Chapter 4
Green Synthesis of Metallic and Metal Oxide Nanoparticles and Their Antibacterial Activities

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Abstract In recent years, the development of metallic and metal oxide nanoparticles in an eco-friendly manner using plant materials has attracted considerable attention. The biogenic reduction of metal ions to the base metal is quite rapid, can be conducted readily at room temperature under sunlight conditions, can be scaled up easily, and the method is eco-friendly. The reducing agents involved include various water-soluble metabolites (e.g., alkaloids, terpenoids, polyphenolic compounds) and coenzymes. Noble metals (silver and gold) have been the main focus of plant-based synthesis. These green synthesized nanoparticles have a range of shapes and sizes compared to those produced by other organisms. The advantages of using plant-derived materials for nanoparticle synthesis have attracted the interest of researchers to investigate the mechanisms of metal ions uptake and bio-reduction by plants. These biosynthesized metallic and metal oxide nanoparticles have a wide range of biological applications. This chapter, however, discusses only the antibacterial activities.

Keywords Green synthesis • Eco-friendly • Antiparticles • Antibacterial activities

4.1 Introduction

Nanotechnology or nanoscale technology normally considers sizes below 100 nm. Nanoscience studies the phenomena, properties and responses of materials at the atomic, molecular, and macromolecular scales, usually at sizes between 1 and 100 nm [1]. At this size range, materials exhibit remarkable properties and are

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gaining importance in the areas of mechanics, optics, biomedical sciences, chemical industry, electronics, drug-gene delivery, energy science, catalysis [2, 3] optoelectronics [4, 5] photo electrochemical applications [6], and nonlinear optical devices [7, 8]. Therefore, the production of nanoparticles with innovative applications can be achieved by controlling the size and shape on the nanometer scale. Nanoparticles can exhibit size and shape-dependent properties, which are of interest for applications ranging from biosensing and catalysts to optics, antimicrobials, and modern electronics. These particles also have applications in a range of fields, such as medical imaging, drug delivery, nanocomposites, and hyperthermia of tumors [9–12]. AuNPs and AgNPs are most common NPs in biomedical applications and interdisciplinary field of nanobiotechnology [13, 14]. AuNPs have been used for a range of purposes, such as markers for biological screening and immunoassay [15], protein assay [16] anticancer [17], antimicrobial activities [18–21], antimelanoma [19] tyrosinase inhibitory [19], and capillary electrophoresis [22]. AgNPs have extensive applications in areas, such as integrated circuits [23], sensors [24], biolabelling filters [24], antimicrobial deodorant fibers [25], antimicrobials [26–29], cell electrodes [30], antioxidants [28], and antiproliferative activities [29]. These AgNPs have potential antimicrobial effects against infectious organisms, such as *Escherichia coli*, *Bacillus subtilis*, *Vibrio cholera*, *Pseudomonas aeruginosa*, *Syphilis typhus*, and *Staphylococcus aureus* [31, 32]. ZnO NPs are used widely in industrial applications, such as pigments [33], dye-sensitized solar cells [34], photo-catalysts [35], and sensors [36]. ZnO is a wide bandgap semiconductor (II–IV) with an energy gap of 3.37 eV at room temperature, and ZnO NPs have a great advantage in their application as catalysts owing to their large surface area and high catalytic activity [37], as well as their antibacterial, antioxidant, and cytotoxic activities [38].

4.2   Synthesis of Nanoparticles

Nanoparticles can be synthesized using two methods: "top-down (Fig. 4.1)" approach or "bottom-up" approach. In the "top-down" approach, the bulk materials are broken down gradually to nanosized materials (lithographic techniques, e.g., grinding, milling), whereas in bottom-up approach, atoms or molecules are assembled to molecular structures in the nanometer range [39].

In bottom-up synthesis, nanoparticles are constructed from smaller entities, e.g., by joining atoms, molecules, and smaller particles [40]. In bottom-up synthesis, the nanostructured building blocks of nanoparticles form first and then assemble to produce the final particle [41]. Figure 4.2 shows the probable mechanism of nanoparticle synthesis by a bottom-up approach.

Of the biological methods of synthesis, methods based on microorganisms have been reported widely [22–25, 42]. Microbial synthesis is of course readily scalable, environmentally benign, and compatible with the use of the product for medical applications. On the other hand, these methods require multiple purification steps, or the maintenance of microbial cell cultures is more cost-incurring.
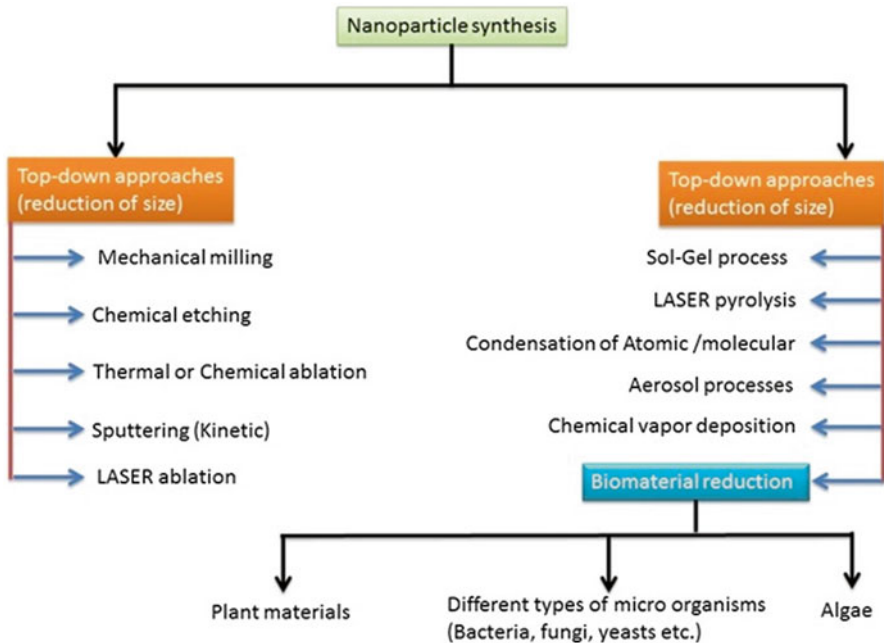
Fig. 4.1 Various approaches for making nanoparticles and cofactor dependent bioreduction with slight modifications. Reprinted from ref. [39] with permission from Elsevier
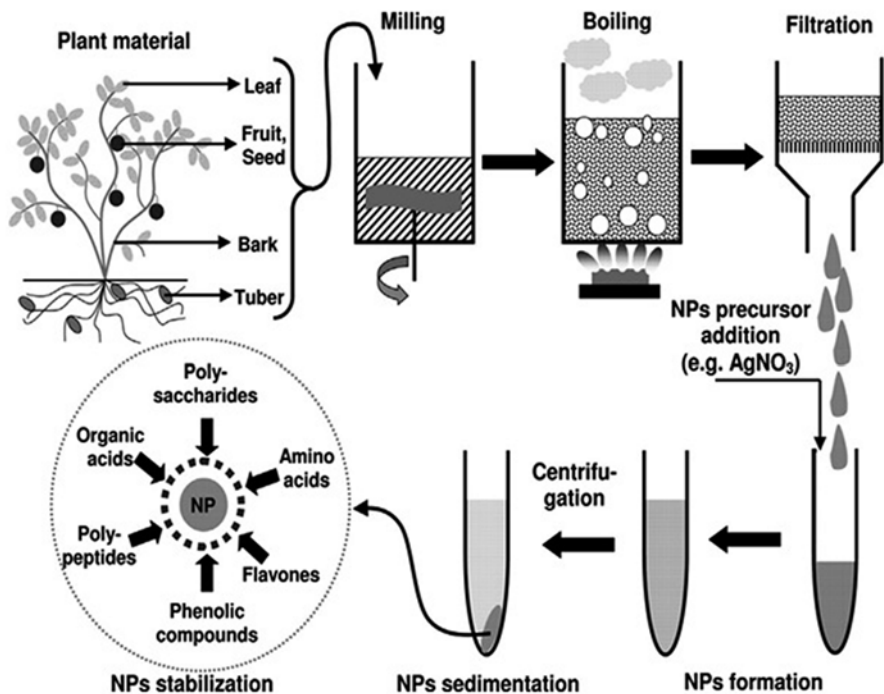

Fig. 4.2 Schematic representation of nanoparticle synthesis from plant extracts

4.2.1 Plant Biomass/Living Plant for Nanoparticle Synthesis (Green Synthesis; Fig. 4.3)

The use of plant materials is considered a green route and a reliable method for green synthesis of nanoparticles owing to its eco-friendly nature. Plants have been exploited successfully for rapid and extracellular biosynthesis of noble metallic and metal oxide nanoparticles [43–50]. Table 4.1 summarizes some of the reports pertaining to metallic and metal oxide nanoparticle synthesized by using various plant extracts, including their size and shape.

4.2.2 Silver Nanoparticles (AgNPs)

AgNPs have been synthesized from various types of plant extracts (Fig. 4.4), yeast, fungi, and bacteria. The use of plant extracts for the synthesis of AgNPs can be advantageous over other environmentally benign biological processes by
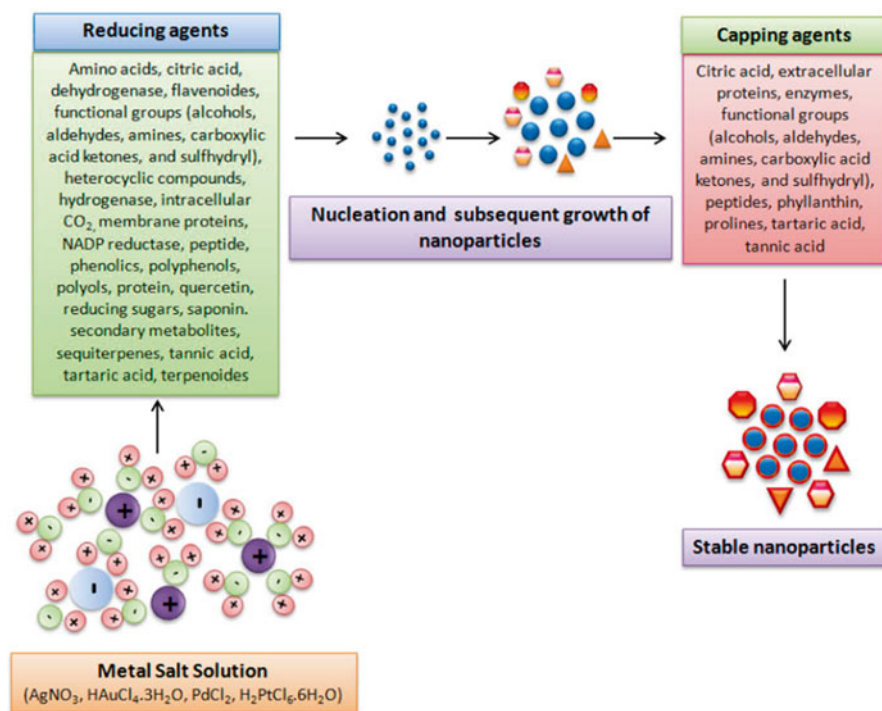

Fig. 4.3 Schematic diagram showing the mechanisms behind the biogenic synthesis of metallic nanoparticles. Reprinted from ref. [46] with permission from the American Chemical Society

Table 4.1 Green synthesis of metallic and metal oxide nanoparticles from different plant extracts and their size and shapes

Plant name	Type of NPs	Shape and size	Tested microorganism	Reference
Soybean–garlic aqueous extract	AuNPs	Spherical (7–12.4 nm)	*S. aureus*, *P. aeruginosa*, and *A. baumannii/ haemolyticus*	El-Batal et al. [124]
Dioscorea batatas rhizome	AuNPs	Triangular, hexagonal, rod, and irregular (18.48–56.18 nm)	*S. aureus*, *S. epidermidis*, and *E. coli*	Sreekanth et al. [125]
Grapes fruit	AuNPs	Spherical (−)	*S. aureus*, *C. koseri*, *B. cereus*, *P. aeruginosa*, *E. coli*, and *C. albicans*	Lokina and Narayanan [126]
Aerva lanata flower	AuNPs	Spherical (48.4 nm)	*S. aureus*, *B. cereus*, and *P. aeruginosa*	Kirubha and Alagumuthu [127]
Senna siamea leaf	AuNPs	Spherical and hexagonal (70 nm)	*S. aureus*, *B. subtilis*, *E. coli*, *P. aeruginosa*, and *K. pneumonia*	Rajasekhar et al. [128]
Tephrosia purpurea leaf	AuNPs	Spherical (~100 nm)	*E. coli*, *E. faecalis*, and *K. pneumonia*	Jisha et al. [129]
Caesalpinia pulcherrima flower	AuNPs	Spherical (10–50 nm)	*A. niger*, *A. flavus*, *E. coli*, and *Streptobacillus* sp.	Nagaraj et al. [130]
Bacopa monnieri plant		Spherical (15–35 nm)	*E. coli*, *S. aureus*, *B. subtilis*, and *Enterococcus*	Mahitha et al. [131]
Plumeria alba flower	AuNPs	Spherical 20–30 nm and 80–150 nm	*A. niger*, *A. flavus*, *E. coli* and *Streptobacillus* sp.	Nagaraj et al. [132]
Ziziphus Jujuba fruit	AuNPs	Spherical (34.8 nm)	*E. coli*, *S. aureus*, *B. cereus*, and *P. aeruginosa*	Kirubha and. Alagumuthu [133]
Abelia grandiflora root	AgNPs	Spherical (10–30 nm)	*E. coli*, *B. megaterium*, *B. subtilis*, *S. aureus*, *K. pneumonia*, and *P. vulgaris*	Sharma et al. [134]
Mimusops elengi leaf	AgNPs	Spherical (55–80 nm)	*K. pneumonia*, *S. aureus*, and *Micrococcus luteus*	Prakash et al. [135]
Vanda Tessellate leaves	AgNPs	Cubic, hexagonal (10–50 nm)	*E. coli*, *Salmonella* sp, and *S. aureus*	Manjunath et al. [136]
Nelumbo nucifera seed	AgNPs	Spherical (2.76–16.62 nm)	*B. cereus*, *S. typhimurium*, *S. epidermidis*, *B. megaterium*	Nguyen Thi Mai Tho et al. [137]
Lycopersicon esculentum	AgNPs	Square (10–45 nm)	*E. coli*	Smaranika Das et al. [138]
Carob leaf	AgNPs	Spherical (5–40 nm)	*E. coli*	Awwad et al. [139]

(continued)

Table 4.1 (continued)

Plant name	Type of NPs	Shape and size	Tested microorganism	Reference
Bacopa monnieri whole plant	AgNPs	Spherical (22 nm)	*S. aureus*, *B. subtilis*, *E. coli*, *K. pneumoniae*	Mahitha et al. [140]
Phyllanthus amarus whole plant	AgNPs	Spherical (8–24 nm)	*P. aeruginosa*	Singh et al. [141]
Pomegranate peel	AgNPs	5–50 nm	*S. aureus*, *P. aeruginosa*, and *E. coli*	Shanmugavadivu et al. [142]
Elaeagnus indica leaves	AgNPs	Mono-dispersive (30 nm)	*E. coli*, *P. putida*, *B. subtilis* and *S. aureus*, *A. flavus* and *F. oxysporum*	Natarajan et al. [143]
Aloe leaf	ZnONPs	Spherical (25–55 nm)	*S. aureus*, *S. marcescens*, *P. mirabilis*, and *C. freundii*	Sangeetha et al. [144]
Solanum nigrum leaf	ZnONPs	Quasi-spherical (29.79 nm)	*S. aureus*, *S. paratyphi*, *V. cholera*, and *E. coli*	Ramesh et al. [145]
Aloe vera	ZnONPs	Irregular, triangular (69 nm)	*S. aureus*, *S. pyogenes*, *P. aeruginosa*, *E. coli*, and *S. typhi*	Ayeshamariam et al. [146]

eliminating the need for elaborate processes of maintaining cell cultures. Plants/plant extracts can act as both reducing and stabilizing agents in the synthesis of nanoparticles. Jose-Yacaman et al. first reported the formation of gold and silver nanoparticles synthesized from living plants [44, 45]. Triangular, hexagonal, cubic, and circular AgNPs were synthesized from a *Pseudocydonia sinensis* fruit extract [46]. Sathiskumar et al. [47] examined the ability of the extracts of powder and bark of *Curcuma longa* towards the formation of AgNPs, and reported that a bark extract can produce a large amount of AgNPs compared to the powder extract. The resulting nanoparticles varied in shape and size but exhibited strong antibacterial activity against *E. coli*. Sreekanth et al. [51] reported the formation of spherical AgNPs using a *Citrus reticulata* juice extract. Similarly, many other studies have synthesized AgNPs from the leaf extract of *Argemone mexicana* [52], bran powder of Sorghum spp., [53], and the leaf extracts of *Allium cepa* [54] and *Euphorbia hirta* [55].

Sreekanth et al. [56] reported the synthesis of AgNPs using *Nelumbo nucifera* root extract, in which AgNPs were predominantly spherical and had a mean size of 16.7 nm. Quite recently, AgNPs have been synthesized using a variety of plants, such as banana (spherical shape), neem (triangular), and tulsi (cuboidal shape) [57], *Abelia grandiflora* (spherical shape and mean size of 10–30 nm) [58], *Malus domestica* fruit extract [59], *Zingiber officinale* root extract (spherical and size ranging from 10 to 20 nm) [60], orange peel extract (spherical and a mean size of 91.89 nm) [61], *Sterculia foetida* L young leaves (spherical, irregular and nanoparticle size
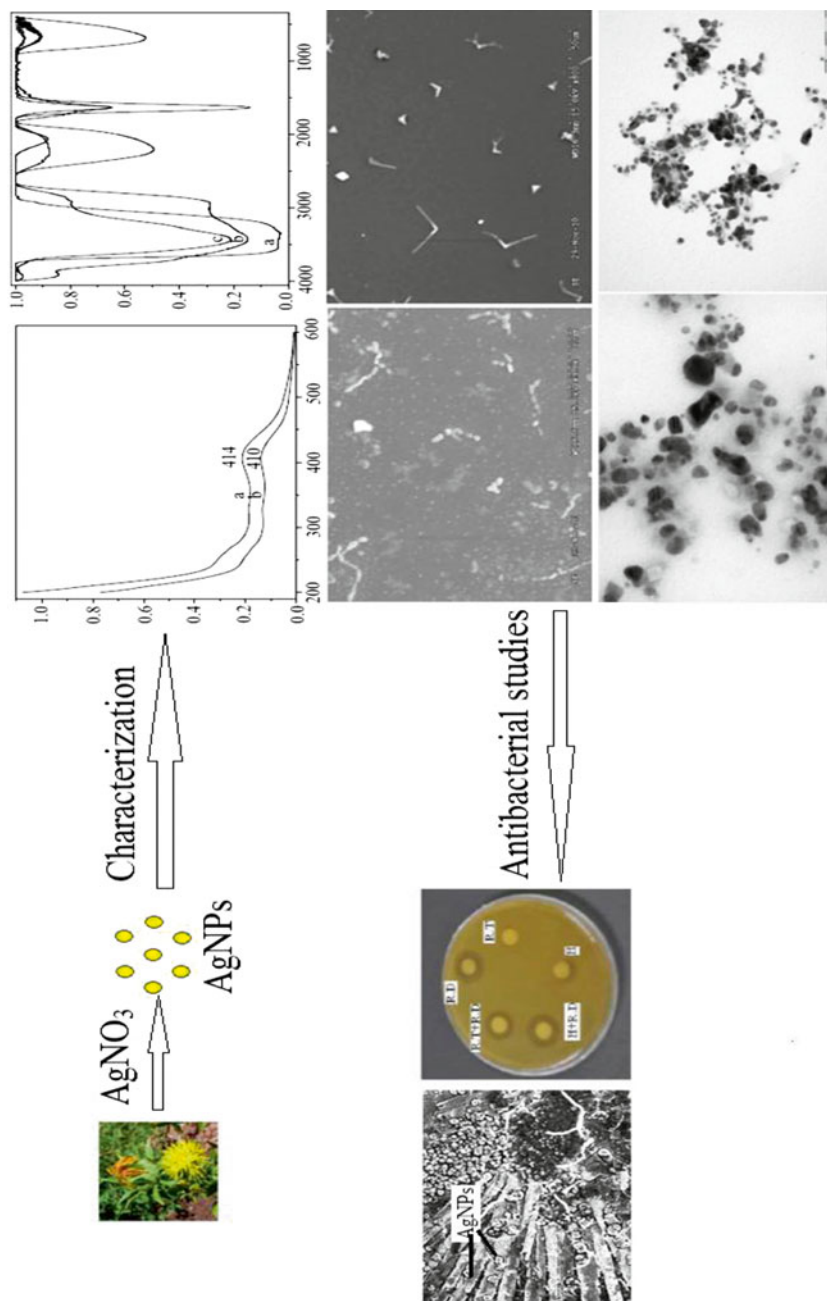
Fig. 4.4 Schematic of the procedure followed to synthesize AgNPs by using *Carthamus tinctorius* flower extracts and to study their antibacterial properties. Reprinted from ref. [51] with permission from the Bentham Sciences

ranges from 30 to 50 nm) [62], *Ocimum tenuiflorum* (spherical particles with a mean size of 29 nm), and *Catharanthus roseus* (spherical shape nanoparticles with a mean size of 19 nm) [63], respectively.

4.2.3 Gold Nanoparticles (AuNPs)

AuNPs are the most attractive noble metal nanoparticles because of their potential applications in catalysis, optics nanoelectronics, gene expression, and clinical diagnosis [64].

Huang et al. [65] reported the formation of gold nanoparticles from the sun-dried leaves of *Cinnamomum camphora*. Narayanan and Sakthivel [66] reported the extracellular synthesis of gold nanoparticles using the leaf extract of *Coriandrum sativum*. They found that the synthesized nanoparticles were triangular, truncated and decahedral in morphology with a mean size of 6.7–57.9 nm. Ramezani et al. [67] tested the leaf extracts of three different plants, i.e., *Eucalyptus camaldulensis, Pelargonium roseum*, and *A. indica*, for the reduction of aqueous chloroaurate solutions. The results indicated that all the leaf extracts tested could produce gold nanoparticles, but a significant increase in reduction was observed when the menthol extracts of *E. camaldulensis* and *P. roseum* were used compared to the *A. indica* leaf extract. Raghunandan et al. [68] reported that the addition of a microwave-exposed aqueous extracellular guava leaf extract to an aqueous gold chloride solution yielded stable poly-shaped gold nanoparticles with high purity. Ankamwar [69] reported the formation of highly stable gold nanoparticles (10–35 nm) when the leaf extract of *Terminalia catappa* was exposed to an aqueous chloroaurate solution. Gold nanoparticles with various sizes were also obtained using the dried leaf extract of *Stevia rebaudiana* [70]. Moreover, Thirumurugan et al. [71] also produced the gold nanoparticles using the leaf extract of *A. indica*.

Recently, Singh and Bhakat [72] reported the synthesis of gold nanoparticles using the leaves and bark of Ficus carica. In addition, the synthesis of spherical gold nanoparticles by the reduction of $AuCl_4^-$ by the leaf extracts of *Sphaeranthus amaranthoides* [73] and *Putranjiva roxburghii* [74] was reported. The syntheses of gold nanoparticles with different sizes in the range of 15–25 nm were obtained by controlling the synthetic parameters using the leaf extract of fenugreek [75].

Several studies have independently reported the reduction of aqueous chloroaurate solution using a variety of plant parts (Fig. 4.5). The formation of gold nanowires was reported from the pulp extract of *Beta vulgaris* [76]. Similarly, spherical-shaped crystalline gold nanoparticles were synthesized using the flower extract of *Nycanthes arbor-tristis* [77] and the leaf extract of *Mangifera indica* [78].
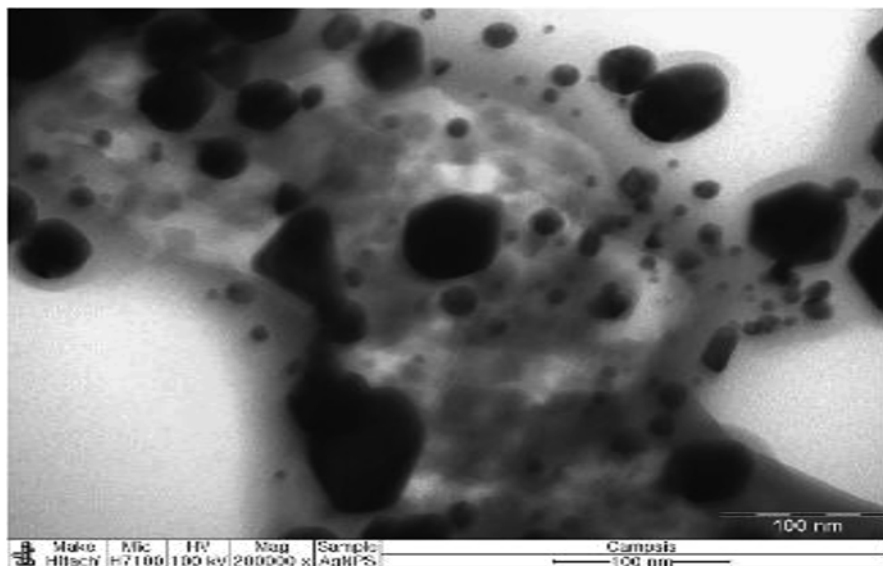
Fig. 4.5 Green-synthesized AuNPs from *Nelumbo nucifera* seed extract. Reprinted from ref. [137] with permission from the Slovenian Chemical Society

4.2.4   Zinc Oxide Nanoparticles (ZnONPs)

ZnONPs is an attractive semiconductor material for nano-electronic and photonic applications [79]. ZnO NPs are used widely in industrial applications, such as pigments, dye-sensitized solar cells [80], photocatalysts [40], and sensors [41]. ZnO is a wide band-gap semiconductor (II–IV) with an energy gap of 3.37 eV at room temperature, and ZnO NPs have great advantage in catalyst applications owing to their large surface area and high catalytic activity [42].

Nagajyothi et al. [38] reported ZnO NPs synthesized using a Coptidis rhizoma root extract and studied the antibacterial, antioxidant, and cytotoxic activities, and observed nanoparticles with a mean size of 8.50 nm and spherical and rod shapes (Fig. 4.6). They also synthesized ZnO NPs from *P. trifoliata* fruits and examined the catalytic activity using the Claisen–Schmidt condensation reaction [81], (Fig. 4.7).

Similarly, there are many reports on ZnO NPs. Divya et al. [48] synthesized ZnO NPs using *Hibiscus rosa-sinensis* leaf extract and examined their antibacterial activity against *Staphylococcus* aureus, *Escherichia coli*, *Pseudomonas aeruginosa, Klebsiella pneumonia*. The ZnO NPs showed the least activity against *Klebsiella pneumonia*. Vijayakumar et al. [49] reported ZnO NPs synthesized using a *Plectranthus amboinicus* leaf extract and studied S*taphylococcus aureus*. An *Eichhornia crassipes* leaf extract was used for the synthesis of ZnONPs with a mean size of 32±4 nm [50]. Salam et al. [82] synthesized ZnONPs from *Ocimum basilicum* L.; the nanoparticles were hexagonal and less than 20 nm in size.
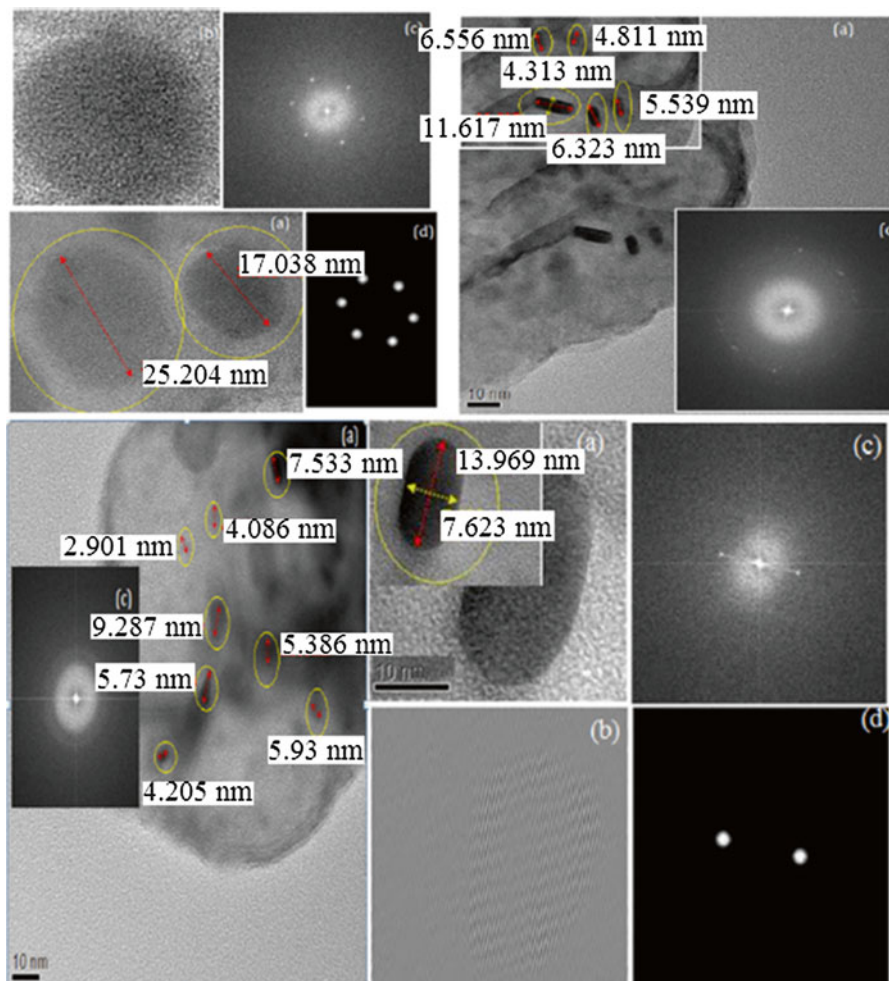
Fig. 4.6 Green-synthesized ZnO NPs from coptidis rhizoma: (**a**) TEM images (**b**) high resolution TEM image (**c**, **d**) selected area electron diffraction (SAED). Reprinted from ref. [38] with permission from Elsevier

Recently, ZnO NPs have been synthesized using different plants, such as *Camellia sinensis* (16 nm) [83], *Borassus flabellifer* fruit extract (rod shape and size ranged from 50 to 60 nm) [84], *Coriandrum sativum* leaf extract (flower shape with a mean size of 66 nm) [85, 86], *Citrus paradisi* peel extract (spherical shape and particle sizes ranging from 12 to 72 nm) [87, 88], leaves of *Adhatoda vasica* (discoid in shape with a mean size of 19–60 nm) [87], *Olea europaea* leaf extract (nanosheets or nanoplatelets with sizes in the range, 18–30 nm) [88], *Azadirachta indica* (nanoflowers of 51 nm), and *Emblica officinalis* (nanoflakes, 16 nm in size) [89] respectively.
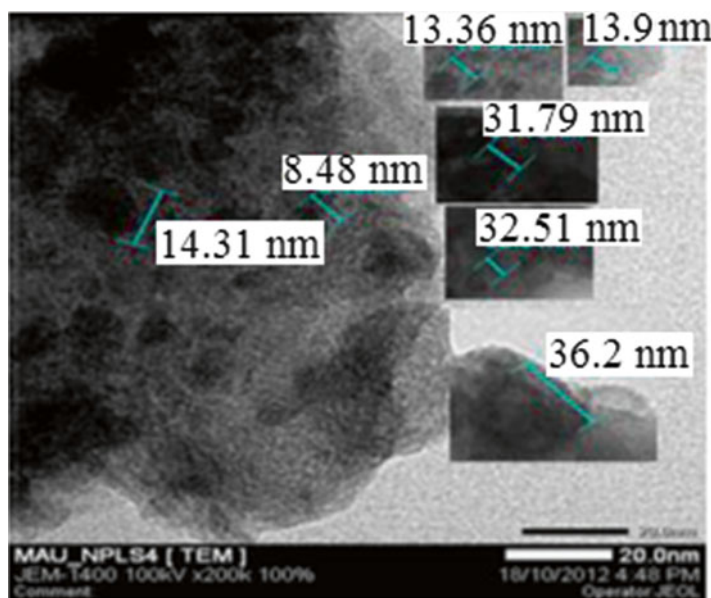
Fig. 4.7 Green-synthesized ZnO NPs from *Poncirus trifoliata.* Reprinted from ref. [81] with permission from Elsevier

4.3 Characterization of Nanoparticles

Nanoparticles are generally characterized by their shape, size, surface area, and disparity. In addition, the homogeneity of these properties is important for many applications. The common instruments used for characterization are as follows [18–21, 28, 29]:

1. UV–Visible spectroscopy.
2. Fourier transform infrared (FT-IR) spectroscopy.
3. Scanning electron microscopy (SEM).
4. Energy dispersive X-ray spectroscopy (EDS).
5. Transmission electron microscopy (TEM).
6. Atomic force microscopy (AFM).
7. X-ray diffraction (XRD).
8. Dynamic light scattering (DLS).

UV–Visible spectroscopy is a commonly used technique. The light wavelengths in the range, 200–700 nm, are generally used to characterize a range of metallic and metal oxide nanoparticles. Spectrophotometric absorption measurements in the wavelength ranges of 400–450, 500–550, and 300–400 nm are used to characterize Ag, Au, and ZnO nanoparticles, respectively.

FTIR spectroscopy is useful for characterizing the surface chemistry. Organic functional groups (e.g., carbonyls, hydroxyls) attached to the surface of nanoparticles and the other surface chemical residues are detected by FTIR spectroscopy.

When the synthesis is completed, the size, shape, and dispersion state of metal and metal oxide nanoparticles are normally measured by SEM, TEM, and AFM.

SEM and TEM are used for morphological characterization on the nanometer to micrometer scale. TEM has a 1,000-fold higher resolution than SEM. The elemental composition of metal nanoparticles is commonly established using energy dispersive spectroscopy (EDS).

AFM offers visualization in three dimensions. The resolution in the vertical, or Z-axis, is limited by the vibration environment of the instrument, whereas the resolution in the horizontal, or X–Y-axis, is limited by the diameter of the tip used for scanning. AFM provides surface characterization on the atomic scale.

XRD is used for phase identification and characterization of the crystal structure of nanoparticles. X-rays penetrate into the nanomaterial and the resulting diffraction pattern is compared with standards to obtain structural information.

DLS is used to characterize the surface charge and size distribution of particles suspended in a liquid.

4.4 Antibacterial Studies of AuNPs

The antibacterial activity of AuNPs with antibiotics exhibit a larger zone of inhibition compared to standard antibiotics [90]. Another study reported similar results for the Marigold flower [91]. The efficacy of the antibacterial activity of AuNPs can be increased by the addition of antibiotics [92]. Williams et al. [93] reported that gold NPs do not affect bacterial growth or functional activity, whereas conjugates of vancomycin to AuNPs decrease the number of growing bacterial cells [94] Gu et al. [95] synthesized gold NPs covered with an antibiotic (vancomycin) and reported significant enhancement of the antibacterial activity for this conjugate compared to the activity of the free antibiotic. Ciprofloxacin with gold nanoshells showed high antibacterial activity against *E. coli* [96, 97]. The coating of aminoglycosidic antibiotics with AuNPs had a significant antibacterial effect on gram-positive and gram-negative bacteria [97]. Cefaclor (a second-generation β-lactam antibiotic)-reduced AuNPs exhibited potent antibacterial activity on both gram-positive (*S. aureus*) and gram-negative bacteria (*E. coli*) compared to Cefaclor and AuNPs alone [98].

4.4.1   Mechanism of the Bactericidal Action of AuNPs

The mechanism of the inhibitory effects of metallic nanoparticles on microorganisms is partially known, but there are still some questions on the mechanism of action along with the spectrum of antibacterial activity [99].

Grace and Pandian [97] reported that the AuNPs have a great bactericidal effect and possess well-developed surface chemistry, chemical stability, and an appropriate smaller size, which make them easier to interact with microorganisms. The nanoparticles bind to the building elements of the outer membrane causing structural changes, degradation, and finally cell death.

Every AuNP is surrounded by a number of stabilizer molecules, which prevent agglomeration and reduce the surface area and interfacial free energy of the nanoparticles, thereby maintaining the particle reactivity [100]. This makes the particles interact easily with the outer membrane components of the cell, and causes significant changes and damage to their surfaces leading to cell death.

Chwalibog et al. [101] reported that the interaction between AuNPs and *Staphylococcus aureus* was prevented by a biofilm and the substance released by the cells caused distortions of the cell wall. AuNPs bind closely to the surface of the microorganisms, causing visible damage to the cells, which can minimize the treatment durations and side effects of drugs [102]. AuNPs generate holes in the cell wall, resulting in leakage of the cell contents and finally cell death. In another way, it can bind to the DNA of bacteria and inhibit DNA transcription [98].

4.5 Antibacterial Studies of AgNPs

Among the non-organic antibacterial agents, Ag ions or Ag NPs are strong antimicrobial agents. A minute amount of silver is harmless to human cells but is biocidal to microbial cells [103].

Priya et al. [104] reported that AgNPs synthesized from banana leaf extract exhibits the maximum inhibition for gram-positive bacteria (*Bacillus*). Silver ions and AgNPs have also inhibitory and lethal effects on both gram-positive and gram-negative bacteria (*E. coli*, and *S. aureus*) [104]. *E. coli* (gram-negative) was less sensitive to Ag NPs compared to gram-positive bacteria *S. aureus*. Similar results were observed by Sondi and Salopek-Sondi [105]. On the other hand, Kim et al. [106] reported that gram-positive *S. aureus* was less affected by AgNPs compared to *E. coli*, even at high concentrations. The AgNPs were tested against both gram-positive and gram-negative bacteria (*E. coli*, *S. aureus*, and *B. substilis*) in liquid systems. The concentrations tested (50, 70, and 90 μg/μL) produced inhibition percentages of 11.43, 40.26, and 51.53; 9.94, 11.95, and 43.64; and 8.69, 12.5, and 44.63 mm, respectively [51]. Agar well diffusion studies showed that green synthesized AgNPs exhibited effective inhibitory action against *S. aureus*, *B. cereus*, *L. monocytogenes*, and *S. flexneri* [107]. The effects of AgNPs, penicillin and tetracycline against both gram-negative (*E. coli and P. mirabilis*) and gram-positive bacteria (*S. epidermidis*) were examined using the disk diffusion method. The antibacterial activity of penicillin against gram-negative and gram-positive bacteria was greater in the presence of AgNPs than tetracycline. The largest increase in the fold area was observed against *S. epidermidis* (75 %) followed in order by *E. coli* (46.66 %) and *P. mirabilis* (13.63 %). Tetracycline in combination with AgNPs

produced an increase in the fold area against *S. epidermidis* (42.85 %) followed in order by *E. coli* (31.57 %) and *P. mirabilis* (9.09 %) [28]. Raffin et al. [108] reported that 16 nm silver nanoparticles were completely cytotoxic to the gram-negative bacteria, *E. coli*, at low concentrations (60 μg/mL).

4.5.1   Mechanism of the Bactericidal Action of AgNPs

The antibacterial mechanism(s) of AgNPs are not completely understood, but various studies have shown that Ag NPs can attach to the surface of the cell membrane, thereby disturbing the permeability and respirational functions of the cell [109]. Furthermore, AgNPs interact with the surface of a membrane and can penetrate the bacterium [110].

Wang et al. [111] reported that AgNPs react with sulfur-containing proteins in the interior of the cells, and those phosphorous-containing compounds, such as vital enzymes and DNA bases, will affect the respiratory chain and cell division in bacteria, ultimately causing cell death.

Key factors such as size (surface area) and particle shape affect the antibacterial activity of AgNPs [112]. Pal et al. reported that triangular AgNPs are more effective against the gram-negative bacteria, *E. coli*, than spherical and rod-shaped AgNPs, suggesting that the shape of AgNPs should be considered when developing highly efficient antibacterial agents [113].

4.6   Antibacterial Studies of ZnO NPs

Among the several metal oxides studied for their antibacterial activity, ZnO NPs exhibit selective toxicity to bacteria but have a minimal effect on human cells [114–117]. Jayaseelan et al. [118] reported that the green synthesized ZnO NPs showed the maximum inhibition zones in the ZnO NPs (25 μg/mL) *P. aeruginosa* (22 ± 1.8 mm) and *A. flavus* (19 ± 1.0 mm). *A. hydrophila*, *E. coli*, *E. faecalis*, and *C. albicans* exhibited a minimum inhibitory concentration at 1.2, 1.2, 1.5, and 0.9 μg/mL. The antibacterial activity of ZnO NPs was evolved against gram-negative and -positive bacteria using the resazurin incorporation method. The MICs of ZnO NPs against *E. coli*, *P. aeruginosa*, and *S. aureus* were observed at 500 μg/mL, 500 μg/mL, and 125 μg/mL, respectively, by the resazurin incorporation method [119]. The MIC of the ZnO nanoparticles against *E. coli* was found to be 500 μg/mL using the disk diffusion method [119]. ZnONPs had a much stronger antibacterial effect on gram-positive bacteria than on gram-negative bacteria [120, 121]. Other reports have confirmed the strong antimicrobial activity of ZnO NPs in the food-borne bacteria, *Salmonella typhimurium* and *Staphylococcus aureus* [122]. In another report, 12 nm ZnO NPs inhibited the growth of *E. coli* by disintegrating the cell membrane and increasing the membrane permeability [123]. Nagajyothi et al. [38] reported the

maximum antibacterial activity against *E. coli* (11 mm) according to the disk diffusion method with much less activity against *B. megaterium* (10 mm), *B. pumilus* (9 mm), and *B. cereus* (9 mm). Sawai [120] reported that ZnO is a biocidal agent and that the antibacterial activity was dependent on the agent concentration.

4.6.1 Mechanism of the Bactericidal Action of ZnO NPs

The production of hydrogen peroxide from the surface of ZnO is considered as an efficient means for the inhibition of bacterial growth [121]. The number of ZnO powder particles per unit volume of powder slurry increases with decreasing particle size, resulting in an increased surface area and increased generation of hydrogen peroxide. The antibacterial activity of ZnO was attributed to the release of Zn^{2+} ions, which damage the cell membrane and interact with the intracellular contents [114]. The antibacterial activity of nanoparticles depends on its size, by which ZnO NPs smaller in size than the pore size in the bacteria can cross the cell membrane without hindrance [121].

4.7 Conclusions

This chapter summarizes recent research works on the synthesis of gold, silver, and zinc oxide nanoparticles using plant extracts and their potential applications in the field of biology. Chemical synthesis methods have enjoyed a colorful history in the production of metallic and metal oxide nanoparticles. These chemically produced nanoparticles have however been implicated in cellular and tissue toxicity. Moreover, the production is environmentally unfriendly and quite expensive. This has made the use of plants the preferred alternatives. Green synthesized gold, silver, and zinc oxide nanoparticles have demonstrated mild to exceptional antimicrobial activities over the past few years. However, one major challenge confronting this biogenic method is the attachment of biological materials onto the nanostructure. Further studies are therefore warranted into the green synthesis of unadulterated nanostructures which may further improve activity and reduce the unlikely probability of toxicity.

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