



Green Synthesis of Metal Nanoparticles: Characterization and their Antibacterial Efficacy

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Abstract

The global emergence and spread of multi-drug resistance in bacterial pathogens has led the researchers to focus on the development of alternative measures or therapeutic agents to combat microbial infections caused by drug-resistant bacteria. Recent advances in nanotechnology have given a new hope for the development of novel nano-based formulations to address the problem. Green synthesis of metal nanoparticles has advantages over other chemical and physical methods such as, reduced toxicity, one-step process, cost-effectiveness, eco-friendliness, and does not require additional capping or stabilizing agents. The techniques employed for the characterization of nanoparticles are UV–Vis spectroscopy, XRD, FTIR, SEM, EDX, TEM, AFM, DLS, zeta potential, and TGA etc. The antibacterial nanoparticles studied most widely are metal nanoparticles. Metal nanoparticles have demonstrated potent antibacterial activity *in vitro* with promising therapeutic potential in wound dressings, medical implant coatings, drug delivery, tumour detection, and photoimaging. Many bioagents such as bacteria, fungi, plant extracts, etc. have been exploited for green synthesis of nanoparticles. Medicinal plants as bio-templates for the synthesis of metal nanoparticles might show an immense impact in biomedicine. However, toxicity of nanoparticles to the host is a major concern that needs to be scrutinized properly to monitor its long-term impact. Still, recently developed nano-based formulations, including metal nanoparticles, are expected to become the next generation therapeutic agents against bacterial pathogens, especially against MDR bacteria. In this chapter, we have reviewed the role of medicinal plants in metal nanoparticle synthesis, characterization techniques, and their efficacy against bacterial pathogens.

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Keywords

Nanoparticles · Antibacterial agents · Toxicity · Nanoparticles synthesis · Characterization

Abbreviations

AFM	Atomic Force Microscopy
DLS	Dynamic light Scattering
FTIR	Fourier Transform Infrared Spectroscopy
SEM	Scanning Electron Microscopy
TEM	Transmission Electron Microscopy
TGA	Thermogravimetric Analysis
UV Vis	UV Visible
XRD	X-ray diffraction

1 Introduction

Over the last few decades, a tremendous increase in the emergence of multi-drug-resistant (MDR) bacterial pathogens has been reported globally. The most common groups of bacteria that are considered problematic includes *S. aureus* (MRSA), *P. aeruginosa*, *Mycobacterium* (MDR), and the extended-spectrum beta-lactamases (ESBL) and carbapenemase-producing *Enterobacteriaceae*. Such MDR bacteria have developed one or more mechanisms by which their susceptibility to antibiotics has been drastically reduced. Therefore, in the era of the emergence of MDR, the efficacy of conventional antibiotics can no longer be trusted for prolonged administration. In the search of novel therapeutic agents to combat MDR bacteria, nanoparticles so far have proven to be a good alternative based on in vitro studies. The recent advances in nanotechnology such as the green route of nanoparticle synthesis has given a new hope for the development of novel nano-based formulations to address the problem. There are numerous bioagents used for the synthesis of nanoparticles. However, the use of plant extracts has clear advantages over other agents (Singh et al. 2016a).

Nanobiotechnology is a fast-developing discipline of science that mainly focuses on synthesis, manipulation, and application of materials at nanometre scale in multiple fields of biology (Shah et al. 2015). Nanoparticles have become of great interest due to their novel physicochemical, optoelectronic, and magnetic properties that are primarily governed by their shape, size, and size distribution (Bogunia-Kubik and Sugisaka 2002; Zharov et al. 2005). Being so small in size and having large surface area-to-volume ratios, they exhibit significant differences in catalytic activity, biological properties, mechanical properties, electrical conductivity etc. in

comparison to the same material in its bulk form or at larger scales (Iravani 2011). The emerging trend of plant-mediated synthesis of metal nanoparticles such as silver, gold etc. has become a focus of attention, leading to the development of multiple methods for their synthesis (Rauwel and Rauwel 2017).

Several efforts have been made to develop environmentally friendly green nanotechnologies and to produce nanoparticles using non-toxic products (Joerger et al. 2000; Chauhan et al. 2012). The synthesis of nanoparticles, using biological entities or green technology, is a one-step procedure resulting in the production of nanoparticles with greater stability and more precise dimensions, which eliminates undesirable processing conditions at a negligible cost (Ingale 2013). Recent advancements in this discipline have been explored for their application in agricultural, biomedical, environmental, and physicochemical areas (Pereira et al. 2015; Rao and Gan 2015; Rai et al. 2016). Silver nanoparticles are the most extensively studied and been found to be potent antibacterial and anti-inflammatory agents that promote faster wound healing. As a result of their therapeutic value, silver nanoparticles are ingredients of pharmaceutical preparations, commercially available wound dressings, and medical implant coatings (Huang et al. 2007b; Pollini et al. 2011; Cox et al. 2011). Similarly, gold nanoparticles have been used in the delivery of certain drugs including paclitaxel, methotrexate, and doxorubicin (Rai et al. 2016). Gold nanoparticles have been also reported for their application in angiogenesis, tumour detection, photoimaging, genetic disorder diagnosis, and photothermal therapy (Singh et al. 2016a). Moreover, being non-toxic, biocompatible, self-cleansing, skin-compatible, antimicrobial, and dermatological in nature, titanium and zinc nanoparticles have been used in cosmetic, biomedical, as UV-blocking agents, and in many other cutting-edge processing technologies (Ambika and Sundrarajan 2015; Zahir et al. 2015). Green synthesis of metal nanoparticles also includes other biological sources such as microbes (bacteria, fungi, algae etc.). In this chapter, we have reviewed plant-mediated synthesis of metal nanoparticles, with a focus on their efficacy against pathogenic bacteria with respect to drug-resistant pathogens. While some excellent review articles are available related to green synthesis of nanoparticles and their antibacterial activity, none of them focussed on MDR bacteria (Shah et al. 2015; Ahmed et al. 2016; Kuppusamy et al. 2016; Singh et al. 2016a).

2 Synthesis of Metal Nanoparticles Using Plants

In last two decades, there has been considerable emphasis on synthesis of nanoparticles using biological agents such as plants, microbes, etc. as these methods are considered less expensive, safe, and eco-friendly alternative to chemical synthesis (Makarov et al. 2014; Gowramma et al. 2015). The use of plants for the synthesis of nanoparticles is even more advantageous than using microorganisms as microbes require culturing, which makes them relatively expensive. In addition, the use of pathogenic microbes or toxic microbial products is often required, adding additional burden (Ahmed et al. 2016). On the other hand, plant metabolites (primary or secondary) such as amino acids, proteins, enzymes, polysaccharides, tannins,

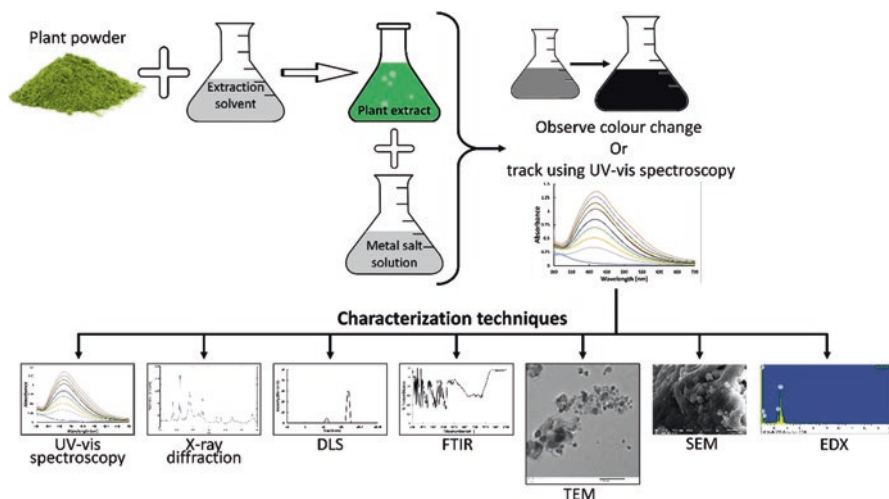


Fig. 1 A representative illustration of metal nanoparticle synthesis and the commonly used techniques for their characterization

alkaloids, phenolics, terpenoids, saponins, and vitamins are used for the reduction of metal ions to make nanoparticles and are environmentally safe (Kulkarni and Muddapur 2014; Kuppusamy et al. 2016). In general, the methods of synthesis of metal nanoparticles have some common stages; (i) collection and washing (distilled water preferably) of the plant's part of interest to remove any debris or dust if present; (ii) shade drying of the sample and then grinding it using a blender to make fine powder; (iii) preparation of plant extract in desirable solvents by dissolving and filtering; and (iv) addition of plant extracts to salt solutions of metals in different combinations for standardization of synthesis (Ahmed et al. 2016). A representative illustration of metal nanoparticle synthesis and the commonly used techniques for their characterization is presented in Fig. 1.

2.1 Silver Nanoparticles

Synthesis of silver nanoparticles using plant material has been commonly reported. To date, a large number of plants have been successfully employed to synthesize silver nanoparticles as mentioned in Table 1, which summarizes the literature from 2009 to 2018 on plant-mediated synthesis, techniques used for their characterization, and organisms against which the nanoparticles were tested, using PubMed and other sources. Some of the recent reports are further elaborated below. Silver nanoparticles have been synthesized using aqueous extract of *Alternanthera dentate* by Kumar et al. (2014a). The nanoparticles were spherical in shape and 50–100 nm in size. It took 10 min to reduce silver ions to silver nanoparticles, validating the technique to be a simple, quick, and economical process. These green synthesized

Table 1 List of plant extract-mediated synthesis of silver nanoparticles, characterization techniques, and the bacteria tested for antimicrobial activity

S. No.	Plant	Part used	Extraction solvent	Average size/ range of NPs	Characterization techniques	Test bacteria	References
1	<i>Acacia leucophloea</i>	Stem bark	Aqueous	17–29 nm	UV–Vis, XRD, TEM, and FTIR	<i>S. aureus</i> , <i>B. cereus</i> , <i>L. monocytogenes</i> , and <i>S. flexneri</i>	Murugan et al. (2014)
2	<i>Acalypha indica</i>	Leaf	Aqueous	20–30 nm	UV–vis, SEM, HRTEM, XRD, EDS, and SEM	<i>E. coli</i> and <i>V. cholerae</i>	Krishnaraj et al. (2010)
3	<i>Actaea racemosa</i>	Leaf	Aqueous	3–15 nm	UV–Vis, TEM, and AFM	<i>E. coli</i> , <i>S. typhimurium</i> , <i>P. aeruginosa</i> , <i>S. aureus</i> , <i>K. rhizophila</i> , and <i>B. thuringiensis</i>	Okafor et al. (2013)
4	<i>Allium sativum</i>	Bulb	Aqueous	7.3 ± 4.4 nm	UV–Vis, XRD, TEM, and FTIR	<i>P. aeruginosa</i> and <i>S. aureus</i>	Rastogi and Arunachalam (2011)
5	<i>Allophylus cobbe</i>	Leaf	Aqueous	2–10 nm	UV–Vis, XRD, DLS, TEM, and FTIR	<i>P. aeruginosa</i> , <i>S. flexneri</i> , <i>S. aureus</i> , and <i>S. pneumonia</i>	Gurunathan et al. (2014)
6	<i>Aloe vera</i>	Leaf	Ethanol	35–55 nm	UV–Vis, SEM, EDX, and FTIR	<i>B. subtilis</i> , <i>K. pneumoniae</i> , and <i>S. typhi</i>	Dinesh et al. (2015)
7	<i>Alternanthera dentata</i>	Leaf	Aqueous	10–80 nm	UV–Vis, XRD, TEM, and FTIR	<i>E. coli</i> , <i>P. aeruginosa</i> , <i>K. pneumoniae</i> , and <i>E. faecalis</i>	Kumar et al. (2014a)
8	<i>Alternanthera sessilis</i>	Leaf	Aqueous	20–30 nm	UV–Vis, FTIR, SEM, TGA, and zeta size	<i>S. aureus</i> and <i>E. coli</i>	Niraimathi et al. (2013)
9	<i>Ananas comosus</i>	Leaf	Aqueous	12.4 nm	UV–Vis, XRD, TEM, and FTIR	<i>S. aureus</i> , <i>S. pneumoniae</i> , <i>P. mirabilis</i> , and <i>E. coli</i>	Emeka et al. (2014)
10	<i>Anogeissus latifolia</i>	Gum	Aqueous	5.7 ± 0.2 nm	UV–Vis, XRD, TEM, and FTIR	<i>S. aureus</i> , <i>E. coli</i> , and <i>P. aeruginosa</i>	Kora et al. (2012)
11	<i>Argemone mexicana</i>	Leaf	Aqueous	10–50 nm	UV–Vis, XRD, SEM, and FTIR	<i>E. coli</i> and <i>P. syringae</i>	Singh et al. (2010)

(continued)

Table 1 (continued)

S. No.	Plant	Part used	Extraction solvent	Average size/ range of NPs	Characterization techniques	Test bacteria	References
12	<i>Artemisia nilagirica</i>	Leaf	Aqueous	70–90 nm	SEM and EDX	<i>S. aureus</i> , <i>E. coli</i> , <i>B. subtilis</i> , and <i>P. mirabilis</i>	Vijayakumar et al. (2013)
13	<i>Artocarpus heterophyllus</i>	Seeds	Aqueous	10.78 nm	UV–Vis, TEM, SEM, EDX, and FTIR	<i>B. cereus</i> , <i>B. Subtilis</i> , <i>S. aureus</i> , and <i>P. aeruginosa</i>	Jagtap and Bapat (2013)
14	<i>Atrocarpus altilis</i>	Leaf	Aqueous	25–43 nm	UV–Vis, XRD, SEM, EDX, DLS, and FTIR	<i>E. coli</i> , <i>P. aeruginosa</i> , and <i>S. aureus</i>	Ravichandran et al. (2016)
15	<i>Azadirachta indica</i>	Bark	Aqueous	90.13 nm	UV–Vis, XRD, SEM, AFM, DLS, and FTIR	<i>E. coli</i> , <i>P. aeruginosa</i> , and <i>B. subtilis</i> , and <i>V. cholerae</i>	Nayak et al. (2016)
16	<i>Azhadirachta indica</i>	Leaf	Aqueous	21 nm	UV–Vis, DLS, and FTIR	<i>K. pneumoniae</i> and <i>S. typhi</i>	Lalitha et al. (2013)
17	<i>Beta vulgaris</i>	Roots	Aqueous	10–15 nm	UV–Vis, XRD, TEM, EDS, and FTIR	<i>E. coli</i> , <i>P. aeruginosa</i> , and <i>S. aureus</i>	Bindhu and Umadevi (2015)
18	<i>Boerhaavia diffusa</i>	Whole plant	Aqueous	25 nm	UV–Vis, XRD, SEM, TEM, and FTIR	<i>A. hydrophila</i> , <i>P. fluorescens</i> , and <i>F. branchiophilum</i>	Kumar et al. (2014b)
19	<i>Caesalpinia coriaria</i>	Leaf	Aqueous	40–52 nm	UV–Vis, XRD, SEM, and FTIR	<i>E. coli</i> , <i>P. aeruginosa</i> , <i>K. pneumoniae</i> , and <i>S. aureus</i>	Jeeva et al. (2014)
20	<i>Cajanus cajan</i>	Leaf	Aqueous	5–60 nm	UV–Vis, SEM, TEM, and FTIR	<i>M. luteus</i> , <i>S. aureus</i> , and <i>E. coli</i>	Nagati et al. (2012)
21	<i>Calotropis gigantea</i>	Leaf	Aqueous	6–12 nm	UV–Vis and AFM	<i>V. Alginolyticus</i>	Baskaralingam et al. (2012)
22	<i>Carica papaya</i>	Fruit	Aqueous	10–50 nm	UV–Vis, XRD, SEM, and FTIR	<i>E. coli</i> and <i>P. aeruginosa</i>	Jain et al. (2009)

23	<i>Centella asiatica</i>	Leaf	Ethanol	42 nm	UV-Vis, XRD, AFR, and FTIR	<i>S. aureus</i> , <i>P. aeruginosa</i> , <i>K. pneumoniae</i> , and <i>E. coli</i>	Logeswari et al. (2013)
24	<i>Centella asiatica</i>	Leaf	Aqueous	24 nm	UV-Vis, XRD, SEM, and AFM	<i>S. aureus</i> , <i>P. aeruginosa</i> , <i>E. coli</i> , and <i>K. pneumoniae</i>	Logeswari et al. (2015)
25	<i>Ceratonia siliqua</i>	Leaf	Aqueous	5–40 nm	UV-Vis, XRD, SEM, AAS, and FTIR	<i>E. coli</i>	Awwad et al. (2013)
26	<i>Ceropegia thwaitesii</i>	Leaf	Aqueous	110.6 nm	UV-Vis, XRD, SEM, EDX, and FTIR	<i>E. coli</i> , <i>V. cholerae</i> , <i>S. aureus</i> , <i>Corynebacterium</i> , <i>P. aeruginosa</i> , <i>S. epidermidis</i> , <i>Mycobacterium</i> , <i>S. typhi</i> , <i>S. flexneri</i> , <i>K. pneumoniae</i> , <i>B. subtilis</i> , <i>M. luteus</i> , and <i>P. mirabilis</i>	Muthukrishnan et al. (2015)
27	<i>Chenopodium murale</i>	Leaf	Aqueous	30–50 nm	UV-Vis and TEM	<i>S. aureus</i>	Abdel-Aziz et al. (2014)
28	<i>Chrysanthemum indicum</i>	Flower	Aqueous	37–72 nm	UV-Vis, XRD, TEM, and EDX	<i>B. subtilis</i> , <i>S. aureus</i> , <i>S. epidermidis</i> , <i>E. coli</i> , <i>K. pneumoniae</i> , and <i>P. aeruginosa</i>	Arokiyaraj et al. (2014)
29	<i>Cinnamon zeylanicum</i>	Bark	Aqueous	31–40 nm	UV-Vis, XRD, TEM, and zeta potential	<i>E. coli</i>	Sathishkumar et al. (2009b)
30	<i>Citrus sinensis</i>	Peel	Ethanol	41 nm	UV-Vis, XRD, AFR, and FTIR	<i>S. aureus</i> , <i>P. aeruginosa</i> , <i>E. coli</i> , and <i>K. pneumoniae</i>	Logeswari et al. (2013)
31	<i>Citrus sinensis</i>	Peel	Aqueous	59 nm	UV-Vis, XRD, SEM, and AFM	<i>S. aureus</i> , <i>P. aeruginosa</i> , <i>E. coli</i> , and <i>K. pneumoniae</i>	Logeswari et al. (2015)
32	<i>Clitoria ternatea</i>	Leaf	Aqueous	20 nm	UV-Vis, XRD, SEM, and FTIR	<i>B. subtilis</i> , <i>S. aureus</i> , <i>S. pyogenes</i> , <i>E. coli</i> , <i>P. aeruginosa</i> , and <i>K. aerogenes</i>	Krithiga et al. (2015)

(continued)

Table 1 (continued)

S. No.	Plant	Part used	Extraction solvent	Average size/ range of NPs	Characterization techniques	Test bacteria	References
33	<i>Cocos nucifera</i>	Inflorescences	Ethyl acetate and methanol (40:60)	22 nm	UV-Vis, TEM, and FTIR	<i>V. alginolyticus</i> , <i>P. shigelloides</i> , <i>K. pneumoniae</i> , <i>S. paratyphi</i> , <i>P. aeruginosa</i> , <i>V. harveyi</i> , <i>B. subtilis</i> , <i>E. coli</i> , <i>V. mimicus</i> , and <i>S. aureus</i>	Maniselvam et al. (2014)
34	<i>Coffea arabica</i>	Seed	Water and ethanol (1:1)	20–30 nm	UV-Vis, XRD, SEM, EDAX, TEM, and FTIR	<i>E. coli</i> and <i>S. aureus</i>	Dhand et al. (2016)
35	<i>Coleus aromaticus</i>	Leaf	Aqueous	40–50 nm	UV-Vis, XRD, SEM, EDAX, and FTIR.	<i>B. subtilis</i> and <i>K. planticola</i>	Vanaja and Annadurai (2013)
36	<i>Coriandrum sativum</i>	Seed	Aqueous	13.09 nm	UV-Vis, XRD, SEM, DLS, and FTIR	<i>B. subtilis</i>	Nazeruddin et al. (2014)
37	<i>Crataegus douglasii</i>	Fruit	Aqueous		UV-Vis and SEM	<i>E. coli</i> and <i>S. aureus</i>	Ghaffari-Moghaddam and Hadi-Dabanlou (2014)
38	<i>Croton sparsiflorus morong</i>	Leaf	Aqueous	22–52 nm	UV-Vis, XRD, SEM, EDX, and FTIR	<i>S. aureus</i> , <i>E. coli</i> , and <i>B. subtilis</i>	Kathiravan et al. (2015)
39	<i>Cymbopogon citratus</i>	Leaf	Aqueous	32 nm	UV-Vis, NTA, TEM, and EDAX	<i>E. coli</i> , <i>S. aureus</i> , <i>P. mirabilis</i> , <i>S. typhi</i> , and <i>K. pneumoniae</i>	Masurkar et al. (2011)
40	<i>Dalbergia spinosa</i>	Leaf	Aqueous	18 ± 4 nm	UV-Vis, TEM, EDS, and FTIR.	<i>B. subtilis</i> , <i>P. aeruginosa</i> , <i>S. aureus</i> , and <i>E. coli</i>	Muniyappan and Nagarajan (2014a)
41	<i>Delphinium denudatum</i>	Root	Aqueous	≤85 nm	UV-Vis, XRD SEM, and FTIR	<i>S. aureus</i> , <i>B. cereus</i> , <i>E. coli</i> , and <i>P. aeruginosa</i>	Suresh et al. (2014)

42	<i>Dioscorea batatas</i>	Rhizome	Aqueous	N.A.	UV-Vis, XRD, SEM, EDX, and FTIR	<i>B. subtilis</i> , <i>S. aureus</i> , and <i>E. coli</i>	Nagajyothi and Lee (2011)
43	<i>Dioscorea bulbifera</i>	Tuber	Aqueous	8–20 nm	UV-Vis, XRD, TEM, DLS, and FTIR	<i>A. baumannii</i> , <i>E. cloacae</i> , <i>E. coli</i> , <i>H. influenzae</i> , <i>K. pneumoniae</i> , <i>N. mucosa</i> , and <i>P. mirabilis</i>	Ghosh et al. (2012)
44	<i>Diospyros paniculata</i>	Root	Methanol	14–28 nm	UV-Vis, XRD, SEM, and TEM	<i>B. subtilis</i> , <i>B. pumilis</i> , <i>S. pyogenes</i> , <i>S. aureus</i> , <i>E. coli</i> , <i>K. pneumoniae</i> , <i>P. vulgaris</i> , and <i>P. aeruginosa</i>	Rao et al. (2016)
45	<i>Dodonaea viscosa</i>	Leaf	Aqueous	16 nm	UV-Vis, XRD, AFM, TEM, and FTIR	<i>E. coli</i> , <i>K. pneumoniae</i> , <i>P. fluorescens</i> , <i>B. subtilis</i> , and <i>S. aureus</i>	Kiruba Daniel et al. (2013)
46	<i>Elettaria cardanmomom</i>	Seeds	Aqueous	40–70 nm	UV-Vis, XRD, SEM, EDAX, and FTIR	<i>B. subtilis</i> and <i>K. planticola</i>	Gnanajobitha et al. (2012)
47	<i>Emblica officinalis</i>	Fruit	Aqueous	10–70 nm	UV-Vis, XRD, SEM, and FTIR	<i>S. aureus</i> , <i>B. subtilis</i> , <i>E. coli</i> , and <i>K. pneumoniae</i>	Ramesh et al. (2015)
48	<i>Erythrina indica</i>	Root	Aqueous	20–118 nm	UV-Vis, XRD, DLS, TEM, EDX, and FTIR	<i>S. aureus</i> , <i>M. luteus</i> , <i>E. coli</i> , <i>B. subtilis</i> , <i>S. typhi</i> , and <i>S. paratyphi</i>	Rathi Sre et al. (2015)
49	<i>Eucalyptus angophoroides</i>	Leaf	Aqueous	3–15 nm	UV-Vis, TEM, and AFM	<i>E. coli</i> , <i>S. typhimurium</i> , <i>S. aureus</i> , <i>P. aeruginosa</i> , <i>K. rhizophila</i> , and <i>B. thuringiensis</i>	Okafor et al. (2013)
50	<i>Eucalyptus chapmaniana</i>	Leaf	Methanol	60 nm	UV-Vis and XRD	<i>S. aureus</i> , <i>S. pneumoniae</i> , <i>E. coli</i> , <i>P. vulgaris</i> , <i>P. aeruginosa</i> , and <i>K. pneumoniae</i>	Sulaiman et al. (2013)

(continued)

Table 1 (continued)

S. No.	Plant	Part used	Extraction solvent	Average size/ range of NPs	Characterization techniques	Test bacteria	References
51	<i>Eucalyptus globulus</i>	Leaf	Aqueous	5–25 nm	UV–Vis, XRD, SEM, EDX, TEM, and FTIR	<i>E. coli</i> , <i>P. aeruginosa</i> , and <i>S. aureus</i>	Ali et al. (2015)
52	<i>Euphorbia hirta</i>	Leaf	Aqueous	40–50 nm	UV–Vis and SEM	<i>S. aureus</i> , <i>E. coli</i> , <i>K. pneumoniae</i> , <i>B. cereus</i> , and <i>P. aeruginosa</i>	Elumalai et al. (2010)
53	<i>Euphorbia milii</i>	Latex	–	105	UV–Vis, particle sizer, and TEM	<i>P. aeruginosa</i> , <i>E. coli</i> , <i>S. aureus</i> , <i>S. epidermis</i> , and <i>M. luteus</i>	Patil et al. (2012)
54	<i>Euphorbia nivulia</i>	Latex from stem	Aqueous	5–10 nm	UV–Vis, TEM, and FTIR	<i>E. coli</i> and <i>S. aureus</i>	Valodkar et al. (2011)
55	<i>Ficus benghalensis</i>	Leaf	Aqueous	16 nm	UV–Vis, XRD, TEM, and EDX	<i>E. coli</i>	Saxena et al. (2012)
56	<i>Ficus benghalensis</i>	Bark	Aqueous	85.95 nm	UV–Vis, XRD, SEM, AFM, DLS, and FTIR	<i>E. coli</i> , <i>P. aeruginosa</i> , and <i>B. subtilis</i> and <i>V. cholerae</i>	Nayak et al. (2016)
57	<i>Garcinia mangostana</i>	Leaf	Aqueous	35 nm	UV–Vis, TEM, and FTIR	<i>E. coli</i> and <i>S. aureus</i>	Veerasamy et al. (2011)
58	<i>Hibiscus cannabinus</i>	Leaf	Aqueous	8–14 nm	UV–Vis, XRD, TEM, EDX, and FTIR	<i>E. coli</i> , <i>P. mirabilis</i> , and <i>S. flexneri</i>	Bindhu and Umadevi (2013)
59	<i>Impatiens balsamina</i>	Leaf	Aqueous	3–15 nm	UV–Vis, TEM, and AFM	<i>E. coli</i> , <i>S. typhimurium</i> , <i>S. aureus</i> , <i>P. aeruginosa</i> , <i>K. rhizophila</i> , and <i>B. thuringiensis</i>	Okafor et al. (2013)
60	<i>Iresine herbstii</i>	Leaf	Aqueous	44–64 nm	UV–Vis, XD, SEM, EDX, and FTIR	<i>S. aureus</i> , <i>E. faecalis</i> , <i>E. coli</i> , <i>K. pneumoniae</i> , and <i>P. aeruginosa</i>	Dipankar and Murugan (2012)
61	<i>Jatropha gossypifolia</i>	Latex	–	62	UV–Vis, particle sizer, and TEM	<i>P. aeruginosa</i> , <i>E. coli</i> , <i>S. aureus</i> , <i>S. epidermis</i> , and <i>M. luteus</i>	Patil et al. (2012)

62	<i>Lactuca sativa</i>	Leaf	Aqueous	40–70 nm	UV–Vis, XRD, SEM, TEM, and FTIR	<i>B. subtilis</i> , <i>E. coli</i> , <i>K. pneumoniae</i> , and <i>P. aeruginosa</i>	Kanchana et al. (2011)
63	<i>Lantana camara</i>	Fruit	Aqueous	12–13 nm	UV–Vis, TEM, and FTIR	<i>M. luteus</i> , <i>B. subtilis</i> , <i>S. aureus</i> , <i>V. cholerae</i> , <i>K. pneumoniae</i> , and <i>S. typhi</i>	Sivakumar et al. (2012)
64	<i>Lycopersicon esculentum</i>	Fruit	Aqueous	10–40 nm	UV–Vis and TEM	<i>E. coli</i>	Maiti et al. (2014)
65	<i>Magnolia grandiflora</i>	Leaf	Aqueous	3–15 nm	UV–Vis, TEM, and AFM	<i>S. typhimurium</i> , <i>P. aeruginosa</i> , <i>S. aureus</i> , <i>K. rhizophila</i> , <i>E. coli</i> , and <i>B. thuringiensis</i>	Okafor et al. (2013)
66	<i>Mangifera indica</i>	Peel	Aqueous	7–27 nm	UV–Vis, XRD, TEM, SEM, and FTIR	<i>E. coli</i> , <i>S. aureus</i> , and <i>B. subtilis</i>	Yang and Li (2013)
67	<i>Mentha piperita</i>	Leaf	Aqueous	90 nm	UV–Vis, SEM, EDS, and FTIR	<i>E. coli</i> and <i>S. aureus</i>	MubarakAli et al. (2011)
68	<i>Mimusops elengi</i>	Leaf	Aqueous	55–83 nm	UV–Vis, XRD, SEM, and FTIR	<i>K. pneumoniae</i> , <i>M. luteus</i> , and <i>S. aureus</i>	Prakash et al. (2013)
69	<i>Moringa oleifera</i>	Leaf	Aqueous	57 nm	UV–Vis and TEM	<i>S. aureus</i> , <i>E. coli</i> , <i>K. pneumoniae</i> , and <i>B. cereus</i>	Prasad and Elumalai (2011)
70	<i>Murraya koenigii</i>	Leaf	Aqueous	40–80 nm	UV–Vis, XRD, SEM, and FTIR	<i>E. coli</i> , <i>S. aureus</i> , and <i>P. aeruginosa</i>	Bonde et al. (2012)
71	<i>Musa balbisiana</i>	Leaf	Aqueous	<200 nm	UV–Vis, SEM, TEM, EDS, and FTIR	<i>E. coli</i> and <i>Bacillus</i> spp.	Banerjee et al. (2014)
72	<i>Musa paradisiaca</i>	Peels	Aqueous	23.7 nm	UV–Vis, XRD, SEM, EDX, TEM, and FTIR	<i>B. subtilis</i> , <i>S. aureus</i> , <i>E. coli</i> , and <i>P. aeruginosa</i>	Ibrahim (2015)
73	<i>Nyctanthes arbor-tristis</i>	Flower	Ethanol	5–20 nm	UV–Vis, XRD, TGA, TEM, and FTIR	<i>E. coli</i>	Gogoi et al. (2015)

(continued)

Table 1 (continued)

S. No.	Plant	Part used	Extraction solvent	Average size/ range of NPs	Characterization techniques	Test bacteria	References
74	<i>Ocimum sanctum</i>	Leaf	Methanol	40–50 nm	UV–Vis and SEM	<i>S. aureus</i> , <i>S. saprophyticus</i> , <i>E. coli</i> , <i>K. pneumoniae</i> , <i>E. faecalis</i> , <i>E. cloacae</i> , and <i>P. vulgaris</i>	Rout et al. (2012)
75	<i>Ocimum tenuiflorum</i>	Leaf	Aqueous	26 nm	UV–Vis, XRD, SEM, and AFM	<i>S. aureus</i> , <i>P. aeruginosa</i> , <i>E. coli</i> , and <i>K. pneumoniae</i>	Logeswari et al. (2015)
76	<i>Olea europaea</i>	Leaf	Aqueous	20–25 nm	UV–Vis, XRD, TEM, and FTIR	<i>S. aureus</i> , <i>P. aeruginosa</i> , and <i>E. coli</i>	Khalil et al. (2014)
77	<i>Opuntia ficus-indica</i>	Leaf	Aqueous	12 nm	UV–Vis, XRD, SEM, and FTIR	<i>E. coli</i> and <i>S. aureus</i>	Gade et al. (2010)
78	<i>Origanum heracleoticum</i>	Leaf	Aqueous	30–40 nm	UV–Vis, XRD, SEM, EDX, and FTIR	<i>E. coli</i> , <i>P. aeruginosa</i> , <i>S. aureus</i> , <i>S. pneumoniae</i> , and <i>K. pneumoniae</i>	Rajendran et al. (2015)
79	<i>Origanum vulgare</i>	Leaf	Aqueous	136 ± 10.09 nm	UV–Vis, FTIR, SEM, XRD, and zeta sizer	<i>A. hydrophilla</i> , <i>Bacillus</i> spp., <i>E. coli</i> , <i>Klebsiella</i> spp., <i>Salmonella</i> spp., <i>S. paratyphi</i> , <i>S. dysenteriae</i> , and <i>S. sonnei</i>	Sankar et al. (2013)
80	<i>Pedaliium murex</i>	Leaf	Aqueous	20–50 nm	UV–Vis, XRD, SEM, EDAX, TEM, DLS, and FTIR	<i>E. coli</i> , <i>K. pneumoniae</i> , <i>M. flavus</i> , <i>P. aeruginosa</i> , <i>B. subtilis</i> , <i>B. pumilus</i> , and <i>S. aureus</i>	Anandalakshmi et al. (2016)
81	<i>Pedilanthus tithymaloides</i>	Latex	–	123	UV–Vis, particle sizer, and TEM	<i>P. aeruginosa</i> , <i>E. coli</i> , <i>S. aureus</i> , <i>S. epidermidis</i> , and <i>M. luteus</i>	Pati et al. (2012)
82	<i>Pelargonium graveolens</i>	Leaf	Aqueous	3–15 nm	UV–Vis, TEM, and AFM	<i>E. coli</i> , <i>S. typhimurium</i> , <i>S. aureus</i> , <i>P. aeruginosa</i> , <i>K. rhizophila</i> , and <i>B. thuringiensis</i>	Okafor et al. (2013)
83	<i>Petroselinum crispum</i>	Leaf	Aqueous	3–50 nm	UV–Vis, XRD, TEM, DLS, and FTIR	<i>K. pneumoniae</i> , <i>E. coli</i> , and <i>S. aureus</i>	Roy et al. (2015)

84	<i>Piper longum</i>	Fruit	Aqueous	15–200 nm	UV–Vis, SEM, DLS, and FTIR	<i>S. aureus</i> , <i>B. cereus</i> , <i>P. aeruginosa</i> , and <i>B. subtilis</i>	Reddy et al. (2014)
85	<i>Piper nigrum</i>	Leaf	Aqueous	5–50 nm	UV–Vis, XRD, TEM, and FTIR	<i>E. coli</i> and <i>S. aureus</i>	Augustine et al. (2014)
86	<i>Pistacia atlantica</i>	Leaf	Aqueous	10–50 nm	UV–Vis, XRD, SEM, TEM, EDAX, and FTIR	<i>S. aureus</i>	Sadeghi et al. (2015)
87	<i>Plectranthus amboinicus</i>	Leaf	Aqueous	20 nm	UV–Vis, XRD SEM, EDS, TEM, and FTIR	<i>E. coli</i> , <i>Staphylococcus</i> spp., <i>Pseudomonas</i> spp., and <i>Bacillus</i> spp.	Ajitha et al. (2014b)
88	<i>Polyalthia longifolia</i>	Leaf	Aqueous	15–50 nm	UV–Vis, TEM, and FTIR	<i>E. coli</i> , <i>P. aeruginosa</i> , and <i>S. aureus</i>	Kaviya et al. (2011b)
89	<i>Prosopis farcta</i>	Leaf	Aqueous	8–11 nm	UV–Vis, XRD, and TEM	<i>S. aureus</i> , <i>B. subtilis</i> , <i>E. coli</i> , and <i>P. aeruginosa</i>	Miri et al. (2015)
90	<i>Rhinacanthus nasutus</i>	Leaf	Aqueous	<22 nm	UV–Vis, XRD, TEM, DLS, and FTIR	<i>S. aureus</i> , <i>B. subtilis</i> , <i>E. coli</i> , <i>P. aeruginosa</i> , and <i>K. pneumonia</i>	Paspuleti et al. (2013)
91	<i>Rhizophora mucronata</i>	Leaf buds	Aqueous	4–26 nm	UV–Vis, XRD, TEM, and FTIR	<i>Proteus</i> spp., <i>P. florescence</i> , and <i>Flavobacterium</i> spp.	Umashankari et al. (2012)
92	<i>Rosmarinus officinalis</i>	Leaf	Aqueous	10–33 nm	UV–Vis, XRD, SEM, TEM, and FTIR	<i>S. aureus</i> , <i>B. subtilis</i> , <i>E. coli</i> , and <i>P. aeruginosa</i>	Ghaedi et al. (2015)
93	<i>Sansevieria trifasciata</i>	Leaf	Aqueous	3–15 nm	UV–Vis, TEM, and AFM	<i>E. coli</i> , <i>S. typhimurium</i> , <i>P. aeruginosa</i> , <i>S. aureus</i> , <i>K. rhizophila</i> , and <i>B. thuringiensis</i>	Okafor et al. (2013)
94	<i>Sargassum wightii</i>	Whole plant	Aqueous	5–22 nm	UV–Vis, XRD, TEM, AFM, and FTIR	<i>P. aeruginosa</i> , <i>V. cholerae</i> , <i>K. pneumoniae</i> , <i>S. aureus</i> , <i>E. coli</i> , <i>S. pneumoniae</i> , and <i>S. typhi</i>	Shanmugam et al. (2014)
95	<i>Securinega leucopyrus</i>	Leaf	Aqueous	11–20 nm	UV–Vis, SEM, EDX, TEM, and FTIR	<i>P. aeruginosa</i> , <i>B. subtilis</i> , <i>K. pneumonia</i> , <i>S. aureus</i> , <i>P. putida</i> , and <i>E. coli</i>	Donda et al. (2013)

(continued)

Table 1 (continued)

S. No.	Plant	Part used	Extraction solvent	Average size/ range of NPs	Characterization techniques	Test bacteria	References
96	<i>Sesbania grandiflora</i>	Leaf	Aqueous	10–25 nm	UV–Vis, XRD, SEM, EDX, TEM, AFM, and FTIR	<i>S. enterica</i> and <i>S. aureus</i>	Das et al. (2013)
97	<i>Skimmia laureola</i>	Leaf	Aqueous	38 nm	UV–Vis, XRD, SEM, and FTIR	<i>E. coli</i> , <i>K. pneumoniae</i> , <i>P. aeruginosa</i> , <i>P. vulgaris</i> , and <i>S. aureus</i>	Ahmed et al. (2015)
98	<i>Solanum nigrum</i>	Leaf	Aqueous	28 nm	UV–Vis, XRD, SEM, and FTIR	<i>B. subtilis</i> , <i>S. aureus</i> , <i>S. pyogenes</i> , <i>E. coli</i> , <i>P. aeruginosa</i> , and <i>K. aerogenes</i>	Kirithiga et al. (2015)
99	<i>Solanum tuberosum</i>	Leaf	Ethanol	52 nm	UV–Vis, XRD, AFR, and FTIR	<i>S. aureus</i> , <i>P. aeruginosa</i> , <i>K. pneumoniae</i> , and <i>E. coli</i>	Logeswari et al. (2013)
100	<i>Solanum tuberosum</i>	Leaf	Aqueous	20 nm	UV–Vis, XRD, SEM, and AFM	<i>S. aureus</i> , <i>P. aeruginosa</i> , <i>E. coli</i> , and <i>K. pneumoniae</i>	Logeswari et al. (2015)
101	<i>Solanum xanthocarpum</i>	Berry	Methanol	4–18 nm	UV–Vis, XRD, and TEM	<i>H. pylori</i>	Amin et al. (2012)
102	<i>Spinacia oleracea</i>	Leaf	Aqueous	40–70 nm	UV–Vis, XRD, SEM, TEM, and FTIR	<i>B. subtilis</i> , <i>E. coli</i> , <i>K. pneumoniae</i> , and <i>P. aeruginosa</i>	Kanchana et al. (2011)
103	<i>Syzygium cumini</i>	Leaf	Ethanol	53 nm	UV–Vis, XRD, AFR, and FTIR	<i>S. aureus</i> , <i>P. aeruginosa</i> , <i>E. coli</i> , and <i>K. pneumoniae</i>	Logeswari et al. (2013)
104	<i>Tagetes erecta</i>	Flower	Aqueous	10–90 nm	UV–Vis, XRD, TEM, EDAX, and FTIR	<i>S. aureus</i> , <i>B. cereus</i> , <i>E. coli</i> , and <i>P. aeruginosa</i>	Padalia et al. (2015)
105	<i>Tephrosia purpurea</i>	Leaf	Aqueous	5–35 nm	UV–Vis, XRD, SEM, EDX, TEM, and FTIR	<i>E. coli</i> , <i>Bacillus</i> spp., <i>Pseudomonas</i> spp., and <i>Staphylococcus</i> spp.	Ajitha et al. (2014a)

106	<i>Terminalia arjuna</i>	Leaf	Aqueous	3–50 nm	UV–Vis, DLS, TEM, and FTIR	<i>E. coli</i> and <i>S. aureus</i>	Ahmed and Ikram (2015)
107	<i>Terminalia chebula</i>	Fruits	Aqueous	<100 nm	UV–Vis, XRD, FTIR, TEM, EDAX, and AFM	<i>E. coli</i> and <i>S. aureus</i>	Mohan Kumar et al. (2012)
108	<i>Trianthema decandra</i>	Roots	Aqueous	10–50 nm	UV–Vis, XRD, SEM, and FTIR	<i>E. coli</i> and <i>P. aeruginosa</i>	Geethalakshmi and Sarada (2010)
109	<i>Tribulus terrestris</i>	Fruit	Aqueous	16–28 nm	UV–Vis, XRD, TEM, AFM, and FTIR	<i>S. pyogenes</i> , <i>E. coli</i> , <i>P. aeruginosa</i> , <i>B. subtilis</i> , and <i>S. aureus</i>	Gopinath et al. (2012)
110	<i>Vitex negundo</i>	Leaf	Methanol	10–30 nm	UV–Vis, XRD, and TEM	<i>S. aureus</i> and <i>E. coli</i>	Zargar et al. (2011)
111	<i>Vitis vinifera</i>	Fruit	Aqueous	30–40 nm	UV–Vis, XRD, SEM, EDX, and FTIR	<i>B. subtilis</i> and <i>K. planticola</i>	Gnanajobitha et al. (2013)
112	<i>Withania somnifera</i>	Root	Aqueous	40–60 nm	UV–Vis, XRD, SEM, EDX, TEM, DLS, and FTIR	<i>S. aureus</i> , <i>S. mutans</i> , <i>P. aeruginosa</i> , and <i>S. typhimurium</i>	Qais et al. (2018)
113	<i>Zingiber officinale</i>	Root	Aqueous	10–20 nm	UV–Vis, SEM, EDS, TEM, and FTIR	<i>Staphylococcus</i> spp., <i>Listeria</i> spp., and <i>Bacillus</i> spp.	Velmurugan et al. (2014a)

silver nanoparticles exhibited antibacterial activity against *P. aeruginosa*, *K. pneumoniae*, *E. coli*, and *E. faecalis* (Kumar et al. 2014a).

Similarly, silver nanoparticles were also synthesized from aqueous extracts of *Withania somnifera* with an average size of 52.19 nm. The silver nanoparticles exhibited broad-spectrum antibacterial and antibiofilm activities against Gram-positive and Gram-negative pathogens. The antibacterial mechanism described was the disruption of cellular membrane and production of intracellular reactive oxygen species in test bacteria (Qais et al. 2018). Rathi Sre et al. (2015) synthesized silver nanoparticles using aqueous root extract of *Erythrina indica*. The 20–118 nm sized silver nanoparticles were characterized using UV–Vis, XRD, DLS, TEM, EDX, and FTIR and exhibited a wide range of antimicrobial activity against *S. aureus*, *M. luteus*, *E. coli*, *B. subtilis*, *S. typhi*, and *S. paratyphi* (Rathi Sre et al. 2015).

Rhizome extracts of *Acorous calamus*, an Indian medicinal plant used in traditional medicine, was used for silver nanoparticle synthesis and showed multifaceted biological activities like antibacterial, antioxidant, and anticancer effects (Nakkala et al. 2014). Extracts from green and black tea (*C. sinensis*) leaves have been used for green synthesis of silver nanoparticles that showed antibacterial activity against Gram-positive bacterial species, i.e. methicillin- and vancomycin-resistant *S. aureus* (Asgar et al. 2018). *Boerhaavia diffusa* extract, a medicinal plant, was used as a reducing agent for the synthesis of silver nanoparticles. Characterization using TEM and XRD revealed the average particle size to be 25 nm and having face-centred cubic (fcc) structure. The nanoparticles exhibited antibacterial activity against fish bacterial pathogens viz. *P. fluorescens*, *A. hydrophila*, and *F. branchiophilum* (Kumar et al. 2014b). Banana peels (*Musa paradisiaca*) have also been shown to reduce AgNO₃ to produce silver nanoparticles. The nanoparticles were characterized using UV–Vis, XRD, SEM, EDX, TEM, and FTIR with an average size of 23.7 nm and exhibited antibacterial activity against *B. subtilis*, *S. aureus*, *P. aeruginosa*, and *E. coli* (Ibrahim 2015). Likewise, leaf extracts of *Ceratonia siliqua*, *Musa balbisiana*, *Azadirachta indica*, *Ocimum tenuiflorum*, *Ocimum sanctum*, *Argemone maxicana* have all been used for synthesizing silver nanoparticles with antibacterial activity against numerous pathogens (Singh et al. 2010; Rout et al. 2012; Awwad et al. 2013; Banerjee et al. 2014).

2.2 Gold Nanoparticles

After silver nanoparticles, gold nanoparticles are the most routinely synthesized nanoparticles using plant extracts. Various plants used in the synthesis of gold nanoparticles are listed in Table 2, which gives the details of the plants used, characterization techniques, and the test organisms against which the gold nanoparticles were active. A brief description of reports is discussed here.

Root extract of *Panax ginseng* has been documented to reduce auric acid to produce gold nanoparticles within 5 min at 80 °C without using any additional capping agent. The gold nanoparticles exhibited antibacterial activity against *B. anthracis*, *B. cereus*, *E. coli*, *S. aureus*, and *V. parahaemolyticus* (Singh et al. 2016b). Gold

Table 2 List of plant extract-mediated synthesis of gold nanoparticles, characterization techniques, and the bacteria tested for antimicrobial activity

S. No.	Plant	Part used	Extraction solvent	Average size/ range of NPs	Characterization techniques	Test bacteria	References
1	<i>Abelmoschus esculentus</i>	Pulp	Aqueous	14 nm	UV-Vis, XRD, SEM, EDX, DLS, and FTIR	<i>B. subtilis</i> , <i>B. cereus</i> , <i>P. aeruginosa</i> , <i>M. luteus</i> , and <i>E. coli</i>	Rahaman Mollick et al. (2014)
2	<i>Anacardium occidentale</i>	Nuts shell	Aqueous	5–20 nm	UV-Vis, XRD, SEM, EDS, TEM, and FTIR	<i>A. hydrophila</i> , <i>A. bestiarum</i> , <i>P. fluorescens</i> , and <i>E. tarda</i>	Velmurugan et al. (2014b)
3	<i>Ananas comosus</i>	Fruit	Aqueous	16 nm	UV-Vis, XRD, SEM, and EDX	<i>S. aureus</i> and <i>P. aeruginosa</i>	Bindhu and Uma Devi (2014)
4	<i>Areca catechu</i>	Nuts	Aqueous	13.7 nm	UV-Vis, XRD, TEM, and FTIR	<i>E. coli</i> , <i>K. pneumoniae</i> , <i>P. aeruginosa</i> , <i>Enterobacter</i> spp., and <i>S. aureus</i>	Rajan et al. (2015)
5	<i>Carica papaya</i>	Leaf	Aqueous	15–28 nm	UV-Vis, XRD, SEM, TEM, and FTIR	<i>B. subtilis</i> , <i>S. aureus</i> , <i>P. vulgaris</i> , and <i>E. coli</i>	Muthukumar et al. (2016)
6	<i>Catharanthus roseus</i>	Leaf	Aqueous	15–28 nm	UV-Vis, XRD, SEM, TEM, and FTIR	<i>B. subtilis</i> , <i>S. aureus</i> , <i>P. vulgaris</i> , and <i>E. coli</i>	Muthukumar et al. (2016)
7	<i>Citrullus lanatus</i>	Rind	Aqueous	20–140 nm	UV-Vis, XRD, SEM, EDX, TGA, and FTIR	<i>B. cereus</i> , <i>L. monocytogenes</i> , <i>S. aureus</i> , <i>E. coli</i> , and <i>S. typhimurium</i>	Patra and Baek (2015)
8	<i>Coleus aromaticus</i>	Essential oil	–	28 nm	UV-Vis, XRD, TEM, EDS, and FTIR	<i>E. coli</i> , and <i>S. aureus</i>	Vilas et al. (2016)
9	<i>Curcuma pseudomontana</i>	Essential oil	–	20 nm	UV-Vis, SEM, TEM, and FTIR	<i>B. subtilis</i> , <i>P. aeruginosa</i> , <i>S. aureus</i> , and <i>E. coli</i>	Muniyappan and Nagarajan (2014b)
10	<i>Dracocephalum kotschy</i>	Leaf	Aqueous	8–22 nm	UV-Vis, XRD, TEM, EDAX, and FTIR	<i>S. aureus</i> , <i>B. subtilis</i> , <i>B. cereus</i> , <i>E. coli</i> , <i>P. aeruginosa</i> , and <i>P. vulgaris</i>	Dorosti and Jamshidi (2016)
11	<i>Euphorbia hirta</i>	Leaf	Aqueous	6–71 nm	UV-Vis, XRD, TEM, EDS, AFM, DLS, and FTIR	<i>E. coli</i> , <i>P. aeruginosa</i> , and <i>K. pneumoniae</i>	Annamalai et al. (2013)

(continued)

Table 2 (continued)

S. No.	Plant	Part used	Extraction solvent	Average size/ range of NPs	Characterization techniques	Test bacteria	References
12	<i>Gloriosa superba</i>	Leaf	Aqueous	20–50 nm	UV–Vis, XRD, TEM, EDX, AFM, and FTIR	<i>S. aureus</i> , <i>S. pneumoniae</i> , <i>E. coli</i> , and <i>K. pneumoniae</i>	Gopinath et al. (2016)
13	Grapes	Fruit	Aqueous	N.A.	UV–Vis, TEM, TGA, and FTIR	<i>S. aureus</i> , <i>C. koseri</i> , <i>B. cereus</i> , <i>E. coli</i> , and <i>P. aeruginosa</i>	Lokina and Narayanan (2013)
14	<i>Hovenia dulcis</i>	Fruit	Aqueous	15–20 nm	UV–Vis, XRD, TEM, EDX, TGA, and FTIR	<i>E. coli</i> and <i>S. aureus</i>	Basavegowda et al. (2014)
15	<i>Jasminum sambac</i>	Leaf	Aqueous	20–50 nm	UV–Vis, XRD, SEM, EDX, TGA, and FTIR	<i>S. aureus</i> , <i>E. coli</i> , <i>S. typhi</i> , and <i>P. aeruginosa</i>	Yallappa et al. (2015)
16	<i>Mentha piperita</i>	Leaf	Aqueous	150 nm	UV–Vis, SEM, EDX, and FTIR	<i>E. coli</i> and <i>S. aureus</i>	MubarakAli et al. (2011)
17	<i>Musa paradisiaca</i>	Peels	Aqueous	N.A.	UV–Vis, XRD, SEM, EDX, and FTIR	<i>Shigella</i> sp., <i>E. aerogenes</i> , <i>Klebsiella</i> spp., and <i>P. aeruginosa</i>	Bankar et al. (2010)
18	<i>Nepenthes khasiana</i>	Leaf	Aqueous	50–80 nm	UV–Vis, XRD, SEM, and FTIR	<i>E. coli</i> and <i>Bacillus</i> spp.	Bhau et al. (2015)
19	<i>Nigella sativa</i> essential oil	Essential oil	–	15–28 nm	UV–Vis, XRD, TEM, and FTIR	<i>S. aureus</i> and <i>V. harveyi</i>	Manju et al. (2016)
20	<i>Panax ginseng</i>	Root	Aqueous		UV–Vis, XRD, SEM, EDX, and TEM	<i>B. anthracis</i> , <i>V. parahaemolyticus</i> , <i>S. aureus</i> , <i>E. coli</i> , and <i>B. cereus</i>	Singh et al. (2016b)
21	<i>Panax ginseng</i>	Root	Aqueous	10–30 nm	UV–Vis, SEM, EDX, and TEM	<i>V. parahaemolyticus</i> , <i>S. aureus</i> , and <i>B. cereus</i>	Singh et al. (2016c)
22	<i>Pistacia integerrima</i>	Gall	Methanol	20–200 nm	UV–Vis, SEM, and FTIR	<i>K. pneumoniae</i> , <i>B. subtilis</i> , and <i>S. aureus</i>	Islam et al. (2015a)
23	<i>Plumbago zeylanica</i>	Root	Aqueous	20–30 nm	UV–Vis, XRD, TEM, EDX, DLS, and FTIR	<i>A. baumannii</i> , <i>E. coli</i> , and <i>S. aureus</i>	Salunke et al. (2014)
24	<i>Plumeria alba</i>	Flower	Aqueous	15.6 ± 3.4 nm	UV–Vis, XRD, TEM, EDX, TGA, and FTIR	<i>E. coli</i>	Mata et al. (2016)

25	<i>Punica granatum</i>	Fruit	Aqueous	5–20 nm	UV–Vis, XRD, TEM, TGA, and FTIR	<i>S. aureus</i> , <i>S. typhi</i> , and <i>V. cholerae</i>	Lokina et al. (2014)
27	<i>Rivea hypocrateriformis</i>	Aerial part	Aqueous	10–50 nm	UV–Vis, XRD, SEM, EDX, TGA, TEM, and FTIR	<i>K. pneumoniae</i> , <i>S. aureus</i> , <i>B. subtilis</i> , <i>P. aeruginosa</i> , and <i>E. coli</i>	Godpurge et al. (2016)
28	<i>Salicornia brachiata</i>	Aerial part	Aqueous	22–35 nm	UV–Vis, XRD, TEM, and FTIR	<i>P. aeruginosa</i> , <i>S. typhi</i> , <i>E. coli</i> , and <i>S. aureus</i>	Ayaz Ahmed et al. (2014)
29	<i>Salix alba</i>	Leaf	Aqueous	50–80 nm	UV–Vis, SEM, AFM, and FTIR	<i>K. pneumoniae</i> , <i>B. subtilis</i> , and <i>S. aureus</i>	Islam et al. (2015b)
30	<i>Solanum nigrum</i>	Leaf	Aqueous	32 ± 6 nm	UV–Vis, XRD, TEM, DLS and FTIR	<i>S. saprophyticus</i> , <i>B. subtilis</i> , <i>E. coli</i> , and <i>P. aeruginosa</i>	Muthuvel et al. (2014)
31	<i>Solanum torvum</i>	Fruit	Aqueous	5–50 nm	UV–Vis, SEM, EDX, and FTIR	<i>B. subtilis</i> , <i>P. aeruginosa</i> , and <i>E. coli</i>	Ramamurthy et al. (2013)
32	<i>Trianthema decandra</i>	Leaf	Aqueous	38–80 nm	UV–Vis, SEM, EDX, and FTIR	<i>E. faecalis</i> , <i>S. aureus</i> , <i>S. faecalis</i> , <i>B. subtilis</i> , <i>Y. enterocolitica</i> , <i>P. vulgaris</i> , <i>E. coli</i> , <i>P. aeruginosa</i> , and <i>K. pneumoniae</i>	Geethalakshmi and Sarada (2013)
33	<i>Trianthema decandra</i>	Root	Aqueous	33–65 nm	UV–Vis, SEM, EDX, and FTIR	<i>S. aureus</i> , <i>S. faecalis</i> , <i>E. faecalis</i> , <i>E. coli</i> , <i>P. aeruginosa</i> , <i>P. vulgaris</i> , <i>B. subtilis</i> , and <i>Y. enterocolitica</i>	Geethalakshmi and Sarada (2012)
34	<i>Turbinaria conoides</i>	Whole plant	Aqueous	2–19 nm,	UV–Vis, XRD, SEM, EDX, TEM, and FTIR	<i>Salmonella</i> spp., <i>E. coli</i> , <i>A. hydrophila</i> , and <i>S. liquefaciens</i>	Vijayan et al. (2014)
35	<i>Zizyphus mauritiana</i>	Leaf	Aqueous	20–40 nm	UV–Vis, XRD, SEM, EDS, TEM, and FTIR	<i>S. aureus</i>	Sadeghi (2015)

nanoparticles (8–22 nm) have also been synthesized using leaf extracts of *Dracocephalum kotschyi*. The nanoparticles were found to be active against *S. aureus*, *B. subtilis*, *B. cereus*, *E. coli*, *P. aeruginosa*, and *P. vulgaris* (Dorosti and Jamshidi 2016). Aqueous extracts of grapefruit have been documented for their ability to synthesize gold nanoparticles and were reported to have dual activities, i.e. antibacterial and anticancer activities (Lokina and Narayanan 2013). In another finding, *Trianthema decandra* root extract-mediated synthesis of gold nanoparticles were reported to be active against Gram-positive and Gram-negative bacteria such as *S. aureus*, *S. faecalis*, *E. faecalis*, *E. coli*, *P. aeruginosa*, *P. vulgaris*, *B. subtilis*, and *Y. enterocolitica* (Geethalakshmi and Sarada 2012). Gold nanoparticles synthesized from *Indigofera tinctoria* leaf extracts were found to exhibit antimicrobial, anticancer, antioxidant, and catalytic properties (Vijayan et al. 2018). Similarly, leaf extracts of *Ziziphus zizyphus* reduced Au^{3+} ions to Au^0 to form gold nanoparticles. The 51.8 ± 0.8 nm sized nanoparticles were characterized using (TEM), scanning electron microscope (SEM), and UV–Vis spectroscopy, AFM, XRD, EDX, and TGA (Aljabali et al. 2018).

2.3 Other Metal Nanoparticles

Apart from silver and gold nanoparticles, many other metal nanoparticles have also been synthesized using different parts of various plants. Zinc oxide nanoparticles synthesized from the leaf extracts of *Tabernaemontana divaricata* have been reported. Antimicrobial studies revealed that the zinc oxide nanoparticles exhibited higher antibacterial activity against *S. aureus* and *E. coli* compared to *S. paratyphi* (Raja et al. 2018). Similarly, zinc oxide nanoparticles were also synthesized from the leaf extracts of *Glycosmis pentaphylla*. XRD revealed the crystalline nature of these nanoparticles and EDAX confirmed the presence of highly pure zinc oxide metal (20.70%). The nanoparticles inhibited the growth of *B. cereus*, *S. aureus*, *S. dysenteriae*, and *S. paratyphi* in agar well diffusion assays (Vijayakumar et al. 2018a). Vijayakumar et al. also reported the synthesis of zinc oxide nanoparticles using leaf extracts of *Atalantia monophylla*. Their study mentioned better bacterial and fungal destruction by zinc oxide nanoparticles than plant extracts and standard drugs (Vijayakumar et al. 2018b). In another report, zinc oxide nanoparticles were synthesized from leaf extracts of *Azadirachta indica*. The MIC values of these synthesized nanoparticles were found to be in 12.5–50 $\mu\text{g/ml}$ range against *S. aureus*, *B. subtilis*, *P. aeruginosa*, *P. mirabilis*, and *E. coli* (Elumalai and Velmurugan 2015).

Copper oxide (CuO) nanoparticles have been synthesized using leaf extracts of *Gloriosa superba*. Characterization revealed the nanoparticles to be in 8–17 nm range. The nanoparticles exhibited antibacterial activity against *K. aerogenes*, *E. coli*, *S. aureus*, and *P. desmolyticum* (Naika et al. 2015). Similarly, the juice of *Citrus medica* has been documented for its ability to produce copper nanoparticles. The nanoparticles were 10–60 nm in size and showed antibacterial activity against *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *P. acne*, and *S. typhi* (Shende et al. 2015). Apart from plant extracts, copper oxide nanoparticles were synthesized using gum

karaya as a biotemplate. The nanoparticles exhibited antibacterial activity against *E. coli* and *S. aureus* with MIC values of 103 ± 4.7 and 120 ± 8.1 $\mu\text{g/ml}$ respectively (Černík and Thekkae Padil 2013).

Titanium dioxide nanoparticles have been reported to be synthesized using ethanolic leaf extracts of *Nyctanthes arbor-tristis* with particle sizes of 150 nm (Sundrarajan and Gowri 2011). Likewise, titanium dioxide nanoparticles were also synthesized using aqueous leaf extracts of *Psidium guajava*. The synthesized nanoparticles exhibited antibacterial activity against *A. hydrophila*, *P. mirabilis*, *E. coli*, *S. aureus*, and *P. aeruginosa* (Santhoshkumar et al. 2014). Many other metal nanoparticles such as nickel, aluminium oxide, magnesium oxide, calcium oxide, vanadium, chromium oxide, manganese oxide, iron, and cobalt have also been synthesized using the green route and tested for their antibacterial efficacy (Ramesh et al. 2012; Ansari et al. 2015; Pandian et al. 2016; Aliyu et al. 2017; Varaprasad et al. 2017; Haneefa 2017; Sharma et al. 2017; Ijaz et al. 2017; Devatha et al. 2018).

3 Factors Affecting Green Synthesis of Metal Nanoparticles

A number of factors are known to influence the synthesis of metal nanoparticles via the green route that are responsible for nucleation and stabilization of nanoparticles. The major controlling factors are concentration of reactants, pH, temperature, and reaction time (Shah et al. 2015). These are discussed below.

3.1 Role of Reactant Concentration

The concentration and nature of phytochemicals present in plant extracts has a remarkable effect on the synthesis and stabilization of metal nanoparticles. The increasing concentration of leaf extract *Cinnamomum camphora* (camphor) in the presence of a fixed amount of precursor (chloroauric acid) resulted in a change of shape of the nanoparticles from triangular to spherical (Huang et al. 2007a). Similarly, Kora et al. (2010) studied the efficacy of silver nanoparticle synthesis at different silver nitrate concentrations and reaction times. A reaction mixture containing 0.1% gum kondagogu and 1 mM AgNO_3 resulted in 55.0 and 18.9 nm sized nanoparticles at 30 and 60 min of reaction time, respectively, with polydispersed morphology. When a reaction mixture of 0.5% gum kondagogu and 1 mM AgNO_3 were tested, the nanoparticles were mostly spherical in shape with an average particle size of 11.2 and 4.5 nm at 30 and 60 min reaction time respectively (Kora et al. 2010). Similarly, it was found that the concentration of *Aloe vera* leaf extract had an influence on the ratio of gold triangular plates to spherical nanoparticles. It was also determined that the carbonyl compounds in the extract assisted in shaping particle growth and the concentration of extract had a modulatory effect on particle size (Chandran et al. 2006). Likewise, silver nanoparticles with hexagonal, decahedral, triangular, and spherical morphology were synthesized by altering *Plectranthus*

amboinicus leaf extract concentration in the reaction mixture (Narayanan and Sakthivel 2010). Hence, the concentration of reactant in reaction mixture affects both the shape and size of the synthesized nanoparticles.

3.2 Role of pH

The pH of the reaction medium also plays a crucial role in the synthesis of nanoparticles (Shah et al. 2015). It has been found that changes in the pH of the reaction medium result in variability in the size and shape of the synthesized nanoparticles. Broadly, nanoparticles synthesized at higher pH are usually smaller compared to the ones produced at lower pH (Dubey et al. 2010; Sathishkumar et al. 2010). For instance, the size of silver nanoparticles synthesized from bark extracts of *Cinnamom zeylanicum* were large and highly dispersed when synthesized at lower pH. However, there was a reduction in particle size when they were synthesized at higher pH. It was speculated that lower pH tends to cause the aggregation of nanoparticles resulting in the formation of larger particles. It has been suggested that the availability of a large number of functional groups at higher pH produces more silver nanoparticles with smaller diameters (Sathishkumar et al. 2009b). Similarly, gold nanoparticles synthesized using *Avena sativa* at pH 2 were larger in size (25–85 nm) compared to the particles synthesized between pH 3 and 4 (5–20 nm). It was suggested that at a lower pH there are fewer functional groups available, resulting in the aggregation of particles to form larger nanoparticles whereas, at pH 3 and 4, there are more accessible functional groups available for particle nucleation (Armendariz et al. 2004). In another finding, gold nanoparticles synthesized using *Elaeis guineensis* extracts at lower pH (4.5) ranged from 4.49 nm to 17.56 nm (average size 9.61 nm). The same nanoparticles, when synthesized at pH 7.5, ranged in size from 4.32 nm to 16.12 nm with an average diameter of 8.51 nm (Irfan et al. 2017). On the contrary, palladium nanoparticles synthesized from bark extracts of *Cinnamom zeylanicum* at lower pH were smaller in size compared to those synthesized at higher pH. The palladium nanoparticles ranged in size from 15–20 nm when synthesized at pH <5 and from 20 to 25 nm at pH >5 (Sathishkumar et al. 2009a).

3.3 Role of Temperature

In the green synthesis of metal nanoparticles, temperature also plays a crucial role. It has been reported that reaction temperature not only determines the size of the nanoparticles, but also in their shape and yield (Song et al. 2009; Sathishkumar et al. 2010). For example, the average size of gold nanoparticles synthesized at 25 °C using peel extracts of *Citrus sinensis* was around 35 nm. However, upon increasing the reaction temperature, the average size of gold nanoparticles became 10 nm (Kaviya et al. 2011a). Similarly, leaf extracts of *Diospyros kaki* were used to synthesize silver nanoparticles at different temperatures (25–95 °C). It was shown that the size and shape of the nanoparticles could be controlled by changing leaf

broth concentration and the reaction temperature (Song et al. 2009). Moreover, variation in thermal conditions for *Avena sativa*-mediated synthesis of gold nanoparticles of varied shape and size (Armendariz et al. 2004). It was revealed by Gericke and Pinches (2006) that higher temperatures promote the formation rate of synthesis of gold nanoparticles. They found that at lower temperatures, spherical-shaped nanoparticles were predominant while plate-like nanoparticles were more common at higher temperatures (Gericke and Pinches 2006). At higher temperatures there is a faster particle formation rate; however, there is also a decrease in average particle size with increasing temperature (Shah et al. 2015).

3.4 Role of Reaction Time

Reaction time also contributes to the green synthesis of metal nanoparticles by influencing the shape, size, and stability of nanoparticles. A study on the green synthesis of silver nanoparticles using *Ananas comosus* (Pineapple) extract showed rapid colour change within 2 min due to the reduction of AgNO_3 to form nanoparticles. When the reaction time was increased to more than 5 min, a slight change in the colour of the reaction mixture was observed (Ahmad and Sharma 2012). Similarly, the green synthesis of silver nanoparticles from silver nitrate using leaf extracts of *Azadirachta indica* exhibited variation in particle size upon changing the reaction time. When reaction time was varied from 30 min to 4 h, the particle size also changed from 10 to 35 nm. Additionally, the zeta potential of nanoparticles was -47 mV at 3.5 h reaction time and increased to -40 mV at 4 h reaction time (Prathna et al. 2011). The *Withania somnifera* (aqueous root extract) synthesized silver nanoparticles also showed a similar trend. The absorption band increased up to 5 h and then became almost constant (Qais et al. 2018). In another similar finding, there was increase in absorbance of UV-Vis spectra and peak sharpening was observed with increasing reaction time. In the synthesis of gold and silver nanoparticles, the absorption band increased up to 2 h and then only slight variations were observed (Dwivedi and Gopal 2010).

4 Advantage of Nanoparticle Synthesis by the Green Route

The green route of metal nanoparticle synthesis has several advantages over chemical or physical synthesis. One of the most advantageous characteristics of biosynthesized metal nanoparticles is their biological compatibility (Singh et al. 2016a). For biomedical applications, nanoparticles with negligible or reduced toxicity is required. Biogenic metal nanoparticles are usually free from the toxic contamination of by-products created during the physical or chemical synthesis of nanoparticles and become attached to particles, consequently limiting their biomedical utility (Baker et al. 2013). Other advantages of biological synthesis of metal nanoparticles include rapid synthesis, eco-friendly nature, and their cost-effective production

(Singh et al. 2016a). In the chemical synthesis of metal nanoparticles, stabilizing agents are required; however, phytoconstituents available in the reaction mixture itself act as capping and stabilizing agents (Makarov et al. 2014). Furthermore, metal nanoparticles contain many functional groups due to the presence of phyto-compounds. These groups progressively interact and adsorb biomolecules when they contact biological fluids, resulting in the formation of a corona, providing added efficacy over bare biological nanoparticles (Monopoli et al. 2012).

Another advantage to biological synthesis of metal nanoparticles is that there are fewer steps required, such as in the attachment of some desired functional groups to the surface to make them biologically active (Baker et al. 2013). Therefore, the nanoparticles produced from biological sources become more therapeutically effective due to the attachment of bioactive phytoconstituents of medicinal importance. For instance, the attachment of metabolites with pharmacological activity from medicinal plants to synthesize nanoparticles can provide an additional benefit with enhanced efficacies (Sintubin et al. 2012; Mukherjee et al. 2012; Makarov et al. 2014).

5 Characterization of Nanoparticles

Recently, nanoparticles are being tailored more precisely as per their application. Therefore, the synthesized nanoparticles must be verified for the presence of desired characteristics before their application especially for biomedical usage. The common attributes for which nanoparticles are usually characterized include shape, particle size or size distribution, surface area, zeta potential, hydrated surface analysis, solubility, adsorption tendency, porosity and pore size, presence of functional groups, stability, etc. (Ingale 2013). Some routinely used techniques for characterization of nanoparticles are UV–Vis spectroscopy, scanning electron microscopy (SEM), transmission electron microscopy (TEM), atomic force microscopy (AFM), X-ray diffraction (XRD), dynamic light scattering (DLS), Fourier transform infrared spectroscopy (FTIR), thermogravimetric analysis (TGA), nuclear magnetic resonance (NMR), matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF), etc. (Khomutov and Gubin 2002; Choi et al. 2007; Gupta et al. 2013; Patra and Baek 2014). Some of these techniques are briefly described below.

5.1 UV–Vis Spectroscopy

UV–Visible spectroscopy is primarily used to track and confirm the formation of various types of metal nanoparticles by measuring the Plasmon resonance and evaluating the collective oscillations of conduction band electrons (Ingale 2013). The technique provides preliminary information about structure, size, stability, and aggregation of the nanoparticles being synthesized (Daniel and Astruc 2004). Metal nanoparticles exhibit specific bands in absorption spectra when the incident light enters into resonance with the conduction band electrons on their surface (Patra and

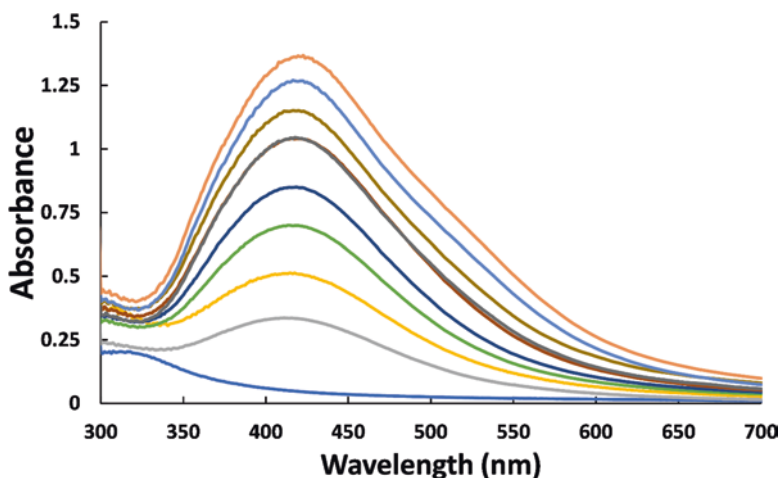


Fig. 2 UV–Visible spectra of silver nanoparticle synthesis using root extract of *Withania somnifera*

Baek 2014). For instance, silver nanoparticles exhibit a specific absorbance band in 400–450 nm range, while gold nanoparticles produce an absorbance maximum from 500 to 550 nm, due to surface plasmon resonance that may vary depending on the size and other related characteristics of the metal nanoparticles (Asharani et al. 2008; Rao and Savithamma 2011; Devi et al. 2012). A representative UV–Visible spectra of silver nanoparticle synthesis using *Withania somnifera* is shown in Fig. 2.

5.2 X-Ray Diffraction

X-ray diffraction (XRD) is a commonly used technique to assess the crystallinity of nanoparticles including metal nanoparticles (Chauhan et al. 2012). The technique is employed for identification and quantification of various crystalline forms or elemental composition materials such as nanoparticles (Tiede et al. 2008). With this technique incident light is diffracted from a powder specimen and then the diffraction pattern is analysed by measuring the angle of diffraction. The width of the particles can be determined by measuring the diffraction peaks and using Scherrer formula (Gupta et al. 2013). A representative X-ray diffraction pattern of silver nanoparticle is shown in Fig. 3.

5.3 Fourier Transform Infrared Spectroscopy

Fourier transform infrared spectroscopy (FTIR) spectroscopy is employed to identify the various functional groups present on nanoparticles. The transmission spectra of nanoparticles are obtained by forming a thin and transparent layer of potassium

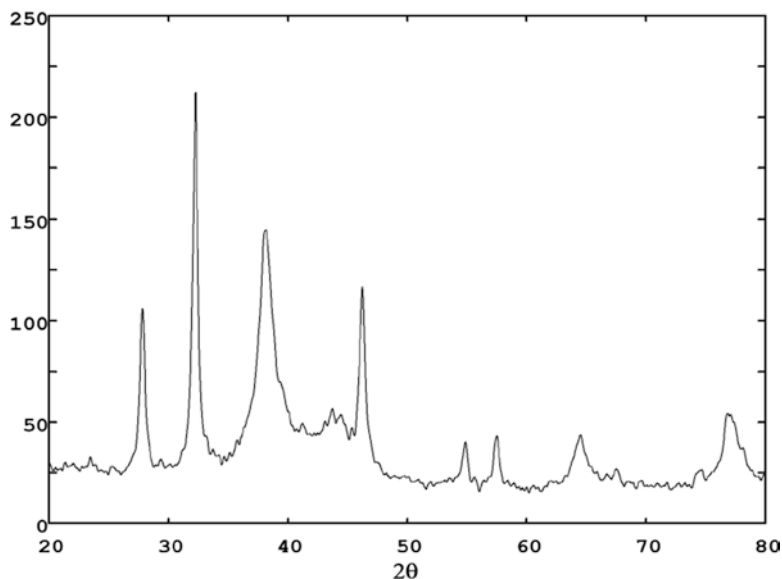


Fig. 3 A characteristic X-ray diffraction pattern of silver nanoparticles

bromide (KBr) pellets along with nanoparticles. The KBr mixtures are then placed in a vacuum line for sufficient time for formation of pellet before use. The transmission spectra are recorded after purging in dry air and the background is corrected using a reference blank sample. i.e. KBr only (Gupta et al. 2013). Using advanced computational software tools, quantitative analysis of nanoparticles can be performed in very short duration (Priya et al. 2011; Kumar et al. 2011). The FTIR spectrum is the representation of fingerprint of absorption or transmission peaks that corresponds to the frequencies of vibrations and rotations between the bonds of atoms present in/on nanoparticles. The functional groups present in the nanoparticles can be identified using FTIR spectroscopy as each bond and functional groups contains a unique combination of atoms (Faraji et al. 2010). The number of functional groups present in nanoparticles can be determined by the size of the peaks in the spectrum (Faraji et al. 2010; Chauhan et al. 2012). A representative FTIR spectrum is shown in Fig. 4.

5.4 Microscopic Techniques

Particle size distribution and morphology of nanoparticles are important traits that can be characterized using microscopic techniques such as SEM, TEM, and AFM (Pal et al. 2011). Particle size or size distribution is a vital attribute of nanoparticles that plays an important role in determining drug release and targeting, toxicity, in vivo distribution, and biological fate (Patra and Baek 2014). It has been reported that nanoparticles are more biologically effective than microparticles owing to their

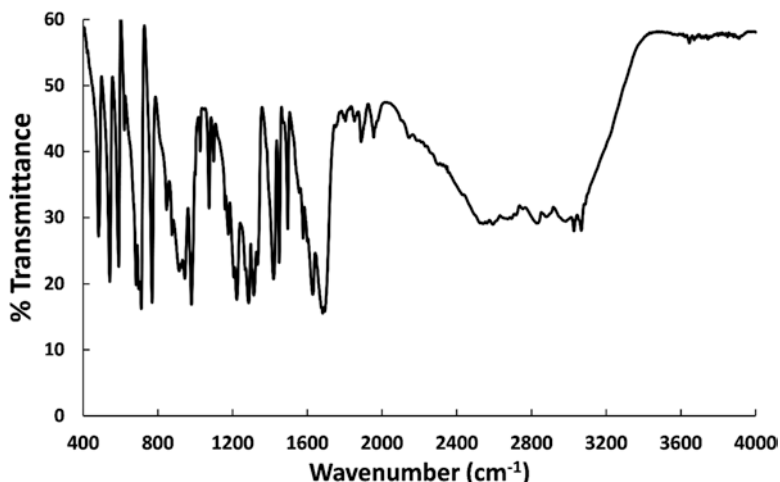


Fig. 4 Representative FTIR spectra

larger size (Kreuter 1991; Panyam et al. 2003). Some common microscopic tools used in size determination of nanoparticle are discussed below:

5.4.1 Scanning Electron Microscopy

SEM characterizes the morphology and size distribution of nanoparticles through direct visualization. This electron microscopic technique has advantages for size and morphological analysis. However, one weakness of this tool is that it provides limited information about true population and size distribution (Pal et al. 2011). Another disadvantage is that only the specimens that can withstand high vacuum pressure and adverse effects of the electron beam can be analysed. With SEM, the particles are dried if they are present in solution and mounted on a sample holder. The sample is then coated with conductive metal such as gold, gold/palladium alloy, osmium, platinum, iridium, chromium, or tungsten (Suzuki 2002). For conducting metal nanoparticles, no coating is required. A beam of high-energy electrons is directed to the sample, which generates a variety of signals on the surface of the specimens (Jores et al. 2004). The signals received from the sample are recorded by a detector that deciphers useful information about the sample such as crystalline structure, external morphology, and chemical composition (Rao and Savithamma 2011; Devi et al. 2012). A two-dimensional image with spatial variations is generated representing the surface of the nanomaterial (Prashanth et al. 2011; Priya et al. 2011). A representative scanning electron micrograph of silver nanoparticles synthesized using *W. somnifera* is shown in Fig. 5.

5.4.2 Transmission Electron Microscopy

TEM is another commonly used method for evaluation of size, shape, and morphology of nanoparticles (Zargar et al. 2011; Chauhan et al. 2012). Sample preparation in TEM is relatively time-consuming and complex as samples must be ultrathin

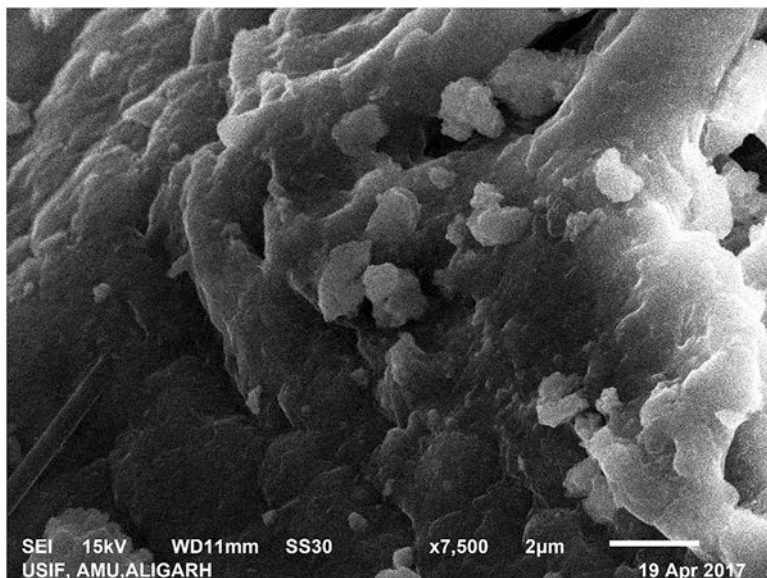


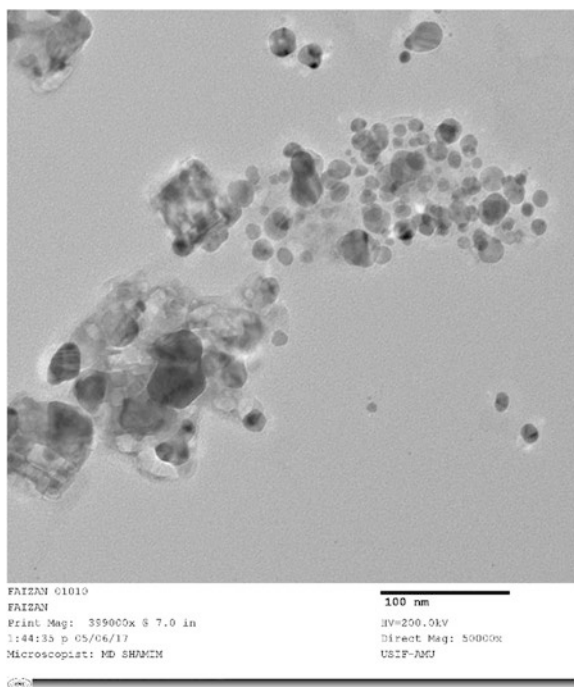
Fig. 5 Representative scanning electron micrograph of silver nanoparticle synthesis using root extract of *Withania somnifera*

such that electrons can be transmitted through them. A thin film containing sample is prepared on copper grids by placing a very small amount of sample solution onto the grid and then removing the excess solution with blotting paper. The sample, including nanoparticles, are fixed with a negative stain followed by embedding in plastic or exposing to liquid nitrogen (Pal et al. 2011). The sample is then allowed to dry under a mercury lamp. In TEM, the sample is exposed to a monochromatic beam of electrons that penetrates through the sample and the beam is projected onto a screen to generate an image (Vahabi et al. 2011; Kumar et al. 2011). Even 0.1 nm sized particles can be visualized, and their crystallographic structure can be obtained at atomic scale (Pal et al. 2011). Using high-resolution transmission electron microscopy (HR-TEM), even the arrangement of atoms as lattice fringe, lattice vacancies, glide plane, and their atomic arrangement can be analysed (Brice-Profeta et al. 2005). A representative transmission electron micrograph of silver nanoparticle synthesized using *W. somnifera* is shown in Fig. 6.

5.4.3 Atomic Force Microscopy

AFM provides three-dimensional images in which the height and volume of nanoparticles can be evaluated (Vesenska et al. 1993; Mucalo et al. 2002). Using a probe tip, physical scanning of the sample is performed to produce an ultra-high-resolution image of nanoparticles (Mühlen et al. 1996). Processing of images with the help of software yields quantitative information regarding nanoparticles such as size (in all three dimensions), morphology, and texture of the surface (Chauhan et al. 2012). An additional advantage of this technique is that it can be performed either in gas or

Fig. 6 Representative transmission electron micrograph of silver nanoparticle synthesis using root extract of *Withania somnifera*



liquid medium. Samples such as nanoparticles or biomolecules are spread on a glass cover slip mounted on the AFM stub and then dried with nitrogen gas. For better interpretation of data, multiples images (6–10) from a single sample are taken. AFM produces a topographical map of the sample by scanning in contact mode which is based on forces between the tip and surface of sample. In non-contact mode, a probe hovers over the conducting surface. Another advantage of this technique is that it is capable of imaging non-conducting samples without any specific treatment such as in case of delicate biological or polymeric nanostructures (Shi et al. 2003).

5.5 Dynamic Light Scattering

DLS is one of the fastest methods used for the determination of particle size and distribution. The technique is also known as photon-correlation spectroscopy and is one of the most popular methods for imaging nanoparticles. DLS is routinely used for the measurement of size of Brownian nanoparticles in colloidal suspensions (De Jaeger et al. 1991; Chauhan et al. 2012). In DLS, a monochromatic beam of light (laser) hits the nanoparticles present in a solution causing a Doppler shift by movement of the particles. Changes in the wavelength of the incident beam of light is related to the size of the particle. DLS computes the size, motion, and distribution of nanoparticles in the medium by measuring the diffusion coefficient of nanoparticles (Saxena et al. 2010).

6 Antibacterial Activity of Metal Nanoparticles

In last two decades, a large number of green synthesized metal nanoparticles have been tested for their antimicrobial efficacy; although, silver and gold nanoparticles are most commonly studied for their antimicrobial activity. Other nanoparticles such as nickel, aluminium oxide, magnesium oxide, calcium oxide, vanadium, chromium oxide, manganese oxide, iron, and cobalt nanoparticles have also proved to possess antibacterial tendency (Ramesh et al. 2012; Ansari et al. 2015; Pandian et al. 2016; Aliyu et al. 2017; Varaprasad et al. 2017; Haneefa 2017; Sharma et al. 2017; Ijaz et al. 2017; Devatha et al. 2018). A list of plant-mediated synthesis of silver nanoparticles and their antibacterial activity is given in Table 1 and some of the reports on drug-resistant bacteria are discussed here briefly.

Due to excellent antimicrobial properties, silver nanoparticles are the most commonly used in food storage, health industry, textile coatings, and many other environmental applications (Ahmed et al. 2016). This has led to the approval of silver nanoparticles for a wide range of uses by numerous accredited bodies such as US EPA, US FDA, Korea's Testing, SIAA of Japan, FITI Testing and Research Institute and Research Institute for Chemical Industry (Veeraputhiran 2013). The medicinal properties of silver (including its antimicrobial properties) have been appreciated for more than 2000 years (Prabhu and Poulouse 2012). Silver has been used for many medical treatments, such as preventing microbial growth in wounded soldiers during World War I (Ankanna et al. 2010). The exact mechanism of action of silver nanoparticles is still under investigation and a debatable topic. According to a widely accepted theory, the antimicrobial action of silver ions is due to their positive charge. When in solution or coming in contact with moisture, the inert form of silver releases silver ions (Klueh et al. 2000). It has been documented that there is an electrostatic attraction between negatively charged bacterial cells and positively charged nanoparticles (Cao et al. 2001). This interaction makes silver nanoparticles a suitable candidate for bactericidal action (Eby et al. 2009).

Silver ions are capable of forming complexes with nucleic acids preferentially by interacting with the nucleosides. All forms of silver with observed antimicrobial properties are the sources of silver ions in one way or another (Sondi and Salopek-Sondi 2004). The nanoparticles penetrate into cells and subsequently accumulate inside the cellular membrane, causing damage to cell membranes and/or cell walls. It is believed that silver ions bind to thiol groups of enzymes with thiol containing compounds, causing the deactivation of enzymes present in cell membranes that are involved in trans-membrane energy generation via the electron transport chain (Ahmed et al. 2016). It has been also proposed that silver ions intercalate between purine and pyrimidine base pairs resulting in disruption of hydrogen bonding between the two strands and ultimately leading to denaturation of DNA. Lysis of bacterial cells might also be a possible reason for the antimicrobial activity of silver nanoparticles. Susceptibility of Gram-positive bacteria are comparatively less than Gram-negative bacteria due to the fact that the cell wall of Gram-positive bacteria is composed of peptidoglycan. More silver ions get stuck in the negatively charged peptidoglycan is thicker in cell wall of Gram-positive bacteria than Gram-negative

bacteria. Other proposed mechanisms of the antibacterial activity of silver nanoparticles include free radical production and interaction between silver and biological macromolecules (enzymes and DNA) through an electron-release mechanism (Sharma et al. 2009; Ankanna et al. 2010). The inhibition of protein and cell wall synthesis and ATP leaking by silver nanoparticles has also been suggested in the literature (Park et al. 2011).

A study conducted by Jeeva et al. (2014) on the synthesis of metallic silver nanoparticles using *C. coriaria* leaf extract-mediated biosynthesis found that they had antimicrobial activity against multi-drug-resistant clinical isolates including *E. coli*, *P. aeruginosa*, *K. pneumoniae*, and *S. aureus* (Jeeva et al. 2014). Similarly, *T. terrestris* fruit extract-mediated synthesis of silver nanoparticles exhibited antibacterial activity against clinically isolated MDR bacteria such as *S. pyogenes*, *P. aeruginosa*, *E. coli*, *B. subtilis*, and *S. aureus* (Gopinath et al. 2012). It has been reported by Prakash and co-workers that silver nanoparticles of 55–83 nm were synthesized using leaf extracts of *Mimusops elengi*. The nanoparticles showed enhanced antibacterial activity against MDR clinical isolates such as *K. pneumoniae*, *M. luteus*, and *S. aureus* (Prakash et al. 2013). Veerasamy et al. (2011) reported that silver nanoparticles synthesized using leaf extracts of *Garcinia mangostana* that were found to be highly effective against MDR human pathogens (Veerasamy et al. 2011). In another study, *Sesbania grandiflora* leaf extract-mediated synthesis of silver nanoparticles exhibited potent antibacterial activity against MDR bacteria including *S. enterica* and *S. aureus* (Das et al. 2013). Green synthesis of silver nanoparticles using *Ananas comosus* leaf extracts showed good antimicrobial activity against *S. aureus*, *S. pneumoniae*, and *E. coli* (Emeka et al. 2014). *Petalium murex* leaf extract-mediated synthesis of silver nanoparticles has also been reported. The nanoparticles were characterized using UV–Vis spectroscopy, XRD, FTIR, FESEM, EDAX, DLS, and TEM. The size distribution was found to be 10–150 nm and exhibited antibacterial activity against *E. coli*, *K. pneumoniae*, *M. flavus*, *P. aeruginosa*, *B. subtilis*, *B. pumilus*, and *S. aureus* (Anandalakshmi et al. 2016). Similarly, silver nanoparticles were synthesized using *Zingiber officinale* root extracts and proved to be both bacteriostatic and bactericidal (Velmurugan et al. 2014a).

Likewise, other metal nanoparticles have also proved to be good antibacterial agents. A list of green synthesized gold nanoparticles exhibiting antibacterial activity is presented in Table 2. Gold nanoparticles synthesized using *Euphorbia hirta* leaf extracts exhibited antibacterial activity against many bacterial pathogens. The growth of *E. coli*, *P. aeruginosa* and *K. pneumonia* were found to be inhibited at 1.25–200 µg/ml concentration range (Annamalai et al. 2013). Biogenic gold nanoparticles synthesized using *Coleus aromaticus* essential oil have also been reported. The nanoparticles demonstrated antibacterial activity against *E. coli* and *S. aureus* (Vilas et al. 2016). Another study reported antibacterial activity of green synthesized silver/gold bimetallic nanoparticles using *Gloriosa superba* leaf extracts (Gopinath et al. 2016). *Mentha piperita* leaf-mediated synthesized gold nanoparticles also exhibited antibacterial activity against *E. coli* and *S. aureus* (MubarakAli et al. 2011).

An eco-friendly synthesis of zinc oxide nanoparticles using the leaves of *Passiflora caerulea* found that amines and alkanes induced the synthesis of particles and exhibited antibacterial activity against urinary tract infection pathogens (Santhoshkumar et al. 2017). A report on *Citrus medica* juice-mediated synthesis of copper nanoparticles found that the nanoparticles were antibacterial against *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *P. acnes* and *S. typhi* (Shende et al. 2015). Biogenic aluminium oxide synthesized nanoparticles exerted bactericidal effects against clinical isolates of multi-drug-resistant *P. aeruginosa* (Ansari et al. 2015). Nickel nanoparticles synthesized using leaf extracts of *Ocimum sanctum* inhibited the growth of *E. coli*, *K. pneumoniae*, *S. typhi*, *B. subtilis*, and *S. epidermidis*. The enhanced antimicrobial activity of nanoparticles was attributed to the formation of ROS that led to the loss of cellular proteins and LDH resulting in cell death (Pandian et al. 2016). Manganese nanoparticles synthesized using lemon fruits were found to inhibit the growth of *S. aureus*, and *B. subtilis*, *E. coli*, and *S. bacillus* (Jayandran et al. 2015). A large number of other metal nanoparticles synthesized using various plants has been reported with potent antibacterial activity.

7 Conclusion and Future Prospects

With advancements in nanobiotechnology, a new hope has arisen regarding the eco-friendly synthesis of metal nanoparticles using plants. Such biogenic nanoparticles are an important aspect of nanotechnology with multiple biomedical applications. The plant-mediated synthesis of metal nanoparticles is a single step and quick way to produce nanoparticles compared to other biological entities such as microbes, which require time-consuming methods to maintain and culture them. Therefore, plants as bio-templates for the synthesis of nanoparticles may show an immense impact in the near future by identifying and standardizing the phytochemicals responsible for this process. Toxicity is the major concern that needs to be properly addressed in suitable animal models to monitor possible adverse effect on long-term administration prior to their therapeutic application. So, it is expected that such nano-based formulations including metal nanoparticles may become the next-generation therapeutic agents against bacterial pathogens with special reference to drug-resistant bacteria. Moreover, large-scale production of nanoparticles using the green route will need to be scaled up to make them commercially available.

Acknowledgements FAQ is thankful to Council of Scientific & Industrial Research (CSIR), New Delhi, India, [File no: 09/112(0626)2k19 EMR] for providing Senior Research Fellowship.

Conflict of Interest The authors declare there are no conflicts of interest.

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