



Green synthesis of metallic nanoparticles as effective alternatives to treat antibiotics resistant bacterial infections: A review



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ABSTRACT

Due to development of bacterial resistance to the conventional antibiotics, the treatment of bacterial infections has become a major issue of concern. The unprescribed and uncontrolled use of antibiotics has led to the rapid development of antibiotic resistance in bacterial strains. Therefore, the development of novel and potent bactericidal agents is of great clinical importance. Interestingly, metallic nanoparticles (NPs) have been proven to be promising alternative to antibiotics. NPs interact with the important cellular organelles and biomolecules like DNA, enzymes, ribosomes, and lysosomes that can affect cell membrane permeability, oxidative stress, gene expression, protein activation, and enzyme activation. Since, NPs target multiple biomolecules concurrently; it becomes very difficult for bacteria to develop resistance against them. Currently, there are different physical and chemical methods utilized for NPs synthesis. However, most of these processes are costly and potentially hazardous for the living organisms and environment. Therefore, there is a need to develop an eco-friendly and cost-effective method of synthesis. Recently, the 'green synthesis' approaches are gaining a lot of attention. It is demonstrated that living organisms like bacteria, yeast, fungi, and plant cells can reduce inorganic metal ions into metal NPs by their cellular metabolites. Both the yield and stability of biogenic NPs are quite satisfactory. In the current article, we have addressed the green synthesis of various metal NPs reported till date and highlighted their different modes and mechanisms of antibacterial properties. It is highly anticipated that biogenic metallic NPs could be viable and economical alternatives for treating drug resistant bacterial infections in near future.

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1. Introduction

The discovery of antibiotics is one of the great and remarkable achievements in the medical field. They have become indispensable in front line medical procedures like surgery, organ transplantation and treatment of various bacterial infections. Unfortunately the rampant use, particularly the misuse of antibiotics causes the development of antibiotic resistance in bacterial species. Such dramatic increase in bacterial resistance to antibiotics is now threatening the therapeutic achievement. As per World Health Organization report, antibiotic resistance has become one of the leading public health threats of 21st century [1]. Both the inhibition of enzyme activity and efficacy of efflux pumps are the main defensive strategies for bacterial cells to become less

susceptible to antibiotics [2]. Since the available antibiotics have become less effective or ineffective against particular bacterial species, it is becoming very tough to treat several bacterial infections.

Nanotechnology has come forth with a fruitful solution for the serious issue of bacterial resistance to antibiotics. NPs can bind efficiently to the bacterial surface and rupture their cell wall that further leads to cell death [2]. It was noticed that NPs with size less than 20 nm can penetrate the bacterial cell wall and in-turn hamper the biochemical pathways through destruction of the cell organelles which ultimately leads to death of bacteria [3]. Biogenic nanoparticles are properly capped with natural flavonoids which inhibit the enzymatic activity that hampers the synthesis of nucleic acids in several micro-organisms [4]. NPs are known to generate reactive oxygen species (ROS) which causes mechanical damage to the bacterial cell membrane [5]. It is reported that NPs can be recycled for repetitive use as antimicrobial agents [6]. It is very difficult for bacteria to develop resistance against NPs, since NPs target several cellular pathways at a time [7]. Therefore, NPs

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can be a great substitute to the conventional antibiotics to treat antibiotic resistant bacterial infections.

The unique properties like large surface area, stability, exceptional mechanical strength and lower melting points make the NPs highly compatible for various clinical applications like drug delivery, cancer therapy, biofilm inhibition and treatment of microbial infections [8]. The property of NPs to hold both the hydrophilic & hydrophobic substances makes them suitable drug carriers [9]. Nanomaterials are fabricated by different physical and chemical methods. However, high energy requirements, complex instrumental design, high expensiveness and low-yield are few crucial shortcomings associated with physical approach [10,11]. The chemical method of synthesis is more economical and provides high yield with simple experimental setup [12]. However, chemical method involves the use of toxic and volatile chemical reactants which are very hazardous and harmful for environment [13]. Due to the high vapour pressure, volatile solvents like aromatic amines and thiols cause air pollution [14]. Uses of reducing agents like sodium borohydride and hydrazine derivatives are highly noxious for the environment [15]. These facts intrigued the researchers to develop alternative routes which are eco-friendly and sustainable for synthesis of NPs. Therefore, the green protocols for synthetic purposes are gaining significant acceptance from scientific community [16].

Currently, synthesis of biogenic NPs has attracted a great deal of attention for its cost-effectiveness, environmental benignity and relative ease of synthesis [17,18]. In this process, cellular extract of living organisms like bacteria, algae, fungi, actinomycetes, and plants are employed as green reaction media as well as capping agents [19]. Aqueous biological extracts have become suitable media for NPs synthesis due to their non-toxic and nonvolatile nature. Biological extracts are generally mixture of different kinds of active biomolecules like proteins, carbohydrates, vitamins, polymers and natural surfactants that provide high stability and enhanced dispersity to the synthesized NPs [20].

Recently, many review articles dealing with the applications of biogenic NPs for inhibiting pathogenic microorganisms have been published. Duran et al. published a review article which focused on antimicrobial potency of silver NPs [21]. The review article published by Ahmad et al. highlighted the plant extract mediated synthesis of silver NPs and their use in antimicrobial applications [22]. Some other articles were also published to mainly focus on the antimicrobial activity of Ag NPs and Zn NPs [23,24]. In the present review, we have tried our level best to summarize and compile the recent research works dealing with the various modes of green synthesis of NPs. Simultaneously, the applications of various biogenic metallic NPs like silver, gold, zinc, iron, palladium, selenium, cerium and tellurium in the treatment of antibiotics resistant bacterial infections have been critically discussed.

2. Modes of synthesis and purification of biogenic NPs

2.1. Fungi assisted synthesis of nanoparticles (NPs)

Fungi are good sources of secondary metabolites and active biomolecules that are very essential for the synthesis of NPs. Some fungal species like *F. oxysporum* secrete proteins, polymers and enzymes that voluntarily help in producing metal NPs [25]. These constituents improve the yield and stability of NPs. In other reports, it was found that several fungal species have capability to synthesize NPs using extracellular amino acid residues. For instance, yeast surface contain glutamic acid and aspartic acid that photo reduces silver ions into silver metal in presence of adequate amount of light [26]. Ahmad et al. (2003) noticed that fungal species like *F. oxysporum* has reductase enzyme in cytosol which reduces silver ions into silver metal in presence of NADH^+ , a

reducing component [27]. Phytochelatin group of compounds which are found mainly in fungus have high capability to reduce silver ion into silver metal [28]. Sanghi and Verma (2009) utilized culture supernatant of fungal species *Coriolus versicolor* to synthesize silver NPs (Ag NPs). In this study, FTIR data confirmed the occurrence of hydroxyl group in the fungal mycelium which donated electrons to silver ion and reduced it into elemental silver to form Ag NPs. It also confirmed the presence of aliphatic and aromatic amines and some proteins in the fungal extract that acted upon as capping agents to stabilize the formed Ag NPs. Furthermore, it was demonstrated that it stabilized silver metal by binding with protein through amide bond [29]. Tan et al. (2002) also reported the involvement of SH group containing protein from fungal extract in the capping and stabilization of Ag NPs [30].

Das et al. (2009) had used mycelia of *R. oryzae* for synthesis of nano conjugate of gold (Au) NPs through *in situ* reduction of chloroauric acid (HAuCl_4) in acidic medium (pH 3) [31]. *Verticillium* fungus is also a good mediator for synthesis of silver NPs. Recently, it has been found that biomass of fungi intracellularly synthesized NPs on exposure of AgNO_3 in acidic medium (pH 5.5–6) [32]. The pictorial representation for green synthesis of various metallic nanoparticles and the mechanism of their anti-bacterial properties is illustrated in Fig. 1.

2.2. Bacteria assisted synthesis of NPs

Bacteria assisted synthesis of NPs takes place in two ways: extracellular and intracellular approaches. Extracellular synthesis of NPs has advantage over intracellular method in terms of being less time-consuming, since it does not need any downstream process for collection of NPs from the organisms [33].

Bacteria contains reductase enzyme inside the cell that catalyze the reduction of metal ions into metal NPs. Bacterial species like *D. radiodurans* has great antioxidant activity and is highly resistant to radiation and oxidative stress [34]. It makes it favourable for use in green synthesis of Au NPs from its ionic form. The fabricated Au NPs were stable for a longer time and showed better antimicrobial activity.

In recent study, Kunoh Tatsuki et al. (2017) used *Leptothrix* bacteria for synthesis of Au NPs by reducing gold salt in aqueous medium [35]. It was noted that that gold salt was reduced by guanine residues of RNA molecules and 2-deoxy guanosine.

2.3. Plant assisted synthesis

Plant assisted synthesis of NPs is more efficient in terms of obtaining a higher yield than the microbial synthesis. Plants have several metabolites and biochemicals (eg. polyphenols) that can function as stabilizing as well as reducing agent in the synthesis of biogenic NPs. Plant mediated synthesis of NPs is eco-friendly (avoiding use of toxic chemicals) and economical. NPs synthesized from plant sources were found to be much more stable than those formed by microbes and fungus [33]. Plant mediated synthesis of NPs can be classified into three groups, viz. extracellular, intracellular and through phytochemicals. Extracellular method is employed when plant extract is used as raw material for NP synthesis. Intracellular synthesis of NPs takes place inside the cells of plant tissue by utilization of cellular enzymes. Post synthesis, the NPs are recovered by rupturing the cell wall of the plant cells. Synthesis of NPs from plant extract is comparatively a cheaper method and it results in higher yield due to presence of larger amount of phytochemicals in plant extract that can either stabilize or reduce the metal ions into metal NPs [36]. Phytochemical mediated synthesis of NPs is not a common process since it requires knowledge of the particular phytochemical needed for synthesis of stabilized NPs [37]. Shakeel Ahmed

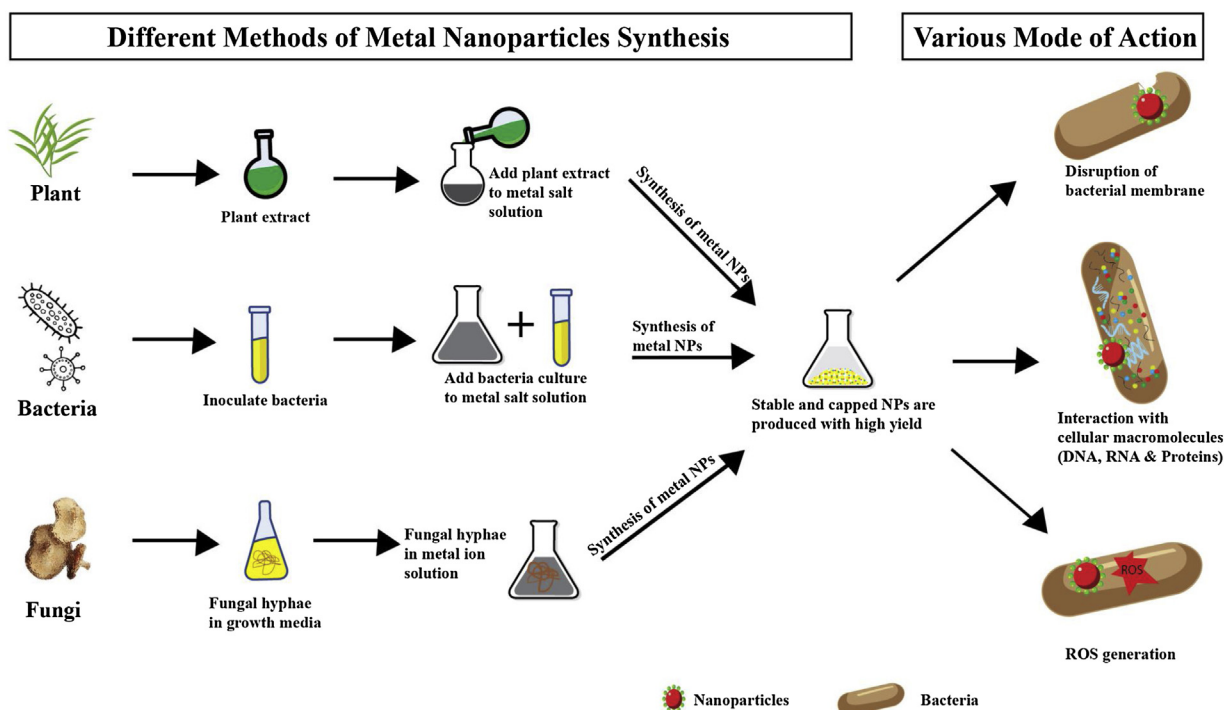


Fig. 1. Pictorial representation for green synthesis of metallic nanoparticles from plant, bacteria and fungi. The various mechanisms of bacterial cell death is also illustrated (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

et al. (2015) synthesized spherical shaped Ag NPs from leaf extract of *A. indica*. The FTIR study demonstrated that flavonoids and phytochemical compounds of the plant extract work as reducing and stabilizing agent during the synthesis of NPs. These NPs exhibited potent antimicrobial activity [38]. T.Y Suman et al. (2013) extracted

Au NPs from root extract of *M. citrifolia* which were also found to be antimicrobial in nature [39].

2.4. Purification of biogenic NPs

Upon synthesis, it is very essential to properly purify the biogenic NPs before employing them in any applications. Since long time, researchers have been using the method of centrifugation to purify the nanoparticles due to ease of operation and less time consuming. Thus, repetitive washing and high speed centrifugation are applied for the separation and purification of biologically produced metallic nanoparticles in order to eliminate the unreacted bioactive molecules [37]. However, this method is having several limitations such as centrifugation may cause agglomeration of NPs, destabilization of NPs due to detachment of the surface passivating agents from NPs and change in the intrinsic property of the NPs. Another very simple method of purification is the method of dialysis by using suitable cut off membrane. Small organic molecules present in the plant extracts can be easily pass through the dialysis membrane, whereas organic molecules present as surface passivating agents remain intact and conjugated with NPs inside the dialysis membrane. However, this method of purification is time consuming and it generally takes more than 24 h. However, the technique of diafiltration is not used for biofabricated nanoparticles as they are insoluble in water. Magnetic nanoparticles like Fe_2O_3 and Fe_3O_4 are easily separated by applying external magnetic force. However, the removal of tightly held biomolecules over the surface of nanoparticles is still a challenge.

3. Factors influencing the synthesis of various NPs

3.1. Temperature

Significant amount of research works are being undertaken worldwide to study the regulation of temperature over NPs. Temperature is one of the most influencing factors that affect the size and shape (morphology) of the NPs and their rate of synthesis. The various shapes (triangle, octahedral platelets, spherical, and rod like), size and synthesis of NPs are dependent on temperature. As temperature increases, there is an increase in reaction rate and formation of nucleation centres [40]. The research group headed by Sneha et al. (2010) evaluated the effect of temperature on morphology of the Au NPs synthesized from Piper betel leaf extract [40]. It was observed that triangular shaped NPs were formed at 20 °C, whereas octahedral shaped NPs with sizes ranging from 5 to 500 nm were fabricated at temperature ranging from 30 to 40 °C. Further, at higher temperature (50–60 °C), the sizes of the NPs were more consistent and spherical in shape. In another notable work, the biological production of Ag NPs from *P. eldarica* bark extract at different temperatures has been reported by Iravani and Zolfaghari (2013) [41]. The synthesis was done at various (25, 50, 100 and 150 °C) temperatures. The findings concluded that the size of the NPs decreased with the rise in temperature while their yield was found to be better. This fact was well supported by scanning electron micrographs of NPs. Fleitas-Salazar et al. (2017) studied the impact of oxidizing atmosphere and temperature on the synthesis of Ag NPs using a biocompatible polymer PEG [42]. The study deciphered that the ability of PEG to reduce silver salt was high at 100 °C. They concluded that at 100 °C the functional groups of PEG actively interacted with Ag molecules that resulted in the formation of more stable Ag NPs. At 60 °C, reduction of Ag ions occurred through oxidation of hydroxyl groups present in PEG. Thus, the authors reported that there are different mechanisms for Ag NP synthesis at various temperatures. Islam et al. (2011) standardized the synthesis

protocol of Au NPs from silver-ammonia complex in aqueous solution of poly(ethylene oxide)-poly(propylene oxide) at different temperatures. It was inferred that at lower temperature, the size and distribution of NPs is controlled by change in morphology of polymer, but at higher temperature it is controlled by change in polymeric chemical composition [43]. Tan et al. (2015) analyzed the effect of temperature on encapsulation and formation of Au NPs with the use of PNIPAm/PEI as template. The TEM analysis revealed that the most optimum encapsulation of Au NPs and that of formation of stable Au-PNIPAm/PEI composite particles had taken place between 25 °C–30 °C and there was homogeneous distribution of particles around the template. But at lower temperature (15 °C), the encapsulation was very poor [44]. In another research work dealt with Manganese doped cobalt ferrite NPs synthesis [45], it was demonstrated that the size of the NPs increases with increase in temperature.

3.2. pH

The pH of reaction plays a pivotal role in the formation of NPs. Like temperature, pH also regulates the formation of nucleation centres. As the pH increases, number of nucleation centres also increases, leading to enhanced formation of metallic NPs. It has been established that pH takes an important role in formulating the morphology as well as size of the NPs. Armendariz et al. (2004) studied the synthesis of Au NPs from *A. sativa* at different pH. It was noticed that at lower pH (pH 2), very less amount of NPs were formed but the size of the NPs was considerably larger (25–85 nm). They postulated that at lower pH, Au NPs do not form more nucleation centres, so they aggregate to form larger sized NPs [46]. On the contrary, at slightly higher pH (pH 3–4), small sized NPs were formed. Another work was done by Fan et al. on pH dependent NPs response. They evaluated the release of Camptothecin loaded NIPAm poly-(N-isopropylacrylamide)/ chitosan NPs on tumors. The authors noticed that the loaded NPs show more release to target when NIPAm and chitosan ratio is 4:1(w/w). It was found that the release rate of Camptothecin was optimum at pH 6.8 whereas; it decreased on increasing or decreasing the pH, at 37 °C. Another novel research work on the effects of pH on the size and their average aspect ratio of gold nanorods was carried out by Okitsu et al. (2009) [47]. It was observed that the size and aspect ratio decreases with increase in pH.

3.3. Reaction time

Along with the temperature and pH, reaction time was also found to be one of the major factors that influence the morphology of NPs. A notable research work to study the effect of reaction time on magnetic NPs was performed by Karade et al. (2018) [48]. Fe NPs were synthesized from Ferric nitrate solution using Green tea extract. It was found that the reaction time influences the magnetic as well as structural properties of magnetic NPs. With the increase in reaction time, the particle size increases from 7.5 nm to 12 nm. It was also found that increase in reaction time can improve the saturation magnetization (Ms) of NPs. Simultaneously, the effects of reaction time on the particle size of cadmium selenide NPs was studied [49]. The measured particle sizes were found to be 15.8, 10.5, 6.7 and 111.7 nm at 4, 8, 12 and 16 h of reaction, respectively. Thus, it was concluded that the particle size of cadmium selenide NPs decreases with increase in reaction time. It was proposed that the unusual increase in size at 16 h was due to aggregation of particles. In another work, the effect of reaction time on particle size of ZnO and Cerium doped ZnO was investigated by Flor et al. (2004) [50]. The authors observed the linear increase in particle size with increase in reaction time. It was also noticed that the Cerium doped particle size is bigger as compared to normal ZnO at

constant reaction time. Another notable research work was done to investigate and understand the influence of reaction time on size, reduction and stability of Au NPs synthesized from oil palm extract (*E. guineensis*) [51]. It was found that the rate of reduction for Ag NPs formation was increasing with the increase in reaction time.

4. Antimicrobial activities of different metallic NPs

4.1. Antimicrobial activity of Ag NPs

Silver is being widely used as an antimicrobial agent since ancient times. Presently it is being employed as a potent antibacterial agent for wound dressing. Although antibiotics are extensively used in medical field, their prolonged exposure enables the bacteria to develop resistance. This may be the result of bacteria's self-defense mechanism due to which mutation in genes might occur. This facilitates the formation of enzymes that inactivate antibiotics [52]. Thus, antibiotic resistance in bacteria has become a major issue of concern. To overcome this challenge, scientists have reported several new approaches; antimicrobial activity of NPs being one of them. Amongst various NPs, silver is the most potent antibacterial agent. Besides, Ag NPs are used as nano carriers for drugs and antibiotics which help in enhancing the activity of antibiotics against resistant microbes [53,54].

The antibacterial activity of Ag NPs depends upon their size and shape. Pal et al. (2007) discovered that if size of Ag NPs decreases, their surface area increases that lead to enhanced binding affinity with molecules. It was also noticed that a triangular shaped Ag NP showed a much more pronounced antimicrobial activity in comparison to a spherical or a rod shaped one [55].

Ag NPs generate reactive oxygen species (ROS) that are responsible for oxidation of cellular components i.e. cellular DNA and protein [56]. Upon oxidation of these components, cell becomes unstable at physiological and genetic level causing failure of metabolism and cell division process. Ag NPs affect the signal transduction in microbial cells of *E. coli* through the alteration of phosphorylation states of tyrosine [57]. Ag NPs also affect biofilm formation by preventing its growth. Ag NPs bind to the membrane of the cells and alter its permeability by changing the membrane potential [57]. Once it enters the cell, these Ag NPs get converted into silver ions and start interacting with cellular biomolecules that cause damage to the cells. It hinders DNA replication by binding with DNA [58]. It interferes with the cell division process by binding with membrane proteins and cellular proteins that assist cell division. [59].

It was found that Ag NPs are more successful against Gram-negative bacteria than Gram-positive ones. This is because the former are capsulated with lipopolysaccharides possessing negative charge which binds with positively charged silver. Contrarily, Gram-positive bacteria are covered by thick layer of peptidoglycans and linear polysaccharides that are cross-linked with each other by embedded proteins, providing rigidity to the cell and thus preventing the binding of NPs to its surface. When silver ions bind with Gram-negative bacteria, holes are created in the cell wall which enables the penetration of Ag NP inside the cell [60].

Kim Soo-Hwan et al. (2011) studied the effect of Ag NPs on *S. aureus* and *E. coli* at various temperature and pH. They concluded that change in temperature and pH did not affect its antimicrobial activity; Ag NPs are highly active at long range of temperature and pH. It was found that these NPs affected cellular morphology, membrane proteins and respiratory enzymes [61].

4.2. Antimicrobial activity of Fe NPs

Like Ag and Au NPs, nano-sized biogenic iron was proven to be a potent anti-microbial agent. The semi-crystalline biogenic iron

oxide nanoparticles (Fe NPs) of size 80–100 nm, that were developed from *T. procumbens* leaf extract demonstrated bactericidal potency against a Gram-negative bacteria like *P.aeruginosa* [62]. Naseem and Farrukh (2015) reported the *G. jasminoides* and *L. inermis* extract mediated production of Fe NPs and studied their repressive effect against *S. aureus*, *S. enterica*, *P. mirabilis* and *E. coli*. Both NPs successfully inhibited the microbial growth under test [63]. Recently, Alam et al. (2019) used *Skimmia laureola* extract for the synthesis of poly-dispersed Fe NPs which showed remarkable antibacterial activity against a wild pathogen *R. solanacearum* through disintegration of cell wall [64]. In another effort, Fe NPs synthesized from *M. oleifera* leaf extract (Aisida et al. 2019) [65] demonstrated antibacterial activity against *P. aeruginosa*, *E. coli*, *S. aureus*, *S. typhi* and *P. multocida*. In another praiseworthy attempt, bioreductive preparation of Fe NPs was reported by using root and leaf extracts of *A. conyzoides* (Madivoli et al.) [66]. Prepared NPs showed astonishing antibacterial potential against both Gram-positive and Gram-negative bacteria. The NPs exhibited maximum activity against *P. aeruginosa* as compared to *E. coli*, *B. subtilis*, *S. aureus* and *C. albicans*. It was perceived that the killing of bacterial strains occurred due to the physical interaction between bacterial cells and NPs. The positively charged NPs easily get attached to the surface of negatively charged bacterial cells that result in rupture of cell wall followed by cell death [66]. Another group synthesized biocompatible Fe NPs from the peel extract of *P. granatum* which exhibited efficient antibacterial effects against *P.aeruginosa* [67]. Khalil et al. (2017) synthesized Fe NPs using aqueous extract of *Sageretia thea* and found its applicability to inhibit growth of *P. aeruginosa*, *S. epidermidis*, *B. subtilis*, *E. coli*, and *K. pneumoniae*. Among them, *P. aeruginosa* and *S. epidermidis* were most susceptible with minimum inhibitory concentration of 7.8 µg/L [68]. Rajiv et al. (2018) biologically produced highly stable Fe NPs from *L. camara* plant extract for inhibition of some pathogenic bacteria. Antibacterial assay showed that the formed NPs effectively inhibited *Pseudomonas* species, *Klebsiella* and *Staphylococcus* [69]. In another work, *E. crassipes* leaf extract were utilized for bio-manufacturing rod shaped Fe NPs which showed good inhibitory effect against *S. aureus* and *P. fluorescens* [69]. Antibacterial assay depicted that 100 µg/ml aqueous Fe NPs showed maximum efficiency against the bacterial strains under investigation. Groiss et al. (2016) prepared spherical shaped and crystalline Fe NPs through bioreduction induced by *C. ramiflora* leaf extract and examined its bactericidal potential against *E. coli* and *S. epidermidis*. Antibacterial assay demonstrated that both the bacterial strains were considerably inhibited at 37 °C [70]. In another approach, *C. guianensis* fruit extract mediated Fe NPs were fabricated and efficiently applied as an inhibitor for some pathogenic microorganisms. Dose-dependent disc diffusion experiment elucidated that the bio-modulated Fe NPs exhibit better antibacterial activity against Gram-negative bacteria like *K. pneumoniae* MTCC 530, *E. coli* MTCC 2939, and *S. typhi* MTCC 3917 than Gram-positive *S. aureus* MTCC 96 [71]. Rufuse et al. (2017) synthesized hematite (α -Fe₂O₃) nanosized materials using *A. occidentale* leaf extract in order to inhibit Gram-positive *Staphylococcus aureus* and Gram-negative *E.coli*. The antibacterial test demonstrated that synthesized NPs were convincingly effective in inhibiting the bacterial strains under trial [72].

Recently, pure hematite phase magnetic Fe NPs were synthesized from floral extract of *C. viminalis* which was further employed as a novel antibacterial agent against some Gram-negative and Gram-positive bacteria [73]. Antibacterial test concluded that these biologically synthesized Fe NPs proved to be most effective against *S. typhi*, *S. aureus*, *S. enterica* and *K. pneumonia* while *S. dysenteriae* was moderately inhibited.

4.3. Bactericidal properties of zinc oxide NPs

Increasing antibiotic resistance and re-emergence of bacterial infections across the world, pose a severe threat to human life. Zinc oxide nanoparticles (ZnO NPs) are widely being explored these days due to its unique antibacterial and antifungal properties. ZnO is known to possess photo-oxidising and photocatalytic effect and is regarded biosafe [74]. ZnO NPs are found to be effective on microorganisms in the size range of nanometers to micrometres.

Due to the nano scaled size of ZnO, it is more convenient for it to interact with bacterial cell by entering inside it. Mostly the antibacterial properties of ZnO NPs are due to their unique physico-chemical properties and high surface area to volume ratios. ZnO NPs were also demonstrated to be biocompatible in human cells in several studies; therefore it is being thoroughly explored by researchers presently [75].

Pathogenic bacteria are known to have cell surface proteins for adhesion and formation of colonies. Polysaccharides and tiechoic acid are also present on the cell wall that protects it from host defence mechanism and environmental conditions. Since, all these are charged macromolecules, the surface modified NPs can be used to induce specific interactions in order to disrupt the cell wall integrity. ZnO NPs directly interact with the cell wall of bacterial cells leading to the disruption of its integrity. ZnO can be used as efficient bactericidal agent for Gram-negative as well as Gram-positive bacteria [76]. Recently, it is reported by Jones et al. that ZnO NPs exhibit higher bactericidal effects on *S. aureus* in comparison to MgO, TiO₂, Al₂O₃, CuO and CeO₂ NPs [77].

ZnO is much more biocompatible than TiO₂ and also has very high photocatalytic efficiency as compared to other inorganic photocatalytic materials [78]. ZnO is known to absorb UV light heavily [7]. This property of ZnO is associated with its conductivity and thus an increase in interaction of ZnO NPs with bacteria [79]. Interaction with UV light causes desorption of these oxygen species from the surface [80]. Thus improved photoconductivity is achieved due to reduction in surface electron depletion region. Significant ROS can be observed on treating bacterial broth with ZnO NP and illuminating it with UV light due to its phototoxic effects. These species help the NPs in entering inside the bacteria and further killing them [78,79].

Shape of ZnO NP also plays a crucial role in its antibacterial properties. Studies have proved that toxicity of these NPs depends upon its morphology. The process and conditions of synthesis decide the morphology of the synthesized NPs [81]. Controlling the synthesis parameters like type of precursor being used, solvents used and physical conditions such as temperature and pH, help in attaining desired antibacterial properties in the ZnO NPs. Studies have revealed the enhanced bactericidal effect of flower shaped NPs against *S. aureus* and *E. coli* as compared to those that are rod shaped [82]. Also it has been proved experimentally that more exposed Zn terminated polar facets show better antibacterial results [83].

Sirelkhatim et al. (2015) revealed that Oxygen annealing of ZnO increased the amount of oxygen atoms on the surface. This leads to high adsorbance of O₂ atoms onto the ZnO surface that causes generation of more ROS leading to more oxidative stress and enhanced antibacterial activity [83]. Another study by Mamat et al. revealed that oxygen annealing caused nanohole formation on the surface, thus increasing the surface area as well as O₂ adsorbance on surface that ultimately results in generation of more ROS and enhanced antibacterial effect [84].

Antibacterial activity of ZnO-NPs is also affected by its size and concentration [85]. Larger the surface area and higher the concentration, more is the bactericidal effect. It has also been found out that smaller the size of the NP, easier it is to penetrate

inside the bacterial cell membrane. This is due to the larger interfacial area in small sized NPs. It was also demonstrated that higher the surface area of the NP, more are the oxygen species present on the surface and thus higher is the ROS generation, resulting in better bactericidal effect [85].

It is reported that ZnO NPs are very promising agents in the array of development of substitute for overcoming antibiotic resistance, because they demonstrate significant bactericidal activity against Gram-negative strains such as *K. aerogenes*, *P. aeruginosa* and *E. coli* etc as well as Gram-positive strain such as *S. aureus* [86].

4.4. Bactericidal properties of Au NPs

Au NPs are well known for their biocompatibility and antimicrobial activity. Au NPs cannot act singly on the target and must be tagged with other biomolecules to show effective antimicrobial property. Cross linking of either collagen, chitosan or gelatin with Au NPs enable it to easily bind with the macromolecules as discussed by Rajendran et al. [87]. Au NPs can also be incorporated with other drug molecules to observe a synergistic antimicrobial effect. Gu et al. demonstrated the effect of Vancomycin conjugated Au NPs on vancomycin resistant *Enterococci* (VRE) and *E. coli*. The antimicrobial effect of Vancomycin was found to be enhanced by 50 folds [88]. Conjugation of Au NPs with pathogen specific antibodies or photosensitizing molecules for photothermal and photodynamic therapy also helps in increasing its antimicrobial activity [89,90].

Au NPs affect bacterial cells following different courses of action. It may enter the cells and alter their membrane potential by inhibiting the activity of ATP synthase. It leads to depletion in the ATP levels that collapses the energy metabolism leading to cell death [91]. Multiple drug resistant (MDR) bacteria are also killed by this non ROS dependent pathway. According to Guerrini, L 2018, multivalency on NPs is enabled due to the high surface/volume ratio, thus incorporating many functional ligands. This multivalency is a way to enhance interaction of NP surface with target bacteria. These features enable NPs to be coupled with antibiotics for the treatment of multiple drug resistance in bacteria. Antibiotics conjugated to NPs were found to be more effective than antibiotics alone [92]. Li et al. (2014) reported that Au NPs (~2 nm size) having cationic surface properties can be efficiently employed as antibacterial agent. This research group investigated its efficacy against Gram-negative bacteria like *P. aeruginosa* (intrinsic resistance to many antibiotics because of low membrane permeability) and *E. cloacae* complex, Gram-positive bacteria *S. aureus* (resistant to almost all therapeutic chemo-agents) and methicillin-resistant *S. aureus* (resistant to most antibiotics used). Effective MIC values for these pathogens were in the range of 8–64 nM and the bacterial cell death was presumed to be due to the loss of membrane integrity [93]. Rajan et al. (2015) demonstrated the effect of size on the antibacterial and anti-cancerous activity of Au NPs. They portrayed that smaller Au NPs would show better bactericidal effect than the larger ones [94].

4.5. Bactericidal activity of other metals

Biogenic palladium, selenium, cerium and tellurium NPs have also shown antimicrobial activity. According to a report, methanolic extract of *M. oleifera* peel was used to produce Pd NPs and harnessed for antimicrobial property against *E. coli* and *S. aureus* [95]. Antimicrobial assay revealed that the NPs effectively inhibited the growth of both bacterial strains. The same group biologically synthesized CeO₂ NPs from *M. oleifera* peel extract and monitored their activity against the above bacterial strains [96]. Antibacterial assay confirmed the successful inhibition of both

strains. Another amnesic work reports the bio-fabrication of CeO₂ NPs from leaf extract of *G. superba* and their potential application against a set of Gram-negative and Gram-positive bacteria [97]. The NPs performed well against *P. aeruginosa*, *P. vulgaris*, *K. pneumonia* and *S. pneumonia*. Simultaneously, the chitosan-cerium oxide hybrid NPs were developed from *Sida acuta* leaf extracts which manifested good repressive effect against *B. subtilis* and *E. coli* [98]. It was anticipated that inhibition occurred due to the nanoparticle induced disintegration of cell membrane. Amarnath et al. (2012) prepared chitosan coated Pd nanocomposites and stabilized them using polyphenols obtained from grape extract and examined their application as a potential inhibitor for *E. coli*. NPs severely damaged the *E. coli* cells at an optimized concentration of 50 µg/L. It was concluded that the NPs bound with bacterial membrane via electrostatic interaction lead to the bacterial cell death [99]. Sharmila et al. (2017) synthesized biogenic Pd NPs from *F. decipiens* leaf extract and reported their high bactericidal activity against both Gram-negative bacteria (*S. aureus* and *B. subtilis*) and Gram-positive bacteria (*P. aeruginosa* and *E. coli*) [100]. In another work, *C. guianensis* fruit extract was employed for the bio-manufacturing of Pd NPs that exhibited extraordinary bactericidal activity against some human pathogens like *S. typhi* MTCC 3917, *V. cholerae* MTCC 3906, *E. coli* MTCC 1687 and *K. pneumonia* MTCC 530 whereas moderately active against *M. luteus* MTCC 1809, *B. cereus* MTCC 1272, *P. mirabilis* MTCC 425, *P. aeruginosa* MTCC 1688, *R. rhodochorus* MTCC 265 and *S. aureus* MTCC 96. Oxidative stress induced by the bio-capped Pd NPs was expected to be responsible for the observed inhibitory effect [101]. Some other workers have convincingly demonstrated the usefulness of biogenic Pd NPs against different microorganisms [102,103].

Sonkusre and Cameotra (2015) produced *B. licheniformis* JS2 derived selenium NPs (Se NPs) which adequately inhibited the growth of microbial colony of *Staphylococcus aureus* onto different surfaces like polystyrene, glass, and catheter [104]. Cremonini et al., (2016) synthesized two different Se NPs from culture supernatant of *S. maltophilia* (Gram-negative bacteria) and *B. mycoide* (Gram-positive bacteria). Both the NPs efficiently prevented the progression of biofilm of *P. aeruginosa* and *Candida spp* [105]. Recently, Se NPs with size of 90–150 nm were prepared by using *S. aureus*, *P. aeruginosa* and *E. coli* which displayed astonishing bactericidal activity against *E. coli* and *S. aureus* [106].

Zare et al. (2012) formed rod shaped elemental Te (Tellurium) NPs by using *Bacillus sp. BZ*. The formed Te NPs showed bactericidal activity against *S. aureus*, *P. aeruginosa*, *S. typhi* and *K. pneumonia* [107]. Rajakumar et al. (2012) also adopted the environmentally benign approach and utilized culture supernatant of *A. flavus* (MTCC no. 7369) to prepare Te NPs [108]. Well diffusion test established that the Te NPs significantly killed *E. coli* at the MIC value of 40 µg/ml. Mohanty et al., (2014) synthesized rod shaped Te NPs through a metal reducing bacteria *S. oneidensis* and reported their exceptional antibacterial property against *P. aeruginosa* [109].

Biologically prepared titanium NPs also find utility as an antimicrobial agent. Santosh kumar et al., (2014) investigated the bactericidal properties of TiO₂ NPs biosynthesized from aqueous leaf extract of *Psidium guajava* [110]. These NPs impressively prevented the growth of *E. coli* and *S. aureus*. *T. foenum-graecum* leaf extract was used in order to synthesize TiO₂ NPs and their antibacterial properties were studied against *Y. enterocolitica*, *P. vulgaris*, *E. faecalis*, *P. aeruginosa*, *S. faecalis*, *S. aureus*, *B. subtilis*, *E. coli* and fungus *C. albicans*. TiO₂ NPs effectively inhibited the test microorganisms [111]. Another important work reports the emphatic bactericidal activity of TiO₂ NPs synthesized by *Prunus yedoensis* leaf extract (Manikandan et al., 2018) against *E. coli* and *S. aureus* [103].

The summary of various biogenic metallic NPs with antibacterial properties is provided in Table 1.

Table 1
Summary of various biogenic metallic NPs with anti-bacterial properties.

Element	Biological source	Used component for synthesis	Size (nm) and shape	Affected Bacterial strains	Ref.
Silver	<i>E. japonica</i>	Leaf extract	46 to 70 spherical	<i>E. coli</i> and <i>S. aureus</i>	[112]
Silver	<i>J. adhatoda L</i>	leaf extract	5-50 spherical	<i>P. aeruginosa</i>	[113]
Silver	<i>A. comosus</i>	Leaf extract	12.4 spherical	<i>S. aureus</i> , <i>S. pneumoniae</i> , <i>P. mirabilis</i> and <i>E. coli</i>	[114]
Silver	<i>M. officinalis</i>	Leaf extract	12 spherical	<i>S. aureus</i> and <i>E. coli</i>	[115]
Silver	<i>Alternaria</i> sp	Fungus	80 spherical	<i>B. subtilis</i> , <i>S. aureus</i> , <i>E. coli</i> and <i>S. marcescens</i> ,	[116]
Silver	Penicillium	Fungus	10-100 spherical	<i>B. cereus</i> , <i>S. aureus</i> , <i>E. coli</i> and <i>P. aeruginosa</i>	[117]
Iron	<i>T. procumbens</i>	Plant extract	80-100	<i>P. aeruginosa</i>	[62]
Iron	<i>G. jasminoides</i> and <i>L. inermis</i>	Plant extract	21, hexagonal	<i>E. coli</i> , <i>S. enterica</i> , <i>P. mirabilis</i> and <i>S. aureus</i>	[63]
Gold	<i>T. harzianum</i>	Fungus		<i>E. coli</i> MTCC 1302	
Gold	<i>H. Cordata</i>	Plant extract	30-90 spherical	<i>B. subtilis</i> , <i>S. typhimurium</i> , <i>B. cereus</i> and <i>E. coli</i>	[118]
Gold	<i>Streptomyces</i> sp. NH21	Fungus	10 spherical	<i>E. coli</i> , <i>K. pneumoniae</i> , <i>P. mirabilis</i> , <i>S. infantis</i> , <i>P. aeruginosa</i> and <i>B. subtilis</i>	[119]
Gold	<i>C. globosum</i>	Fungus	12 spherical	<i>E. coli</i> , ATCC 25922; <i>K. pneumoniae</i> , IMS/GN9; <i>P. mirabilis</i> , IMS/GN13 and <i>S. aureus</i>	[120]
Gold	<i>A. catechu</i>	Plant extract	13.7 spherical	<i>E. coli</i> , <i>K. pneumoniae</i> , <i>P. aeruginosa</i> , Enterobacter and <i>S. aureus</i>	[94]
Gold	<i>C. cujete L</i> (Calabash tree)	Plant extract	32.89 spherical	<i>E. Coli</i> (MTCC 1687), <i>P. aeruginosa</i> (MTCC 1688), <i>V. cholerae</i> (MTCC 3906), <i>Salmonella typhi</i> (MTCC 531), <i>S. flexneri</i> (MTCC 9543) and <i>B. subtilis</i> (MTCC 441)	[121]
Gold	<i>A. terreus</i>	Fungus	10-19 spherical	<i>Escherichia coli</i>	[122]
Zinc oxide	<i>E. faecalis</i>	bacteria	16 to 96 spherical	<i>E. Coli</i> , <i>K. pneumoniae</i> and <i>S. aureus</i>	[123]
Zinc oxide	<i>R. graveolens</i>	Herb	28 wurtzite	<i>K. aerogenes</i> , <i>P. aeruginosa</i> , <i>E. coli</i> , <i>S. aureus</i>	[86]
Zinc oxide	<i>E. faecalis</i>	bacteria	16 to 96 spherical	<i>E. coli</i> 03, <i>K. Pneumonia</i> 125 <i>S. aureus</i> 20 <i>E. coli</i> MTCC 9537, <i>K. pneumoniae</i> MTCC 109, <i>S. aureus</i> MTCC 96, <i>P. Aeruginosa</i> MTCC 741, <i>S. flexneri</i> MTCC 1457 and <i>E. faecalis</i> NCIM 5025	[124]
Zinc oxide	<i>C. pictus D. Don</i>	Plant extract	11–25 hexagonal	<i>S. aureus</i> (NCIM 2079), <i>B. subtilis</i> (NCIM 2063) <i>E. coli</i> (NCIM 2065) and <i>S. paratyphi</i> (NCIM 2501)	[125]
Zinc oxide	<i>C. halicacabum</i>	Plant extract	30-80 spherical & rod	<i>E coli</i> and <i>S. aureus</i>	[126]

5. Mode and mechanism of action of biogenic nanoparticles

5.1. Mechanism of action of metal NPs and metal oxide NPs

The mechanism of action of metal and metal oxide nanoparticles against bacteria is still not very clear and lot of studies is currently going on across the world. Researchers have shown that free metal nanoparticles exert toxic effects in dissolution of the outer membrane of bacterial surface. While oxidative stress induced by reactive oxygen species (ROS) is the principle mechanism in case of metal oxide nanoparticles [127]. Studies have shown that Ag NPs induced formation of pits and gaps on the membrane surface of bacteria by the release of ions which further interact with disulfide or sulfhydryl groups of enzymes causing interruption of metabolic pathway that finally leads to bacterial cell death [128]. Some studies revealed that ZnO NPs augmented the ROS generation on the membrane surface, thereby causing the dysfunction of membrane and death of bacterial cell [129]. Similar mechanism of oxidative stress driven by ROS generation was also observed in case of TiO₂ NPs [130]. It was shown that TiO₂ NPs can cause lipid peroxidation that affect the fluidity and integrity of the cell wall of bacteria through ROS generation.

5.2. Difference in mode of action of biogenic NPs under aerobic and anaerobic condition

The inhibitory and toxic effect of nanoparticles against bacterial strains depends on the flux of ions released by the nanoparticles. The extent of this ion flux is directly proportional to the amount of oxygen available. It was an interesting observation that Ag NPs showed better toxicity against aerobic *E. coli* under adequate oxygen supply, while the same nanoparticles exhibit lower toxicity against anaerobic bacteria with low oxygen supply. Accordingly, it was found that the minimum inhibitory concentration (MIC) for Ag

NPs against anaerobic bacterial strains was quite high compared to aerobic bacteria [131]. While the MIC values for anaerobic bacteria like *F. nucleatum*, *S. sanguis* and *S. mutans* were 25, 50 and 50 µg/mL, respectively, it was 6 µg/mL in case of aerobic bacterial strain like *E. coli*. Hence, by manipulating the accessibility of oxygen, we can control the toxicity of nanoparticles.

5.3. Different ways that are targeted by biogenic NPs

The exact pathways that are targeted by nanoparticles for antibacterial activity are still not deciphered. However, biogenic nanoparticles follow two distinct ways to demonstrate their antibacterial potency; either by generating ions (in case of metallic NPs) or by releasing reactive oxygen species (in case of metallic oxide NPs). The released ions and ROS damage the cell wall, genetic material or membrane lipid through oxidation [132]. During the synthesis, biogenic nanoparticles get reduced by bioactive compounds like vitamins, proteins, polyphenols, carbohydrates, polymers etc. that are present in the cellular extract of living plants and microbes [133]. These molecules not only provide high degree of stability to the synthesized NPs but also add different functional groups to the surface of NPs. These functional groups allow the establishment of chemical bonds and offers active sites for physical interaction between nanoparticles and bacterial cell which is necessary for their inhibition [134]. Electrostatic and lipophilic interactions are the principle mechanisms through which biogenic NPs get attached with the bacterial cell. Accordingly, it was noticed that biogenic nanoparticles show better antimicrobial property than chemically synthesized ones. In a study, it was reported that biogenic NPs showed remarkable inhibitory effect against all the bacterial strains studied like *P. vulgaris*, *K. pneumoniae*, *P. aeruginosa*, *S. aureus* and *V. cholera* while chemically synthesized nanoparticles were found to be ineffective against *P. vulgaris*, *P. aeruginosa* and *K. pneumoniae* [135].

5.4. Effects of biogenic NPs over clinically resistant bacterial strains

Development of antibiotics resistance in bacterial strains has become a major issue of concern. Therefore, a lot of research work is going on in the field of Nanobiotechnology to overcome this health issue. It was reported that biogenic Ag NPs could effectively inhibit the growth of clinical resistant strain of *P. aeruginosa* isolated from wound site of patient (MIC: 2.0 mg/ml) [136]. In another noticeable approach, it was noticed that biogenic ZnO NPs in combination with antibiotics can effectively kills methicillin resistant *S. aureus* (MRSA) clinical isolates (MIC: 2000 µg/mL, MBC: 2200 µg/mL) [137]. In addition, it was reported that biogenic nanoparticles can overcome the issues of antibiotics resistance associated with the formation of bacterial biofilm [138].

6. Concluding remarks and future perspectives

In recent times, antibiotic resistance of bacteria has become one of the major concerns in the human healthcare. Most of the antibiotics kill bacteria by disrupting membrane permeability, inhibiting membrane synthesis and modulating enzymatic pathways that are involved in cellular transcription, translation and replication process. It prevents the action of the antibiotics by degrading them or by decreasing their binding affinity towards target enzymes due to mutation in target genes. To overcome this challenge, scientists have focused to develop alternative approaches. The metal nanoparticles have proven to be one of best choices to treat drug resistant bacterial infections. The high surface to volume ratio of NPs increases their affinity towards the cellular membrane. In addition, the positive charge on the NPs can further enhance the affinity for the negatively charged membrane due to the presence of either lipopolysaccharide or teichoic acid. It is not easy for bacteria to develop the resistance against NPs, since NPs simultaneously target several pathways to kill bacterial cell.

Scientists have developed several methods for synthesis of metal nanoparticles. The physical method is very expensive and the yield of NPs is also very low. The chemical synthesis methods which were extensively used in the last decades are not eco-friendly and pollutes the environment. Chemical synthesis is indeed costly and time consuming process. In order to overcome these challenges, scientists have recently developed a method of green synthesis which is environmental friendly and cost effective. In green synthesis, plants, bacteria and fungi are extensively used. These organisms are the major sources of metabolites that can work as stabilizing and reducing agent during NPs synthesis. NPs can be synthesized both extracellularly and intracellularly. Extracellular method is more preferred over intracellular method, since it does not need any downstream process for harvest of NPs from the organisms. Plant assisted synthesis is cheaper and more proficient in getting higher yields of NPs than the microbes. Plants have numerous metabolites and biochemicals that can function as stabilizing and/or reducing agent in green synthesis of NPs.

Among the various reaction parameters, temperature, pH and reaction time greatly influence the morphology (size and shape), chemical properties and rate of synthesis of the NPs by monitoring the formation of nucleation centres. The antibacterial property of the NPs changes on altering the size, shape and oxidation state of NPs. It is reported that antibacterial activity is greater with smaller size and decrease with increase in size of the NPs. When we talk about shape, triangular and spherical shaped nanoparticles have great antibacterial property, but the reason behind is not yet well understood. Most of the metal nanoparticles belonging to d-block element are reported as potent antibacterial agents. This is due to the fact that they contain high oxidation number and get easily reduced and oxidized in presence of reducing and oxidizing agent, respectively.

During the study, we observed that the formation of biogenic metallic nano-materials with antimicrobial property is quite simple and economical. Many biologically produced NPs showed excellent inhibition against several pathogenic microorganisms. Some of the nano-materials remarkably killed the various microbial species which developed high resistance to available drugs. Moreover, very small-sized nano-materials can even act as a carrier to deliver the antimicrobial drugs to the internal organs of the body which got affected by microbes. It is highly anticipated that these biogenic NPs will be able to successfully replace the available drugs against which the bacteria have developed resistance. Apart from biomedical applications, these ecofriendly NPs can even solve the problem of microbial contamination of potable water. Water contamination by bacteria causes millions of death every year worldwide, particularly in third world countries. In this regard, these NPs may be harnessed as an effective water disinfectant in order to provide contamination-free drinking water which is safe for human consumption.

Declaration of Competing Interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

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