovery of new therapeutic agents, but because such evidence may be of value in disclosing new sources of already known biologically active compounds.

Keywords: antimicrobial resistance, biological activities, drug delivery, green synthesis, health, silver nanoparticles

1. Introduction

The antimicrobial potential of silver (Ag) and Ag-based solutions has long been established, however, their application was considered obsolete upon the discovery of antibiotics [1, 2]. In recent years, the developing crisis of multi-drug resistant pathogenic infections has led to the resurgence in this metal, however, with the use of nanotechnology to generate its nanoparticle form. For this reason, tremendous efforts have been extended in nanotechnology, particularly in the development of green synthetic strategies for silver nanoparticle (AgNPs) production to facilitate their use in antimicrobial therapeutic applications [3].

The interest in silver nanoparticles (AgNPs) as an alternative to current antibiotics has increased profoundly over the last few years. This is owed to the cumulative incidence of microbial drug-resistant infections and the lack of appropriate treatment thereof [4]. The World Health Organisation report of 2014 highlighted the probability of a post-antibiotic era in which common infections and minor injuries

could potentially result in fatalities [5]. Accordingly, concerted efforts have been extended by global pharmaceuticals to formulate new or improved antibiotics. However, despite high research cost-intensive investment in the last decade or so only two new classes of antibiotics have been introduced into the market [6, 7]. The imperative need for the uncovering of novel antimicrobial scaffolds has led to the resurgence of silver, however, in its nano-particulate form [8].

The antimicrobial activities of AgNPs are well established and currently researchers are striving to develop greener synthetic strategies for their production [1, 9]. The use of nanotechnology for the synthesis of AgNPs from environmentally compatible biomaterials is evolving into an important branch of science and technology [10]. To this end, a variety of biological extracts have been explored for the bottom-up synthesis of AgNPs [11]. However, there is an ongoing search to identify novel capping structures to produce AgNPs with increased bio-efficacies. In this context, this chapter points to highlight the use of plants as an alternative green technology for nanoparticle synthesis and their biomedical applications as potential biofactories for antibacterial, antifungal and anti-cancer agents.

2. Preparation of nanosilver

2.1 Conventional nanoparticle synthetic strategies

Established technologies for AgNP synthesis and other metal preparations can be categorised distinctly into two approaches, namely: "top to bottom", which is normally employed by physicists and "bottom to up", a construction favourite of chemists [12, 13]. Both approaches converge at the nanodimension but vary drastically in the synthetic technology. "Top to bottom" approaches apply various physical methods such as grinding, milling, sputtering, evaporation-condensation and thermal/laser ablation to break down bulk solid materials to their nanoparticulate form. "Bottom to up" approaches entail various chemical and biological methods to synthesise nanoparticles by the self-assembly of atoms such as Ag^+ into nuclei that further develop into nano-sized particles [9].

Important physical "top to bottom" methods for nanoparticle preparation include evaporation-condensation and laser ablation techniques [14]. Evaporation-condensation applies a tube furnace at atmospheric temperature wherein primary material (metal Ag) contained in a boat; is centred in the furnace and vaporised into a carrier gas [9]. Several inadequacies have been identified with this technique, for example, the furnace occupies a large space, requires high energy input whilst raising the environmental temperature around the source material and requires long durations to achieve thermal stability. Additionally, a major drawback to this type of synthesis is the resulting imperfections in the surface structure of the derived nanoparticles which can ultimately alter their physical properties [9, 15]. In laser ablation, irradiation is used to remove material from a bulk metal in solution. The efficacy of this technique and characteristics of nascent particles is largely dependent on a number of parameters including the wavelength of the laser, duration of laser pulses, laser fluence, ablation duration and the effective liquid medium with or without surfactants [16, 17]. An important advantage of laser ablation for AgNP preparation is the absence of chemicals in solution which could potentially contaminate the nanoparticle preparation [18].

Regarding "bottom to up" approaches, wet chemical reduction is the most frequently practiced method for nanoparticle preparation [15] although, several other methods have been reported [19–22]. As the name suggests, wet chemical reduction

involves the reduction of a metal salt precursor in aqueous or organic solution. Various organic and inorganic compounds successfully utilised as reducing agents in the synthesis of AgNPs include: ascorbate; borohydride; citrate; elemental hydrogen; formaldehyde; N-N-dimethyl formamide (DMF); Tollen's reagent; and polyethylene glycol blocks [15, 23, 24]. In addition to reducing agents, protective stabilising agents are also included in the reaction solution to prevent agglomeration of nascent nanoparticles [25, 26]. With stability achieved, this method can be useful to produce high nanoparticle yields with low preparation costs [27]. However, the efficacy of this method is challenged by the potential contamination of nascent nanoparticles by precursor chemicals, the use of toxic solvents and the generation of hazardous by-products [13, 28].

Evidently, the aforementioned physical and chemical methods have certain limitations that restrict their use in the preparation of nanoparticles for biological applications [29]. In this regard, concerted efforts have been extended to develop nanoparticle synthetic strategies that are environmentally sound. Essentially, this would entail the use of benign, biotechnological tools and has given rise to the concept of green technology. This technology can best be described as the use of biological routes such as plants and microorganisms or their byproducts in the synthesis of nanoparticles [29–31]. These bio-inspired methods **(Figure 1)** are not only environmentally welcoming but are cost effective and can be easily up-scaled for large productions [32].

2.2 Biological nanoparticle synthetic strategies

As previously eluded, biological approaches for AgNP synthesis employ the use of living organisms or their extracts as capping/reducing agents in a synthetic reaction. To date, a variety of biological entities have been explored for their Ag^+ reducing abilities and include viruses, bacteria, plants, algae, fungi, yeast and mammalian cells [11, 13, 34–36]. Biological synthesis can be divided into two strategies, specifically: bioreduction and biosorption. Bioreduction occurs when metal ions undergo chemical reduction into biologically stable complexes. Many organisms have displayed dissimilatory metal reduction involving the coupling of reduction with oxidation of an enzyme. The resulting stable, inert nanoparticles can then be safely extracted from the reaction mixture. Alternatively, biosorption involves the attachment of metal ions onto an organism itself, such as on the cell wall. Various bacteria, fungi and plant species express peptides or possess modified cell wall structures that are capable of binding metal ions, thereby forming stable complexes in the form of nanoparticles [36].

In this review, the use of plant and bacterial biological material for AgNP synthesis will be discussed. For a review on the use of alternative biological entities as AgNP factories, studies by the following authors are recommended [11, 36, 37].

2.3 AgNP synthesis from plants

Plants have shown the capacity to hyper-accumulate metals as a means to protect themselves from insects and herbivores. This observation has paved way for the technology known as phytoextraction, wherein plants are employed to extract minerals from various groundwater and soil sediments. Major applications of phytoextraction include the mining of precious metals from unfeasible ground sites (phytomining), stabilisation or recovery of non-naturally occurring contaminants (phytoremediation) and the addition of essential metals to growing crops. Interestingly, studies have unveiled that metals accumulated by

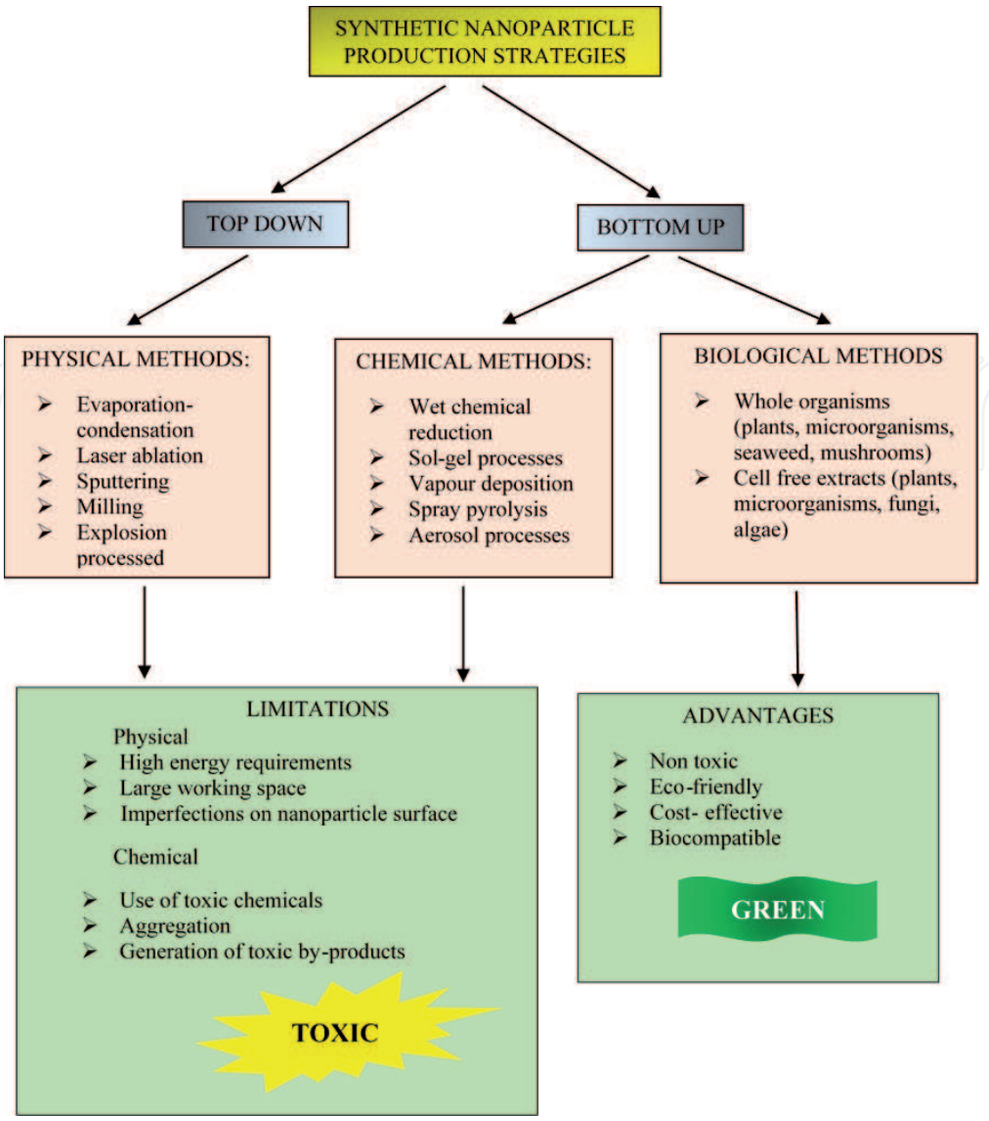

Figure 1.
Different approaches for AgNP synthesis. Adapted from [9, 33].

the plant are usually deposited in the form of nanoparticles. This has stimulated interest for the use of plants as factories for nanoparticle synthesis [35]. Whole plants have been explored for the synthesis of nanoparticles when grown on the appropriate metal enriched substrates. Species such as *Brassica juncae* (mustard greens) and *Medicago sativa* (alfalfa) have demonstrated the ability to accumulate AgNPs. For example, 50 nm sized AgNPs, at a high yield (13.6% of total plant weight) were reported for *M. sativa* when grown on silver nitrate ($AgNO_3$) [38]. Additionally, icosahedral gold nanoparticles of 4 nm size were observed in *M. sativa* and semi-spherical copper nanoparticles of 2 nm size were observed in *Iris pseudacorus* when the plants were grown on gold and copper salt enriched substrates, respectively [39, 40].

Although whole plants can potentially serve as factories for nanoparticle synthesis, several disadvantages have been identified with this technology especially when up-scaling for industrial applications. For example, physical attributes of nanoparticles such as size and shape vary upon the localisation of the particles in the plant due to the differences in metal ion content in different plant tissues and the possibility of nanoparticle movement and penetration [39]. This heterogeneity of important bioactivity-determinants such as size and shape [41, 42] limit the use of these nanoparticles and especially in applications where mono-dispersed nanoparticle preparations are required. Furthermore, recovery of nanoparticles from living

plants entails laborious extraction, isolation and purification procedures and may potentially result in low yields [35].

The use of plant broths/extracts in nanoparticle synthesis was introduced by Shankar *et al*., (2003). In their study, compounds responsible for the reduction of metal ions were extracted and used as reducing agents in a synthetic reaction mixture, resulting in the extracellular production of nanoparticles [43]. This strategy tentatively offers several advantages compared to the use of whole plants. For example, nanoparticle formation occurs considerably faster as opposed to whole plants which require diffusion of metal ions throughout the plant body. Additionally, the use of extracts would be more economical due to the ease of purification [35].

This *in vitro* approach has been actively developed and applied to a variety of plant flora for the synthesis of AgNPs [28]. Various organ extracts: stem, root, leaf, bark, fruit and fruit peel have demonstrated the ability to reduce Ag^{+}. Particularly, biomolecules **(Figure 2)** such as proteins, amino acids, enzymes, polysaccharides, alkaloids, tannins, phenolics, saponins, terpenoids and vitamins present in the extracts act as both reducing and stabilising agents [9].

Terpenoids are a class of diverse organic polymers manufactured in plants from five-carbon isoprene units and display strong antioxidant activities. In a previous study by Shankar *et al*., involving gold nanoparticle synthesis from geranium leaf extracts, it was suggested that these polymers were actively involved in the reduction of gold ions into stable nanoparticles [44]. Later Singh

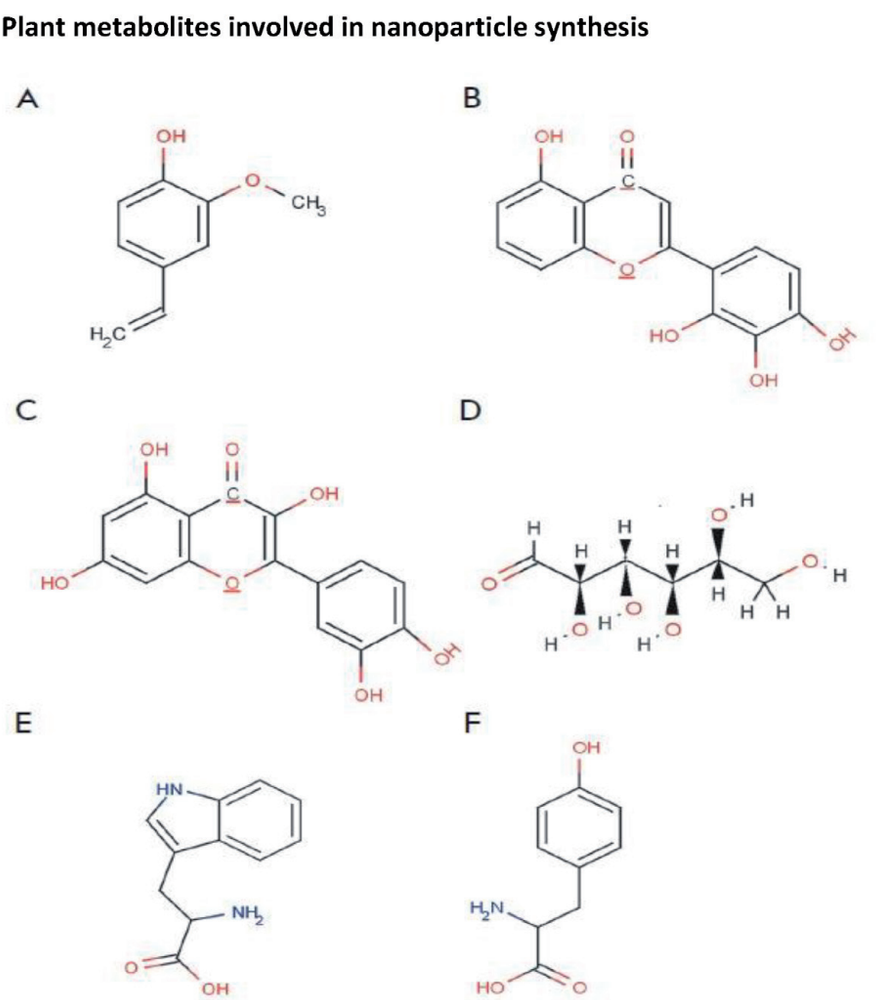

Figure 2.
Major plant metabolites involved in the synthesis of metal nanoparticles: (A)-terpenoids (eugenol); (B & C)-flavonoids (luteolin, quercetin); (D)-a reducing hexose with the open chain form; (E & F)-amino acids (tryptophan, tyrosine). Adapted from [35].

et al. reported that eugenol, the main terpenoid found in *Szyygium aromaticum* (clove), played an important role in reducing $AgNO_3$ and $HAuCL_4$. The Fourier transform infrared (FTIR) spectroscopy analysis of their study suggests that the dissociation of the proton from the OH group in eugenol leads to the formation of intermediate resonance structures which can undergo further oxidation. This latter reaction may be coupled to the reduction of Ag^+ and subsequent formation of stable AgNPs [45].

Flavonoids are made up of a large group of polyphenolic compounds containing various classes such as anthocyanins, isoflavonoids, flavonols, chalcones, flavones and flavanones. There are several functional groups present on flavonoid compounds that can participate in nanoparticle formation. It has been hypothesised that the tautomerization of flavonoids from the enol to keto form releases a reactive hydrogen atom that can participate in the reduction of metal ions. For example, studies involving AgNP synthesis from *Ocimum sanctum* extracts indicate that synthesis is likely to be the result of tautomerization of the flavonoids luteolin and rosmarinic acid [46]. Additionally, some flavonoids can chelate metal ions with their carbonyl groups or π-electron. Quercetin is an example of a flavonoid with strong chelating activity [35]. These mechanisms may explain the prevalence of flavonoid groups adsorbed on to the surface of AgNPs derived in previous studies [47, 48]. Further indication of flavonoid involvement in nanoparticle synthesis is provided by a study using *Lawsonia inermis,* in which the flavonoid apiin was extracted and successfully employed in the synthesis of gold and Ag nanoparticles [49].

Sugars contained in plant extracts are also capable of inducing nanoparticle formation. It is known that monosaccharides in the linear form containing an aldehyde (e.g. glucose), are capable reducing agents [35]. Monosaccharides harbouring a keto-group may act as antioxidants upon tautomeric transformation from a ketone to an aldehyde (e.g. fructose). In this regard, glucose is reportedly more efficient at metal ion reduction than fructose due to the kinetics of tautomerism from a ketone to an aldehyde which limits the reducing potential of fructose. Disaccharides and polysaccharides may also participate in the reduction of metal ions however, this is largely dependent on the ability of their monosaccharide components to take on an open chain configuration within an oligomer. Examples include lactose and maltose. In contrast, sucrose is unable to participate in metal ion reduction because the linkage of its glucose and fructose monomers restrict the formation of open chains. However, when sucrose was placed in tetrachloroauric and tetrachloroplatinic acids, nanoparticle formation proceeded [50]. This may be due to the acidic hydrolysis of sucrose yielding glucose and fructose. In general, it is suggested that nanoparticle formation by sugars occurs by the oxidation of an aldehyde group into a carbonyl group which subsequently leads to the reduction of metal ions and nanoparticle formation [44].

FTIR analysis of plant derived metal nanoparticles have revealed the presence of proteins on their surface, suggesting that proteins may also possess metal ion reducing ability. However, amino acids have displayed differences in their potential for metal ion reducing and binding efficiencies. For example, lysine, cysteine, arginine and methionine have been shown to bind Ag^+. In a separate study, aspartate was used to reduce tetrachloroauric acid forming nanoparticles, whilst valine and lysine did not possess this ability. Amino acids capable of binding metal ions are thought to do so through their amino or carboxyl groups or through side chain groups: carboxyl groups of aspartic and glutamic acid, imidazole ring of histidine, thiol of cysteine, thioether of methionine, hydroxyl group of serine; threonine and tyrosine, carbonyl groups of asparagine and glutamine [35].

Linkage of amino acids in a peptide chain may also affect the ability of individual amino acids to bind and reduce metal ions. For example, the R-carbon of amines and carboxylic acids in a peptide bond are inaccessible for association with metal ions. However, the free side chains of individual amino acids can still participate in binding and reduction of metal ions although, this is largely dependent on the amino acid sequence. Tan *et al*. demonstrated that synthesised peptides derived from amino acids with strong binding abilities and high reducing activities displayed lower reduction than expected [51]. A previous study suggested that protein molecules capable of nanoparticle formation display a strong attraction of metal ions to the regions on the molecule responsible for reduction however, their chelating activity is limited [52]. It was also suggested that the amino acid sequence of a protein can influence the size, shape and yield of derived nanoparticles. For example, the synthetic peptide GASLWWSEKL was found to rapidly reduce metal ions forming a large number of small nanoparticles (˂10 nm), however, replacement of the N- and C- terminal residues forming the peptide SEKLWWGASL led to slower reduction and formation of larger nanospheres and nanotriangles (40 nm). These findings seemingly suggest that peptides and proteins present in plant extracts probably play a vital role in determining nanoparticle size and shape and potentially affect the overall yield of the nanoparticles [51].

2.4 AgNP synthesis from bacteria

There exists a vast array of literature pertaining to the use of bacteria as factories for nanoparticle synthesis [53, 54]. Bacteria have a marked advantage over other microbial systems such as fungi due to their abundance, rapid growth rate, cheap cultivation and the relative ease of their manipulation [55]. Their ubiquitous nature has led to their exposure and proliferation in many environmental extremes and ultimately depends on the natural defence mechanisms of these microorganisms to resist the effects posed by environmental stresses [56]. Bacteria have demonstrated these defence mechanisms in a few non-optimal growth conditions including environments contaminated with metal ions.

AgNP synthesis by bacteria can occur intracellularly or by the use of their extracts [53]. Several studies have reported intracellular synthesis by a variety of bacterial species and as similarly reported for the use of whole plants, this technology is associated with long duration periods for nanoparticle synthesis. For example, Pugazhenthiran *et al*. reported an incubation time of 7 days for AgNP synthesis from *Bacillus* sp. [57]. Kalimuthu *et al*. reported a reaction time of 24 hours for AgNP synthesis by *Bacillus licheniformis* [58]. Although this reaction time was more industrially significant, the authors reported an additional extraction to acquire the derived nanoparticles. Synthesis of AgNPs by the use of bacterial cell free supernatant (CFS) extracts was reported by Shahverdi *et al*., (2007). Interestingly, nanoparticle synthesis occurred within five minutes of Ag^{+} coming into contact with the CFS [59]. Thus, this method presents the greatest potential for industrial production of AgNPs from bacteria. Several other studies have reported on the production of AgNPs from bacterial CFS extracts but not at the previously stated formation rate [60, 61]. This seemingly suggests that bacterial extracts differ in their metal ion reducing abilities and may require an external energy source to accelerate nanoparticle formation.

2.4.1 Bacterial metabolites involved in nanoparticle synthesis

As previously stated, metal nanoparticle synthesis in bacteria may potentially occur through resistance mechanisms attained by these organisms to overcome the

toxic effects of metals. These strategies include redox state changes, efflux systems, intracellular precipitation, metal accumulation and extracellular formation of complexes **(Figure 3)** [56]. In an early study, Slawson *et al*. observed that the Ag resistant strain *Pseudomonas stutzeri* AG259, was capable of accumulating AgNPs (35–46 nm) within its periplasmic space. The formation of these nanoparticles was thought to have occurred by a mechanism involving the NADH-dependent reductase enzyme which undergoes oxidation to form NAD^+. The lost free electron may potentially reduce Ag^+ to AgNPs [62]. Later, He *et al*. reported that the NADH-dependent reductase enzyme may similarly participate in the extracellular formation of gold nanoparticles by the bacterium *Rhodopseudomonas capsulata* [63]. Other studies have reported nanoparticle formation without the use of biological enzymes. Non-enzymatic nanoparticle synthesis by a *Corynebacterium* sp. was reported by Sneha *et al*. [64]. Organic functional groups present at the cell wall were thought to induce metal ion reduction [64]. Sintubin *et al.* proposed a two-step mechanism for AgNP formation by several lactic acid bacteria, involving biosorption of Ag^+ on the cell wall which is coupled to the subsequent reduction of these ions to form the nanoparticles [65]. Parikh *et al*. identified a gene homologue in a Ag-resistant *Morganella* strain with a 99% nucleotide sequence similarity to a periplasmic Ag-binding protein-encoding gene [66]. Johnston *et al*. further reported the production of a small non-ribosomal peptide, delftibactin by *Delftia acidovorans* which they believed to be associated with a resistance mechanism. By producing inert gold nanoparticles bound to delftibactin, gold ions no longer caused toxicity to the cells [67].

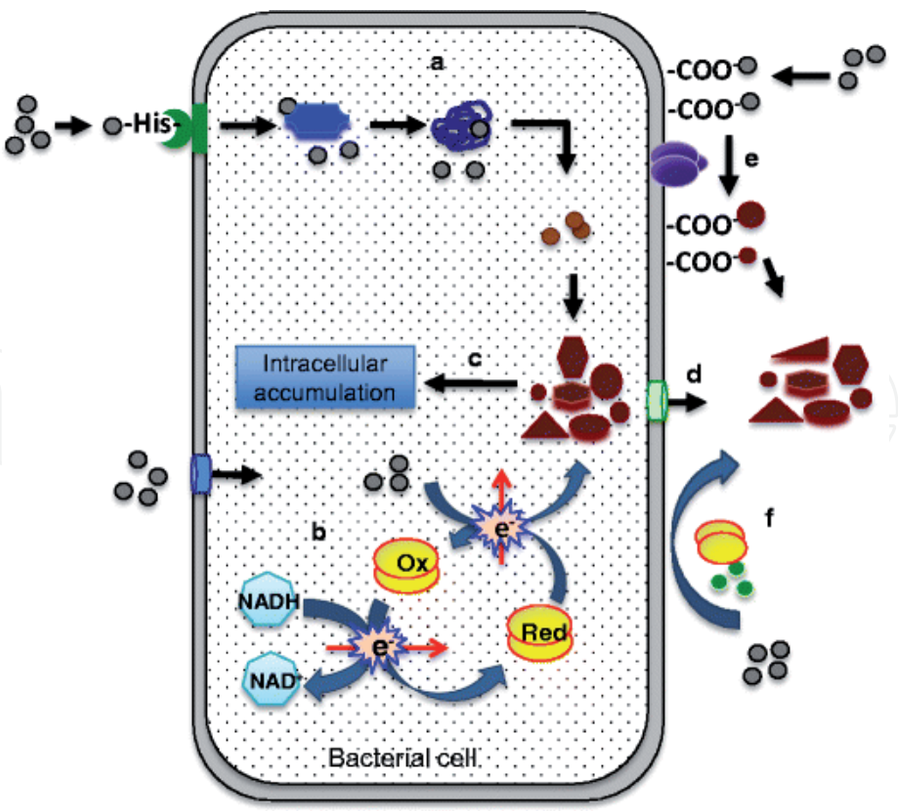

Figure 3.
Metabolites and mechanisms involved in AgNP synthesis in bacteria: (a)-uptake of Ag^+ and activation of reduction machinery; (b)-electron shuttle system involving various cofactors and enzymes; (c & d)- intra or extracellular localisation of AgNPs; (e)-electrostatic interaction between Ag^+ and cell wall peptides/proteins & (f)-extracellular reduction by enzymes or other metabolites released in solution. Adapted from [53].

3. Mechanism of nanoparticle synthesis using plants and microbes

There are three main phases in the synthesis of metal nanoparticles from plants and plant extracts. Initially, an activation phase takes place during which metal ions are reduced from mono or divalent oxidation states to zero-valent states, followed by nucleation of the reduced atoms. This step is immediately followed by a growth phase where small neighbouring nanoparticles coalesce into larger particles with greater thermodynamic stability while further biological reduction occurs. As growth proceeds nanoparticles aggregate to form various shapes such as: cubes, spheres, triangles, hexagons, pentagons, rods and wires [68]. Lastly, a termination phase follows in which nanoparticles acquire the most energetically favourable conformation, which ultimately determines the final shape of the particles **(Figure 4)** [69]. This step is largely influenced by the ability of the plant extract to stabilise the resulting nanoparticles. For example, the high surface energy of nanotriangles results in their decreased stability. Such nanoparticles would then acquire a more stable morphology such as a truncated triangle to minimise Gibbs free energy unless the stability is supported by the given extracts. It can be tentatively suggested that a similar mechanism occurs by the use of bacterial extracts since proteins and metabolites may also participate in Ag^+ reduction as previously stated.

Several controlling factors affect the synthesis and morphology of derived nanoparticles. Several researchers have associated these variations with the choice of adsorbate and catalyst used in the synthetic process [29, 70]. However, reaction parameters have also been shown to strongly affect the synthesis of nanoparticles from biological extracts.

Studies have revealed that the pH of a reaction solution strongly influences the formation of the produced nanoparticles. Variances in reaction pH tend to induce variability in the shape and size of the produced nanoparticles. Lower acidic pH values tend to produce larger particles when compared to higher pH values. In a study employing *Avena sativa* (oat) biomass for the production of gold nanoparticles, larger particles (25–85 nm) where formed at pH 2 whilst smaller particles (5–20 nm) were formed at pH 3 and 4 [71]. The researchers suggested that at pH 2, fewer functional groups were available for particle nucleation resulting in aggregation of the particles. A similar finding was observed in the synthesis of gold nanoparticles from the bacterium *Rhodopseudomonas capsulate.* At an increased pH of 7, spherical particles in the range of 10–20 nm in size were observed. In contrast, lowering the reaction pH to 4 resulted in the formation of nanoplates [63].

Temperature is an important factor in any synthesis. With respect to nanoparticle formulation with the use of biological entities, temperature elevation has demonstrated catalytic behaviour by increasing the reaction rate and efficiency of nanoparticle formation. For example, a study on the influence of reaction temperature in the synthesis of AgNPs from neem leaf extracts suggested that temperature elevation (10–50°C) was correlated with enhanced reduction of Ag^+ [72]. It was also noted that smaller sized AgNPs were produced at 50°C, similar to the finding of Kaviya *et al.* in the production of AgNPs from *Citrus sinensis* peel extracts using varying temperatures [73]. Similarly, this trend was observed in the production of AgNPs from the spent culture supernatants of *Escherichia coli* [61]. The authors tentatively suggested that the increased reaction rate might be because of temperature on a key enzyme participating in nanoparticle synthesis. However, the study importantly revealed that temperature elevation above 60°C contrastingly favoured the production of larger sized particles. The reason for this observation was reported as follows: at high temperatures, kinetic energy of the molecules increase resulting in rapid reduction of Ag^+ (facilitating reduction and nucleation), to the

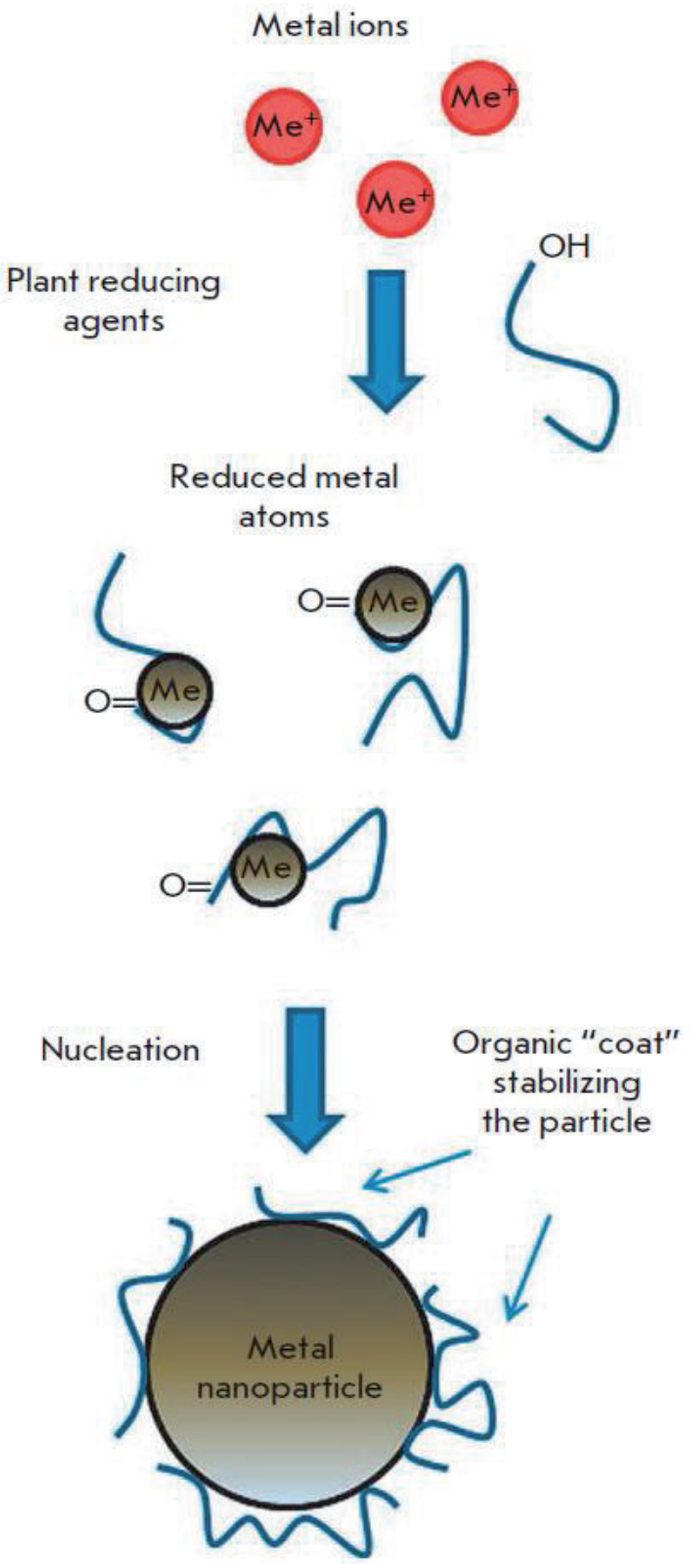

Figure 4.
Schematic representation of nanoparticle synthesis using a plant extract. Adapted from [35].

detriment of secondary reduction on the surface of nascent particles in the growth phase. However, higher temperatures beyond the optimum are thought to increase the growth of the crystal around the nucleus, resulting in the production of larger particles [48, 61].

Temperature has also been demonstrated to affect the structural form of nanoparticles. For example, AgNP synthesis using *Cassia fistula* extracts resulted in the formation of Ag nanoribbons at room temperature whilst spherical AgNPs

were formed at temperatures above 60°C [74]. High temperatures in the study were thought to alter the interaction of plant biomolecules with the faces of Ag, inhibiting the coalescence of adjacent nanoparticles.

Sunlight irradiation, a recently reported primary energy source for nanoparticle formation, has been observed to derive AgNPs with desired physical attributes. Recent studies on sunlight driven AgNP synthesis using *Allium sativum* (garlic extract) and *Andrachnea chordifolia* ethanol leaf extract revealed that sunlight rapidly enhanced nanoparticle formation to produce spherical AgNPs with average diameters of 7.3 nm and 3.4 nm, respectively [75, 76]. In addition, this use of sunlight has also been used in AgNP synthesis from *Bacillus amyloliquefaciens* CFS to produce circular and triangular crystalline AgNPs with an average diameter of 14.6 nm [77].

A variety of literature reports on the synthesis of AgNPs with differing morphologies. Understanding the effects of these morphological characteristics on bioactivity is therefore an important consideration when deriving nanoparticles for therapeutic purposes. Characteristically, AgNPs are small (1–100 nm) and therefore possess a large surface area that facilitates their interaction with bacterial cell membranes [41, 78]. However, it has been suggested that within this confined size range, AgNPs present a size-dependent inhibition spectrum. Martinez-Castanon *et al*. reported that AgNPs of 7 nm in size had minimum inhibitory concentration (MIC) values of 6.25 μg ml^{-1} and 7.5 μg ml^{-1} for *E. coli* and *Staphylococcus aureus*, respectively. In contrast, larger nanoparticles (29 nm) capped with the same reducing agent displayed higher MIC values for the respective strains [79]. These results are in accordance with other studies that report nanoparticles of $<$ 10 nm in size display improved bactericidal activities [42, 80].

The interaction of AgNPs of varying shapes with *E. coli* cells has unveiled that shape plays an important factor in bioactivity. Pal *et al*. reported that at a low Ag content of 1 μg, truncated triangular nanoparticles showed nearly complete inhibition of *E. coli* cells, whilst spherical nanoparticles with a total silver content above 12.5 μg displayed a reduction in colony forming units. Rod-shaped particles and $AgNO_3$ presented inferior activities when compared to truncated triangular and spherically shaped AgNPs [41].

Considering these factors and the aforementioned factors affecting synthesis of nanoparticles, it can tentatively be suggested that the fine tuning of reaction parameters such as pH or temperature may be applied in producing AgNPs with these desired physical attributes. However, the use of sunlight irradiation provides a promising alternative in this regard.

3.1 Anti-microbial properties of silver nanoparticles

There exists an abundance of literature reporting on antimicrobial activities of biologically derived AgNPs [81–84]. Most of these studies utilise the disc diffusion assay [85] or agar well diffusion assay [86] to establish inhibitory effects. Positive indication of inhibitory activities are visualised by zones of inhibition on a microbial lawn. Veersamy *et al*. reported zones of inhibition of *S. aureus* and *E. coli* to be 15 mm and 20 mm respectively for AgNPs (20 μg ml^{-1}) derived from mangosteen leaf extracts [48]. Similarly, Logeswari *et al*. reported zones of inhibition of AgNPs synthesised from various plant extracts against several bacterial strains [81]. Although diffusion techniques are preferred amongst researchers, they seem to be labour-intensive. In addition, many researchers do not establish the initial concentration of AgNP solution prior to antimicrobial evaluation [82, 87]. Such disparities make comparison between published data inapplicable [88].

Determination of minimum inhibitory concentration (MIC) by the broth microdilution or macrodilution method [89, 90] is easy to access and provides accurate

information with respect to microbial susceptibility. Moreover, MIC values are reported in various concentration units such as μg ml^{-1}, μg l^{-1} or ppm thereby facilitating comparison between publications [53]. These methods are therefore attractive for AgNP bioactivity analysis. Furthermore, determination of MICs is an important consideration for any therapeutic agent in development to assess their toxicity at the specified concentration range. As previously mentioned, the antimicrobial effects of AgNPs are well established. However, a relatively confined amount of studies has been conducted to elucidate their mechanisms of antimicrobial action. These mechanisms are poorly understood and have failed to achieve consensus amongst researchers. Despite this, three common mechanisms of bactericidal activity have been proposed by various studies. These include the uptake of Ag^+ (1), generation of reactive oxygen species (ROS) (2) and cell membrane disruption (3) (**Figure 5**) [91].

Since Ag^+ are known to possess antibacterial activities, their release from AgNPs may potentially aid to the bioactivity of the nanoparticles. It is therefore fitting to consider the mechanistic action of Ag^+ on bacterial cells.

The NADH–ubiquinone reductase has been established as one of the major targets for Ag^+. Specifically, the binding of Ag^+ to this enzyme may be responsible for their bactericidal effect even at minute concentrations [92]. Later, Dibrov *et al*. reported the binding of Ag^+ to transport proteins leads to the leakage of protons and ultimately induces the collapse of the proton motive force [93]. Such interactions with transport proteins may be attributed to the strong affinity of Ag^+ to thiol groups found on cysteine residues of these molecules [94]. Ag^+ has also been reported to inhibit phosphate uptake and additionally causes an efflux of intracellular phosphate [95]. It has also been hypothesised that the antimicrobial effect of Ag^+ is correlated with the disruption of DNA replication. DNA molecules in a relaxed conformation can be replicated effectively. However, when Ag^+ are present in bacterial cells, DNA molecules enter a condensed form and replicating ability diminishes which ultimately leads to cell death [8].

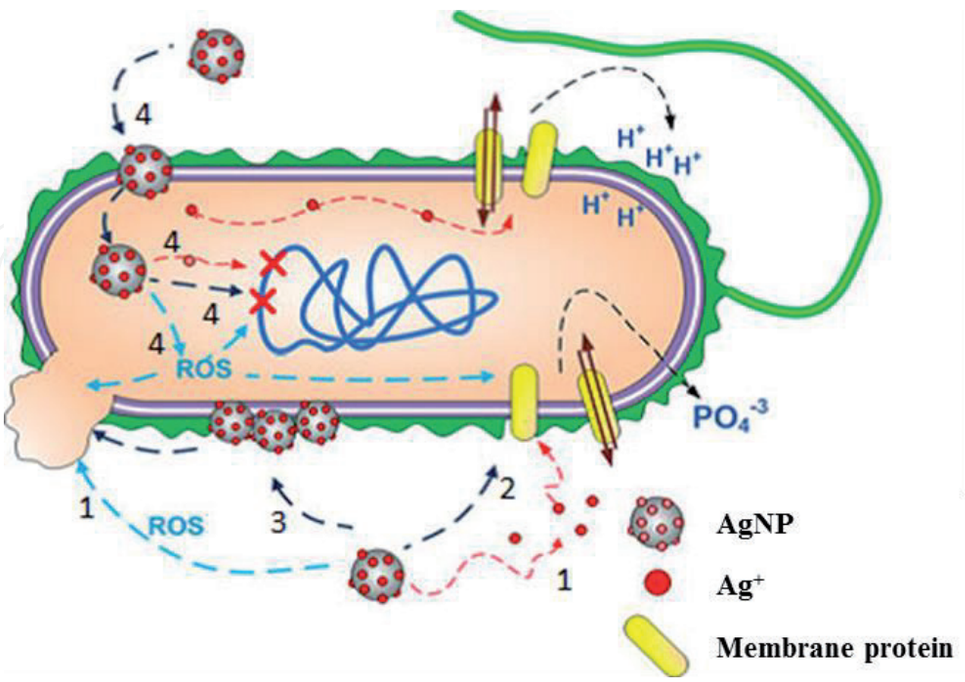

Figure 5.
Interactions of AgNPs with bacterial cells: (1) release of Ag^+ and generation of ROS; (2) interaction with cell membrane proteins; (3) accumulation in cell membrane and disruption of permeability; (4) entry into the cell and release of Ag^+, leading to generation of ROS and damage of cellular DNA. In turn, generated ROS may affect DNA, cell membrane and membrane proteins whilst released Ag^+ may affect cell membrane proteins and DNA. Adapted from [91].

The exposure of bacterial cells to AgNPs leads to the generation of ROS [96]. Naturally, ROS are metabolic by-products of respiring beings. Whist low levels of these species are skilfully controlled by various antioxidant defence mechanisms, high levels of ROS results in oxidative stress which is detrimental to any living organism. Metals can serve as catalysts and produce ROS in an oxygen containing environment [97]. AgNPs are therefore likely to catalyse reactions with oxygen leading to the production of excess free radicals. Kim *et al*. demonstrated the generation of free radicals from AgNPs by means of spin resonance measurements. Toxicity of AgNPs and $AgNO_3$ diminished upon addition of an antioxidant suggesting that the mechanism of action against bacterial strains was associated with the formation of free radicals from AgNPs. The generation of excess free radicals attack membrane lipids resulting in the breakdown of the membrane and cause damage to DNA [1].

The release of Ag^+ from nanoparticles attached to the membrane and nanoparticles inside the cell also play a role in the generation of ROS. Ag^+ released on the membrane are capable of ROS generation by acting as electron acceptors whilst those present inside the cell more likely to interact with thiol groups of respiratory chain enzymes as previously stated, or scavenging superoxide dismutase enzymes [98]. The effect of ROS scavengers on *E. coli* cells was reported by Inoue *et al*.. Specifically, ROS such as superoxide anions, hydroxyl radicals, hydrogen peroxide and singlet oxygen contributed to the bactericidal activity against *E. coli* [99]. According to literature, the bactericidal effect of AgNPs may also be the result of damage to the outer membrane of bacterial cells. Previous studies by Sondi and Salopek-Sondi suggested that treatment of *E. coli* cells with AgNPs induced changes in the membrane morphology (**Figure 6a**). This resulted in increased membrane permeability and shifts in normal transport through the plasma membrane [100]. Morones *et al*. hypothesised that these mechanisms could explain the number of nanoparticles found inside *E. coli cells* (**Figure 6b**). AgNPs with oxidised surfaces were also reported to induce the formation of holes on the surface of *E. coli* cells and portions of the cellular surface were observed to be eaten away [101]. The attachment and penetration of AgNPs has also been observed in *P. aeruginosa* (**Figure 6c**), *V. cholera* and *S. typhus* [80].

The mechanism of AgNP adhesion and penetration of bacterial cell membranes remains to be elucidated. Literature reports indicate that electrostatic interactions between positively charged particles and negatively charged cell membranes is essential for the bioactivity of these particles [102, 103]. However, this strategy does

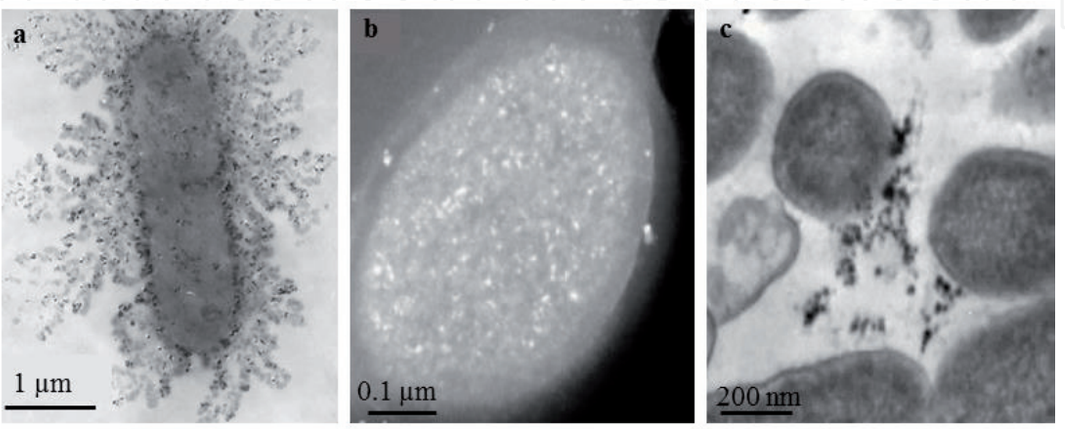

Figure 6.
Transmission electron micrographs of (a) E. coli cell after 1 h treatment with 50 µg cm^{-3} AgNPs; (b) E. coli cell after 30 min treatment with 100 µg ml^{-1} AgNPs (c) P. aeruginosa cells after 30 min treatment with 100 µg ml^{-1} AgNPs [80, 100].

not validate the adhesion and penetration abilities of negatively charged nanoparticles [104]. The researchers argued that although the particles were negatively charged, interactions between the particles and building elements of the membrane are likely to have occurred causing structural changes and degradation of the membrane. Morones *et al*. proposed that the interaction of AgNPs and bacterial membranes could be attributed to the strong affinity of the particles to sulphur containing proteins present on the membrane [80]. These interactions are thought to be conserved in the interaction of Ag^+ and thiol groups on respiratory enzymes and transport proteins [80, 91].

Sondi and Salopek-Sondi [104] further reported that damage to *E. coli* cell membranes might also occur due to the incorporation of AgNPs into their membrane structure. Scanning electron microscopy revealed the formation of "pits" on the surface of the membrane [100]. Similar findings were observed by [102]. Amro *et al*. [109] additionally reported the formation of irregularly shaped "pits" on the outer membrane of *E. coli* cells through the progressive release of lipopolysaccharide molecules. This release of LPS molecules was induced by metal depletion in the cells [105]. A membrane with such morphological changes would display a high increase in permeability, rendering the cell incapable of regulating proper transport through the membrane as previously described.

Although these studies have been conducted on Gram-negative bacteria, AgNPs have also been reported to exert inhibitory activities against Gram-positive bacteria which differ from their counterparts based on differences in cell wall structure [106]. It can be tentatively suggested that AgNPs may form interactions with Gram-positive bacteria through surface proteins present on the cell wall. Once penetrated, the mechanisms of bacterial activity are conserved with that of Gram-negative bacteria.

A relatively confined amount of literature focuses on the mechanisms of antifungal activity exerted by AgNPs. However, based on the studies that have been reported, it seems that inhibition of fungal growth by AgNPs may be the result of damage to fungal cellular membranes. Kim *et al*. demonstrated the effect of AgNPs on *Candida albicans*. Transmission electron microscopy (TEM) analysis revealed that the treatment of cells with AgNPs lead to the formation of "pits" on the cell membrane which ultimately disrupts membrane potential [107]. A similar finding was made by Nasrollahi *et al*. who reported that AgNP incubation with *C. albicans* led to damage of the cell membrane [108]. Endo *et al*. reported that disruption of membrane integrity inhibits the normal budding process of daughter cells. Therefore, the authors suggested that AgNPs exert their inhibitory activity by inhibiting the budding of daughter cells due to the destruction of the cell membrane [109].

AgNPs may also disrupt antioxidant defences in fungal cells. Eukaryotic cell studies suggest that AgNPs directly interact with gluthathione, gluthathione reductase or enzymes responsible for maintaining proper levels of gluthathione [110]. With respect to fungal cells, it has been hypothesised that Ag^+ largely affect the function of membrane bound enzymes such as those in the respiratory chain. It has also been reported that exposure of fungal cells to Ag^+ led to the loss of DNA replication ability. This results in the deactivation of ribosomal subunit protein expression and synthesis of non-functional enzymes and cellular proteins [111].

From these findings it can be tentatively suggested that bactericidal mechanisms of AgNPs are conserved in their inhibition of fungal cells. In summary, AgNPs exert their antimicrobial effects by releasing Ag^+, disrupting the cell membrane/wall, generating ROS and inhibiting proper DNA replication.

3.2 Cytotoxicity of silver nanoparticles

The unique physico-chemical and biological properties of AgNPs have extremely promising industrial and medical applications, as previously mentioned. However, there exists a dearth of knowledge regarding the effects of prolonged exposures to nanoparticles on human health and the environment [112]. It is therefore imperative to establish the *in vitro* and *in vivo* cytotoxic effect of AgNPs in mind for therapeutic purposes.

3.2.1 In vivo *studies*

Human contact with nanoparticles occurs in the form of intravenous injection, oral administration, inhalation and dermal contact [113]. Injection of AgNPs *in vivo* results in short circulation times and broad tissue distribution. Target sites often include the liver (main target), spleen, lungs and kidneys [114]. Inhalation studies suggest that AgNPs become deposited in the olfactory mucosa and olfactory nerves which can potentially induce impairment and dysfunction of brain cells [115] in addition to immunotoxicity [116]. With regard to oral administration, migration of AgNPs to the gastrointestinal tract promotes dissolution of the particles which subsequently releases Ag^+ [117]. A recent study on oral exposure to Ag^+ indicated that these ions interact with sulphur leading to the formation of sulphur containing Ag granules in the intestinal epithelium [118]. The authors suggested that during intestinal digestion, Ag^+ give rise to particle formation, possibly in the form of Ag_2S or AgCl salt. They further added that this formation might influence their uptake and reduce the toxic effects of Ag^+, however the effects of Ag salts on the intestine are yet to be elucidated [118, 119]. Reports on the exposure of workers to low doses of Ag dust indicated no significant changes in health status.

3.2.2 In vitro *studies*

Many researchers have demonstrated the cytotoxic effects of AgNPs *in vitro*, however there is still a lack of consistent and reliable data amongst publications. For example, in a recent review, Kim and Ryu (2013) attributed oxidative stress, apoptosis and genotoxicity to be the main *in vitro* outcome of AgNP exposure [120]. Later, Gliga *et al*. identified a major drawback of this review, highlighting that the AgNPs were different in each study, *i.e.* synthesised by different techniques, of varying size distributions and coatings, tested on different cell lines under different cell culture conditions and often without the use of appropriate controls [121]. Additionally, Hackenberg *et al*. reported cytotoxicity of human mesenchymal stem cells at a concentration of 10 μg ml^{-1} AgNPs (˂50 nm), whereas Samberg *et al*. reported no toxicity of progenitor human adipose-derived stem cells at concentrations up to 100 μg ml^{-1} AgNPs (10–20 nm) [122, 123]. To determine the effect of size on cytotoxicity, Liu *et al*. compared the cytotoxicity of AgNPs ranging in size from 5 to 50 nm on four different cell lines (A549, HepG2, MCF-7 and CGC-7901) and reported that 5 nm AgNPs were most toxic [124]. On the contrary, Kim *et al*. reported the enhanced release of lactate dehydrogenase (LDH) and reduced cell viability in the presence of 100 nm sized AgNPs when compared to smaller AgNPs (10–50 nm) [125]. It can be noted that the variation in parameters in these studies makes it difficult to observe trends and come to accurate assumptions. To achieve some consensus in this regard, Gliga *et al*. studied the cytotoxic effect of varying sized AgNPs capped by various agents on the normal bronchial epithelial cell line (BEAS-2B). They reported that 10 nm sized AgNPs induced cytotoxicity

irrespective of the capping agents, at high concentrations (20–50 μg ml^{-1}), whilst larger AgNPs did not display significant cytotoxic effects at all tested concentrations. The group additionally reported that at non-cytotoxic concentrations (10 μg ml^{-1}), significant DNA damage was observed for all AgNPs independent of size and coating. In contrast, panda *et al*. reported no genotoxicity of AgNPs capped with protein at 20–80 μg ml^{-1} for 24–55 nm sized particles [126].

Overall, it is difficult to establish the cytotoxic effect of AgNPs due to the differences in nanoparticle synthetic methods, their various sizes and capping agents and lastly the diverse evaluation tests used to determine toxicity. In fact, by using different organisms and/or culture cells there is no conclusive evaluation of AgNP toxicity [127]. However, bearing in mind the results presented in this review, it can be tentatively suggested that smaller sized AgNPs are more cytotoxic than larger sized particles at higher concentrations.

4. Potential applications of biologically derived nanoparticles

The physiochemical characteristics of metal nanoparticles render them applicable across a genre of multi-disciplinary fields for a variety of uses including catalysis [128]; micro-electronics [129]; solar energy conversion [130] amongst many others [131]. They have also been recognised for their potential in a number of medical applications [132]. However, the use of nanoparticles derived from physical and chemical synthetic routes raises health and toxicity concerns due to the nature of the reaction conditions which may ultimately affect the properties of the derived particles [133].

Biologically derived nanoparticles provide a greener alternative to nanoparticles derived from the aforementioned routes since, the synthesis methods used to derive these particles are clean and non-toxic [9]. As a result, they are suitable for a number of biomedical applications (**Table 1**) including: cancer therapy; drug delivery; tumour detection; genetic disorder diagnosis; tissue repair; cell labelling; antimicrobial development; targeting and immunoassays and yet to be discovered applications [37, 114, 132, 134–136].

With respect to biologically derived AgNPs, their major exploitation exists in the development of antimicrobial agents due to their renowned microbial inhibitory activities and with the current status on antimicrobial drug resistance, these particles are being extensively sought after as possible alternatives to antibiotics [1, 8].

Plant	Applications	Reference
Moringa oleifera	Anti-microbial	[137]
Eclipta prostrata	Anti-protozoal	[138]
Gelidiella acerosa	Anti-fungal	[139]
Melia azedarach	Anti-cancer	[140]
Lampranthus coccineus	Anti-viral	[141]
Malephora lutea	Anti-Alzheimer	[142]
Melia azedarach	Wound healing	[143]
Ocimum sanctum	Anti-diabetic	[144]
Allium sativum	Antioxidant	[145]

Table 1.
Selective applications of silver nanoparticles synthesised using plant extracts.

5. Conclusion

In conclusion, it can be established that green synthetic strategies using plant and bacterial based extracts are promising alternatives to produce AgNPs. However, to produce AgNPs with enhanced bioactivities, morphological characteristics such as size and shape need to be finely tuned. Furthermore, the use of extracts with known medical value provides with attractive capping substrates that may potentially enhance the bioactivities of the produced particles.

Acknowledgements

This study was made possible through financial support from the National Research Foundation. The research facilities were provided by the University of KwaZulu-Natal.

Conflict of interest

Authors declare no conflict of interest.

Author details

Jerushka S. Moodley[1], Suresh Babu Naidu Krishna[2], Karen Pillay[1] and Patrick Govender[1*]

1 Department of Biochemistry, School of Life Sciences, University of KwaZulu Natal, Private Bag X54001, Durban, South Africa

2 Department of Biomedical and Clinical Technology, Durban University of Technology, Durban, South Africa

*Address all correspondence to: govenderpt@ukzn.ac.za

References

[1] Kim, J.S., et al., *Antimicrobial effects of silver nanoparticles.* Nanomedicine: Nanotechnology, Biology and Medicine, 2007. **3**(1): p. 95-101.

[2] Alexander, J.W., *History of the medical use of silver.* Surgical infections, 2009. **10**(3): p. 289-292.

[3] Peng, G., et al., *Nanosilver at the interface of biomedical applications, toxicology, and synthetic strategies*, in *Metal Nanoparticles for Drug Delivery and Diagnostic Applications*. 2020, Elsevier. p. 119-139.

[4] Ventola, C.L., *The antibiotic resistance crisis: part 1: causes and threats.* Pharmacy and Therapeutics, 2015. **40**(4): p. 277.

[5] Organization., W.H., *Antimicrobial resistance global report on surveillance: 2014 summary*. 2014, World Health Organization.

[6] Walsh, C., *Where will new antibiotics come from?* Nature Reviews Microbiology, 2003. **1**(1): p. 65-70.

[7] Projan, S.J., *Why is big Pharma getting out of antibacterial drug discovery?* Current Opinion in Microbiology, 2003. **6**(5): p. 427-430.

[8] Rai, M., A. Yadav, and A. Gade, *Silver nanoparticles as a new generation of antimicrobials.* Biotechnology Advances, 2009. **27**(1): p. 76-83.

[9] Ahmed, S., et al., *A review on plants extract mediated synthesis of silver nanoparticles for antimicrobial applications: A green expertise.* Journal of Advanced Research, 2016. **7**(1): p. 17-28.

[10] Ponarulselvam, S., et al., *Synthesis of silver nanoparticles using leaves of Catharanthus roseus Linn. G. Don and their antiplasmodial activities.* Asian Pacific Journal of Tropical Biomedicine, 2012. **2**(7): p. 574-580.

[11] Velusamy, P., et al., *Bio-inspired green nanoparticles: synthesis, mechanism, and antibacterial application.* Toxicological Research, 2016. **32**(2): p. 95.

[12] Biswas, A., et al., *Advances in top–down and bottom–up surface nanofabrication: Techniques, applications & future prospects.* Advances in colloid and interface science, 2012. **170**(1): p. 2-27.

[13] Thakkar, K.N., S.S. Mhatre, and R.Y. Parikh, *Biological synthesis of metallic nanoparticles.* Nanomedicine: Nanotechnology, Biology and Medicine, 2010. **6**(2): p. 257-262.

[14] El-Nour, K.M.A., et al., *Synthesis and applications of silver nanoparticles.* Arabian journal of chemistry, 2010. **3**(3): p. 135-140.

[15] Iravani, S., et al., *Synthesis of silver nanoparticles: chemical, physical and biological methods.* Research in pharmaceutical sciences, 2014. **9**(6): p. 385.

[16] Kim, S., et al., *Catalytic effect of laser ablated Ni nanoparticles in the oxidative addition reaction for a coupling reagent of benzylchloride and bromoacetonitrile.* Journal of Molecular Catalysis A: Chemical, 2005. **226**(2): p. 231-234.

[17] Chen, Y.-H. and C.-S. Yeh, *Laser ablation method: use of surfactants to form the dispersed Ag nanoparticles.* Colloids and Surfaces A: Physicochemical and Engineering Aspects, 2002. **197**(1): p. 133-139.

[18] Tsuji, T., et al., *Preparation of silver nanoparticles by laser ablation in solution: influence of laser wavelength on particle size.* Applied Surface Science, 2002. **202**(1): p. 80-85.

[19] Amin, S.A., M. Pazouki, and A. Hosseinnia, *Synthesis of TiO 2–Ag*

nanocomposite with sol–gel method and investigation of its antibacterial activity against E. coli. Powder Technology, 2009. **196**(3): p. 241-245.

[20] Yang, J. and J. Pan, *Hydrothermal synthesis of silver nanoparticles by sodium alginate and their applications in surface-enhanced Raman scattering and catalysis.* Acta Materialia, 2012. **60**(12): p. 4753-4758.

[21] Yin, B., et al., *Electrochemical synthesis of silver nanoparticles under protection of poly (N-vinylpyrrolidone).* The Journal of Physical Chemistry B, 2003. **107**(34): p. 8898-8904.

[22] Saion, E., E. Gharibshahi, and K. Naghavi, *Size-controlled and optical properties of monodispersed silver nanoparticles synthesized by the radiolytic reduction method.* International journal of molecular sciences, 2013. **14**(4): p. 7880-7896.

[23] Pillai, Z.S. and P.V. Kamat, *What factors control the size and shape of silver nanoparticles in the citrate ion reduction method?* The Journal of Physical Chemistry B, 2004. **108**(3): p. 945-951.

[24] Chou, K.-S. and C.-Y. Ren, *Synthesis of nanosized silver particles by chemical reduction method.* Materials Chemistry and Physics, 2000. **64**(3): p. 241-246.

[25] Kapoor, S., et al., *Reduction and aggregation of silver ions in aqueous gelatin solutions.* Langmuir, 1994. **10**(9): p. 3018-3022.

[26] Bai, J., et al., *One-pot synthesis of polyacrylamide-gold nanocomposite.* Materials Chemistry and Physics, 2007. **106**(2): p. 412-415.

[27] Song, K.C., et al., *Preparation of colloidal silver nanoparticles by chemical reduction method.* Korean Journal of Chemical Engineering, 2009. **26**(1): p. 153-155.

[28] Iravani, S., *Green synthesis of metal nanoparticles using plants.* Green Chemistry, 2011. **13**(10): p. 2638-2650.

[29] Patra, J.K. and K.-H. Baek, *Green nanobiotechnology: factors affecting synthesis and characterization techniques.* Journal of Nanomaterials, 2014. **2014**: p. 219.

[30] Dauthal, P. and M. Mukhopadhyay, *Noble metal nanoparticles: Plant-mediated synthesis, mechanistic aspects of synthesis, and applications.* Industrial & Engineering Chemistry Research, 2016. **55**(36): p. 9557-9577.

[31] Rajput, S., et al., *Fungal isolate optimized for biogenesis of silver nanoparticles with enhanced colloidal stability.* Langmuir, 2016. **32**(34): p. 8688-8697.

[32] Dhuper, S., D. Panda, and P. Nayak, *Green synthesis and characterization of zero valent iron nanoparticles from the leaf extract of Mangifera indica.* Nano Trends: J Nanotech App, 2012. **13**(2): p. 16-22.

[33] Mittal, A.K., Y. Chisti, and U.C. Banerjee, *Synthesis of metallic nanoparticles using plant extracts.* Biotechnology advances, 2013. **31**(2): p. 346-356.

[34] Mohanpuria, P., N.K. Rana, and S.K. Yadav, *Biosynthesis of nanoparticles: technological concepts and future applications.* Journal of Nanoparticle Research, 2008. **10**(3): p. 507-517.

[35] Makarov, V., et al., *"Green" nanotechnologies: synthesis of metal nanoparticles using plants.* Acta Naturae (англоязычная версия), 2014. **6**(1 (20)).

[36] Pantidos, N. and L.E. Horsfall, *Biological synthesis of metallic nanoparticles by bacteria, fungi and plants.* Journal of Nanomedicine & Nanotechnology, 2014. **5**(5): p. 1.

[37] Singh, P., et al., *Biological synthesis of nanoparticles from plants and microorganisms.* Trends in biotechnology, 2016.

[38] Harris, A.T. and R. Bali, *On the formation and extent of uptake of silver nanoparticles by live plants.* Journal of Nanoparticle Research, 2008. **10**(4): p. 691-695.

[39] Gardea-Torresdey, J., et al., *Formation and growth of Au nanoparticles inside live alfalfa plants.* Nano letters, 2002. **2**(4): p. 397-401.

[40] Manceau, A., et al., *Formation of metallic copper nanoparticles at the soil–root interface.* Environmental science & technology, 2008. **42**(5): p. 1766-1772.

[41] Pal, S., Y.K. Tak, and J.M. Song, *Does the antibacterial activity of silver nanoparticles depend on the shape of the nanoparticle? A study of the gram-negative bacterium Escherichia coli*. Applied and environmental microbiology, 2007. **73**(6): p. 1712-1720.

[42] Jeong, Y., D.W. Lim, and J. Choi, *Assessment of size-dependent antimicrobial and cytotoxic properties of silver nanoparticles.* Advances in Materials Science and Engineering, 2014. **2014**.

[43] Shankar, S.S., A. Ahmad, and M. Sastry, *Geranium leaf assisted biosynthesis of silver nanoparticles.* Biotechnology progress, 2003. **19**(6): p. 1627-1631.

[44] Shankar, S.S., et al., *Bioreduction of chloroaurate ions by geranium leaves and its endophytic fungus yields gold nanoparticles of different shapes.* Journal of Materials Chemistry, 2003. **13**(7): p. 1822-1826.

[45] Singh, A.K., et al., *Biosynthesis of gold and silver nanoparticles by natural precursor clove and their functionalization with amine group.* Journal of Nanoparticle Research, 2010. **12**(5): p. 1667-1675.

[46] Ahmad, N., et al., *Rapid synthesis of silver nanoparticles using dried medicinal plant of basil.* Colloids and Surfaces B: Biointerfaces, 2010. **81**(1): p. 81-86.

[47] Gannimani, R., et al., *Synthesis and antibacterial activity of silver and gold nanoparticles produced using aqueous seed extract of Protorhus longifolia as a reducing agent.* 2014.

[48] Veerasamy, R., et al., *Biosynthesis of silver nanoparticles using mangosteen leaf extract and evaluation of their antimicrobial activities.* Journal of Saudi Chemical Society, 2011. **15**(2): p. 113-120.

[49] Kasthuri, J., S. Veerapandian, and N. Rajendiran, *Biological synthesis of silver and gold nanoparticles using apiin as reducing agent.* Colloids and Surfaces B: Biointerfaces, 2009. **68**(1): p. 55-60.

[50] Panigrahi, S., et al., *General method of synthesis for metal nanoparticles.* Journal of Nanoparticle Research, 2004. **6**(4): p. 411-414.

[51] Tan, Y.N., J.Y. Lee, and D.I. Wang, *Uncovering the design rules for peptide synthesis of metal nanoparticles.* Journal of the American Chemical Society, 2010. **132**(16): p. 5677-5686.

[52] Glusker, J., A. Katz, and C. Bock, *Metal ions in biological systems.* The Rigaku Journal, 1999. **16**(2): p. 8-16.

[53] Singh, R., et al., *Bacteriagenic silver nanoparticles: synthesis, mechanism, and applications.* Applied microbiology and biotechnology, 2015. **99**(11): p. 4579-4593.

[54] Iravani, S., *Bacteria in nanoparticle synthesis: current status and future prospects.* International Scholarly Research Notices, 2014. **2014**.

[55] Pantidos, N. and L.E. Horsfall, *Biological synthesis of metallic nanoparticles by bacteria, fungi and plants.* Journal of Nanomedicine & Nanotechnology, 2014. **2014**.

[56] Shah, M., et al., *Green synthesis of metallic nanoparticles via biological entities.* Materials, 2015. **8**(11): p. 7278-7308.

[57] Pugazhenthiran, N., et al., *Microbial synthesis of silver nanoparticles by Bacillus sp.* Journal of Nanoparticle Research, 2009. **11**(7): p. 1811-1815.

[58] Kalimuthu, K., et al., *Biosynthesis of silver nanocrystals by Bacillus licheniformis.* Colloids and Surfaces B: Biointerfaces, 2008. **65**(1): p. 150-153.

[59] Shahverdi, A.R., et al., *Rapid synthesis of silver nanoparticles using culture supernatants of Enterobacteria: a novel biological approach*. Process Biochemistry, 2007. **42**(5): p. 919-923.

[60] Saifuddin, N., C. Wong, and A. Yasumira, *Rapid biosynthesis of silver nanoparticles using culture supernatant of bacteria with microwave irradiation*. Journal of Chemistry, 2009. **6**(1): p. 61-70.

[61] Gurunathan, S., et al., *Biosynthesis, purification and characterization of silver nanoparticles using Escherichia coli*. Colloids and Surfaces B: Biointerfaces, 2009. **74**(1): p. 328-335.

[62] Slawson, R.M., et al., *Germanium and silver resistance, accumulation, and toxicity in microorganisms.* Plasmid, 1992. **27**(1): p. 72-79.

[63] He, S., et al., *Biosynthesis of gold nanoparticles using the bacteria Rhodopseudomonas capsulata.* Materials Letters, 2007. **61**(18): p. 3984-3987.

[64] Sneha, K., et al., *Corynebacterium glutamicum-mediated crystallization of silver ions through sorption and reduction processes.* Chemical Engineering Journal, 2010. **162**(3): p. 989-996.

[65] Sintubin, L., et al., *Lactic acid bacteria as reducing and capping agent for the fast and efficient production of silver nanoparticles.* Applied microbiology and biotechnology, 2009. **84**(4): p. 741-749.

[66] Parikh, R.Y., et al., *Extracellular synthesis of crystalline silver nanoparticles and molecular evidence of silver resistance from Morganella sp.: towards understanding biochemical synthesis mechanism*. ChemBioChem, 2008. **9**(9): p. 1415-1422.

[67] Johnston, C.W., et al., *Gold biomineralization by a metallophore from a gold-associated microbe.* Nature chemical biology, 2013. **9**(4): p. 241-243.

[68] Kim, J., et al., *Peptide-mediated shape-and size-tunable synthesis of gold nanostructures.* Acta biomaterialia, 2010. **6**(7): p. 2681-2689.

[69] Si, S. and T.K. Mandal, *Tryptophan-Based Peptides to Synthesize Gold and Silver Nanoparticles: A Mechanistic and Kinetic Study.* Chemistry–A European Journal, 2007. **13**(11): p. 3160-3168.

[70] Ajayan, P.M., *Nanotechnology: How does a nanofibre grow?* Nature, 2004. **427**(6973): p. 402-403.

[71] Armendariz, V., et al., *Size controlled gold nanoparticle formation by Avena sativa biomass: use of plants in nanobiotechnology.* Journal of Nanoparticle Research, 2004. **6**(4): p. 377-382.

[72] Verma, A. and M.S. Mehata, *Controllable synthesis of silver nanoparticles using Neem leaves and their antimicrobial activity.* Journal of Radiation Research and Applied Sciences, 2016. **9**(1): p. 109-115.

[73] Kaviya, S., et al., *Biosynthesis of silver nanoparticles using Citrus sinensis peel extract and its antibacterial activity.* Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 2011. **79**(3): p. 594-598.

[74] Lin, L., et al., *Nature factory of silver nanowires: Plant-mediated synthesis using broth of Cassia fistula leaf.* Chemical Engineering Journal, 2010. **162**(2): p. 852-858.

[75] Karimi Zarchi, A., et al., *A sunlight-induced method for rapid biosynthesis of silver nanoparticles using an Andrachnea chordifolia ethanol extract.* Applied Physics A: Materials Science & Processing, 2011. **103**(2): p. 349-353.

[76] Rastogi, L. and J. Arunachalam, *Sunlight based irradiation strategy for rapid green synthesis of highly stable silver nanoparticles using aqueous garlic (Allium sativum) extract and their antibacterial potential.* Materials Chemistry and Physics, 2011. **129**(1): p. 558-563.

[77] Wei, X., et al., *Synthesis of silver nanoparticles by solar irradiation of cell-free Bacillus amyloliquefaciens extracts and AgNO 3.* Bioresource Technology, 2012. **103**(1): p. 273-278.

[78] Christian, P., et al., *Nanoparticles: structure, properties, preparation and behaviour in environmental media.* Ecotoxicology, 2008. **17**(5): p. 326-343.

[79] Martinez-Castanon, G., et al., *Synthesis and antibacterial activity of silver nanoparticles with different sizes.* Journal of Nanoparticle Research, 2008. **10**(8): p. 1343-1348.

[80] Morones, J.R., et al., *The bactericidal effect of silver nanoparticles.* Nanotechnology, 2005. **16**(10): p. 2346.

[81] Logeswari, P., S. Silambarasan, and J. Abraham, *Synthesis of silver nanoparticles using plants extract and analysis of their antimicrobial property.* Journal of Saudi Chemical Society, 2012. **19**(3): p. 311-317.

[82] Prasad, T. and E. Elumalai, *Biofabrication of Ag nanoparticles using Moringa oleifera leaf extract and their antimicrobial activity.* Asian Pacific Journal of Tropical Biomedicine, 2011. **1**(6): p. 439-442.

[83] Awwad, A.M., N.M. Salem, and A.O. Abdeen, *Green synthesis of silver nanoparticles using carob leaf extract and its antibacterial activity.* International Journal of Industrial Chemistry, 2013. **4**(1): p. 29.

[84] Sharma, V.K., R.A. Yngard, and Y. Lin, *Silver nanoparticles: green synthesis and their antimicrobial activities.* Advances in colloid and interface science, 2009. **145**(1): p. 83-96.

[85] Biemer, J.J., *Antimicrobial susceptibility testing by the Kirby-Bauer disc diffusion method.* Annals of Clinical & Laboratory Science, 1973. **3**(2): p. 135-140.

[86] Perez, C., M. Pauli, and P. Bazerque, *An antibiotic assay by the agar well diffusion method.* Acta Biol Med Exp, 1990. **15**(1): p. 113-5.

[87] Jagtap, U.B. and V.A. Bapat, *Green synthesis of silver nanoparticles using Artocarpus heterophyllus Lam. seed extract and its antibacterial activity.* Industrial Crops and Products, 2013. **46**: p. 132-137.

[88] Allahverdiyev, A.M., et al., *Coping with antibiotic resistance: combining nanoparticles with antibiotics and other antimicrobial agents.* Expert review of anti-infective therapy, 2011. **9**(11): p. 1035-1052.

[89] Eloff, J., *A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria.* Planta medica, 1998. **64**(08): p. 711-713.

[90] Schwalbe, R., L. Steele-Moore, and A.C. Goodwin, *Antimicrobial susceptibility testing protocols*. 2007: Crc Press.

[91] Marambio-Jones, C. and E.M. Hoek, *A review of the antibacterial effects of silver nanomaterials and potential implications for human health and the environment.* Journal of Nanoparticle Research, 2010. **12**(5): p. 1531-1551.

[92] Semeykina, A.L. and V.P. Skulachev, *Submicromolar Ag+ increases passive Na+ permeability and inhibits the respiration-supported formation of Na+ gradient in Bacillus FTU vesicles.* FEBS letters, 1990. **269**(1): p. 69-72.

[93] Dibrov, P., et al., *Chemiosmotic mechanism of antimicrobial activity of Ag+ in Vibrio cholerae.* Antimicrobial agents and chemotherapy, 2002. **46**(8): p. 2668-2670.

[94] Liau, S., et al., *Interaction of silver nitrate with readily identifiable groups: relationship to the antibacterialaction of silver ions.* Letters in applied microbiology, 1997. **25**(4): p. 279-283.

[95] Schreurs, W. and H. Rosenberg, *Effect of silver ions on transport and retention of phosphate by Escherichia coli*. Journal of Bacteriology, 1982. **152**(1): p. 7-13.

[96] Ninganagouda, S., et al., *Growth kinetics and mechanistic action of reactive oxygen species released by silver nanoparticles from Aspergillus niger on Escherichia coli*. BioMed research international, 2014. **2014**.

[97] Stohs, S. and D. Bagchi, *Oxidative mechanisms in the toxicity of metal ions.* Free radical biology and medicine, 1995. **18**(2): p. 321-336.

[98] Park, H.-J., et al., *Silver-ion-mediated reactive oxygen species generation affecting bactericidal activity.* Water research, 2009. **43**(4): p. 1027-1032.

[99] Inoue, Y., et al., *Bactericidal activity of Ag–zeolite mediated by reactive oxygen species under aerated conditions.* Journal of inorganic biochemistry, 2002. **92**(1): p. 37-42.

[100] Sondi, I. and B. Salopek-Sondi, *Silver nanoparticles as antimicrobial agent: a case study on E. coli as a model for Gram-negative bacteria.* Journal of colloid and interface science, 2004. **275**(1): p. 177-182.

[101] Smetana, A.B., et al., *Biocidal activity of nanocrystalline silver powders and particles.* Langmuir, 2008. **24**(14): p. 7457-7464.

[102] Stoimenov, P.K., et al., *Metal oxide nanoparticles as bactericidal agents.* Langmuir, 2002. **18**(17): p. 6679-6686.

[103] Hamouda, T. and J. Baker, *Antimicrobial mechanism of action of surfactant lipid preparations in enteric Gram-negative bacilli*. Journal of applied microbiology, 2000. **89**(3): p. 397-403.

[104] Sondi, I., D.V. Goia, and E. Matijević, *Preparation of highly concentrated stable dispersions of uniform silver nanoparticles.* Journal of colloid and interface science, 2003. **260**(1): p. 75-81.

[105] Amro, N.A., et al., *High-resolution atomic force microscopy studies of the Escherichia coli outer membrane: structural basis for permeability.* Langmuir, 2000. **16**(6): p. 2789-2796.

[106] Dipankar, C. and S. Murugan, *The green synthesis, characterization and evaluation of the biological activities of silver nanoparticles synthesized from Iresine herbstii leaf aqueous extracts.* Colloids and Surfaces B: Biointerfaces, 2012. **98**: p. 112-119.

[107] Kim, K.-J., et al., *Antifungal activity and mode of action of silver nano-particles on Candida albicans.* Biometals, 2009. **22**(2): p. 235-242.

[108] Nasrollahi, A., K. Pourshamsian, and P. Mansourkiaee, *Antifungal activity of silver nanoparticles on some of fungi*. International Journal of Nano Dimension, 2011. **1**(3): p. 233-239.

[109] Endo, M., et al., *Fungicidal action of aureobasidin A, a cyclic depsipeptide antifungal antibiotic, against Saccharomyces cerevisiae.* Antimicrobial agents and chemotherapy, 1997. **41**(3): p. 672-676.

[110] Carlson, C., et al., *Unique cellular interaction of silver nanoparticles: size-dependent generation of reactive oxygen species.* The journal of physical chemistry B, 2008. **112**(43): p. 13608-13619.

[111] Elgorban, A.M., et al., *Antifungal silver nanoparticles: synthesis, characterization and biological evaluation.* Biotechnology & Biotechnological Equipment, 2016. **30**(1): p. 56-62.

[112] Ravishankar Rai, V. and A. Jamuna Bai, *Nanoparticles and their potential application as antimicrobials.* Science against microbial pathogens, communicating current research and technological advances. Formatex, Badajoz, 2011: p. 197-209.

[113] Chen, X. and H. Schluesener, *Nanosilver: a nanoproduct in medical application.* Toxicology letters, 2008. **176**(1): p. 1-12.

[114] Ahamed, M., M.S. AlSalhi, and M. Siddiqui, *Silver nanoparticle applications and human health*. Clinica chimica acta, 2010. **411**(23): p. 1841-1848.

[115] Oberdörster, G., et al., *Translocation of inhaled ultrafine particles to the brain.* Inhalation toxicology, 2004. **16**(6-7): p. 437-445.

[116] Rahman, M., et al., *Expression of genes related to oxidative stress in the mouse brain after exposure to silver-25 nanoparticles.* Toxicology letters, 2009. **187**(1): p. 15-21.

[117] Zhang, T., et al., *Cytotoxic potential of silver nanoparticles.* Yonsei medical journal, 2014. **55**(2): p. 283-291.

[118] Loeschner, K., et al., *Distribution of silver in rats following 28 days of repeated oral exposure to silver nanoparticles or silver acetate.* Particle and fibre toxicology, 2011. **8**(1): p. 18.

[119] Walczak, A.P., et al., *Behaviour of silver nanoparticles and silver ions in an in vitro human gastrointestinal digestion model.* Nanotoxicology, 2012. **7**(7): p. 1198-1210.

[120] Kim, S. and D.Y. Ryu, *Silver nanoparticle-induced oxidative stress, genotoxicity and apoptosis in cultured cells and animal tissues.* Journal of Applied Toxicology, 2013. **33**(2): p. 78-89.

[121] Gliga, A.R., et al., *Size-dependent cytotoxicity of silver nanoparticles in human lung cells: the role of cellular uptake, agglomeration and Ag release.* Particle and fibre toxicology, 2014. **11**(1): p. 11.

[122] Hackenberg, S., et al., *Silver nanoparticles: evaluation of DNA damage, toxicity and functional impairment in human mesenchymal stem cells.* Toxicology letters, 2011. **201**(1): p. 27-33.

[123] Samberg, M.E., et al., *Silver nanoparticles do not influence stem cell differentiation but cause minimal toxicity.* Nanomedicine, 2012. **7**(8): p. 1197-1209.

[124] Liu, W., et al., *Impact of silver nanoparticles on human cells: effect of particle size.* Nanotoxicology, 2010. **4**(3): p. 319-330.

[125] Kim, T.H., et al., *Size-dependent cellular toxicity of silver nanoparticles.*

Journal of Biomedical Materials Research Part A, 2012. **100**(4): p. 1033-1043.

[126] Panda, K.K., et al., *In vitro biosynthesis and genotoxicity bioassay of silver nanoparticles using plants.* Toxicology in vitro, 2011. **25**(5): p. 1097-1105.

[127] Lima, R., A.B. Seabra, and N. Durán, *Silver nanoparticles: a brief review of cytotoxicity and genotoxicity of chemically and biogenically synthesized nanoparticles.* Journal of Applied Toxicology, 2012. **32**(11): p. 867-879.

[128] Crooks, R.M., et al., *Dendrimer-encapsulated metals and semiconductors: synthesis, characterization, and applications*, in *Dendrimers III*. 2001, Springer. p. 81-135.

[129] Gittins, D.I., et al., *A nanometre-scale electronic switch consisting of a metal cluster and redox-addressable groups.* Nature, 2000. **408**(6808): p. 67-69.

[130] Kamat, P.V., *Meeting the clean energy demand: nanostructure architectures for solar energy conversion.* The Journal of Physical Chemistry C, 2007. **111**(7): p. 2834-2860.

[131] Fedlheim, D.L. and C.A. Foss, *Metal nanoparticles: synthesis, characterization, and applications*. 2001: CRC press.

[132] Rai, M., et al., *Strategic role of selected noble metal nanoparticles in medicine.* Critical reviews in microbiology, 2016. **42**(5): p. 696-719.

[133] Baker, S., et al., *Plants: emerging as nanofactories towards facile route in synthesis of nanoparticles.* BioImpacts: BI, 2013. **3**(3): p. 111.

[134] Khlebtsov, N. and L. Dykman, *Biodistribution and toxicity of engineered gold nanoparticles: a review of in vitro and in vivo studies.* Chemical Society Reviews, 2011. **40**(3): p. 1647-1671.

[135] Huang, X., et al., *Gold nanoparticles: interesting optical properties and recent applications in cancer diagnostics and therapy.* Nanomedicine, 2007. **2**(5): p. 681-693.

[136] Bhadra, M.P., B. Sreedhar, and C.R. Patra, *Potential theranostics application of bio-synthesized silver nanoparticles (4-in-1 system).* 2014.

[137] Prasad, T. and E. Elumalai, *Biofabrication of Ag nanoparticles using Moringa oleifera leaf extract and their antimicrobial activity.* Asian Pacific Journal of Tropical Biomedicine, 2011. **1**(6): p. 439.

[138] Rajakumar, G. and A.A. Rahuman, *Larvicidal activity of synthesized silver nanoparticles using Eclipta prostrata leaf extract against filariasis and malaria vectors.* Acta tropica, 2011. **118**(3): p. 196-203.

[139] Vivek, M., et al., *Biogenic silver nanoparticles by Gelidiella acerosa extract and their antifungal effects.* Avicenna Journal of Medical Biotechnology, 2011. **3**(3): p. 143.

[140] Sukirtha, R., et al., *Cytotoxic effect of Green synthesized silver nanoparticles using Melia azedarach against in vitro HeLa cell lines and lymphoma mice model.* Process Biochemistry, 2012. **47**(2): p. 273-279.

[141] Haggag, E.G., et al., *Antiviral potential of green synthesized silver nanoparticles of Lampranthus coccineus and Malephora lutea.* International journal of nanomedicine, 2019. **14**: p. 6217-6229.

[142] Youssif, K.A., et al., *Anti-Alzheimer potential, metabolomic profiling and molecular docking of green synthesized silver nanoparticles of Lampranthus*

coccineus and Malephora lutea aqueous extracts. PLoS One, 2019. **14**(11): p. e0223781.

[143] Chinnasamy, G., S. Chandrasekharan, and S. Bhatnagar, *Biosynthesis of Silver Nanoparticles from Melia azedarach: Enhancement of Antibacterial, Wound Healing, Antidiabetic and Antioxidant Activities.* Int J Nanomedicine, 2019. **14**: p. 9823-9836.

[144] Malapermal, V., et al., *Enhancing antidiabetic and antimicrobial performance of Ocimum basilicum, and Ocimum sanctum (L.) using silver nanoparticles.* Saudi Journal of Biological Sciences, 2017. **24**(6): p. 1294-1305.

[145] Otunola, G.A., et al., *Characterization, Antibacterial and Antioxidant Properties of Silver Nanoparticles Synthesized from Aqueous Extracts of Allium sativum, Zingiber officinale, and Capsicum frutescens.* Pharmacognosy magazine, 2017. **13**(Suppl 2): p. S201-S208.