



Synthesis of Nanomaterials by Biological Route

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Atul Thakur, Deepika Chahar, and Preeti Thakur

Abstract

Nanotechnology has become one of the most important and emerging technologies in all areas of science. Various metallic and nonmetallic nanoparticles synthesized using nanotechnology have received global attention due to their potential applications in the biomedical and physiochemical areas. From the past years, the synthesis of nanoparticles using plants, bacteria, microorganisms, and various other biosources have been extensively studied and have been accepted as a green and efficient way for producing nanostructures. Various naturally occurring biodegradable materials like vitamins, sugars, and tea- or polyphenol-rich agricultural residues, which act as reducing and capping agents, are used for synthesizing nanoparticles with no toxicity. The green synthesis of nanostructures means plant- and other biosource (like fungi, flower, fruit, bacteria, starch, etc.)-mediated synthesis of nanoparticles. Various physiochemical and greener methods are available for the eco-friendly synthesis of nanoparticles that also require use of synthetic compounds for the assembly of nanostructures. The green approach of nanoparticle synthesis has many advantages like low cost, the lack of dependence on the use of any toxic materials, and the environmental friendliness for the sustainable assembly of stable nanostructures. The nanoparticles synthesized using green technology have

A. Thakur (✉)

Centre of Nanotechnology, Amity University Haryana, Gurugram, India

e-mail: athakur1@ggn.amity.edu

D. Chahar · P. Thakur

Department of Physics, Amity School of Applied Sciences, Amity University Haryana, Gurugram, India

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numerous applications in fields like health care, food and feed, cosmetics, biomedical science, energy science, drug-gene delivery, environmental health, and so on. This technique is economic and sustainable, and hence an ideal way of production of natural nanoparticles. This chapter highlights the synthesis of biosource-mediated nanostructures and their applications in various fields. The aim is to provide insight into the use of plants, fruits, flowers, fungi, etc. as a bio-renewable, sustainable, diversified resource and platform for the production of useful nanoparticles having applications in various fields, including medicine, industry, defense, water purification, agriculture, and pharmaceuticals.

Keywords

Green synthesis · Flowers · Plants · Agriculture · Environment

5.1 Introduction

The green synthesis of nanoparticles by using plant extracts has drawn a lot of attention in recent years because it is environment friendly, highly efficient, and economical and is a simple synthesis method that can produce nanoparticles at industrial scale (Khan et al. 2017a; Khan et al. 2018a; Ambika and Sundrarajan 2015; Momeni and Nabipour 2015; Singh et al. 2016; Jagtap and Bapat 2013; Ghoreishi et al. 2011; Song and Kim 2008; Rao et al. 2016; Moteriya and Chanda 2016; Giljohann et al. 2010; Pereira et al. 2013). The nanoparticles that are synthesized based on secondary metabolites of plants like phenolics, alkaloids, saponins, terpenes, lipids, and carbohydrates are used for the treatment of toxic organic contaminations existing in the environment, and used for environmental remediation (Bhainsa and Souza 2006; Bagher et al. 2018; Gholami et al. 2018). Among the various eco-friendly methods available for the synthesis of nanomaterials, the use of medicinal/herbal plant extracts seems to be a better choice for chemical factories (Abdel-aziz et al. 2013; Dipankar and Murugan 2012; Khatami et al. 2016; Patil et al. 2012; Ramar et al. 2014). Natural compounds extracted from medicinal/herbal plants are used for capping of nanoparticles and exhibited a suitable platform for the development of newly discovered treatment selection switch with improved features (Patil and Kim 2016; Ahn et al. 2016; Khatami 2018; Dhanuskodi and Prabukumar 2018; Karthik et al. 2018a). There are many advantages of using natural plant products, for example, these are cheap, safe, and suitable primary materials for the synthesis of nanoparticles (Singh et al. 2016; Gnanasangeetha and Saralathambavani 2013; Singh et al. 2015; Zhang et al. 2011; Taylor et al. 2015; Golinska et al. 2014; Seshadri et al. 2011; Noruzi 2014; Elango and Roopan 2015). A number of secondary metabolites can be exploited in medicinal plant extracts regarding the green synthesis of nanoparticles (Sayed et al. 2018; Khatami 2018). This does not require the expensive and harmful chemicals; moreover in comparison with other microbial synthesis techniques, the rate of synthesizing nanoparticles by plant extracts is much higher and faster (Oh et al.

2017; Devi et al. 2017; Hamed et al. 2016; Miri and Sarani 2018). Industrialization and urbanization lead to generation of excess of harmful and unwanted substances causing damage to the environment. A variety of such pollutants in the environment (including water and soil) are exposed by the microorganisms. Therefore, the microbial growth is inhibited due to persistence metal ions as they are nonbiodegradable in nature and often cause toxicity. However, microorganisms can survive even at high metal ion concentration and grow due to their ability to fight against the metal stress. The mechanisms include efflux systems; alteration of solubility and toxicity via reduction or oxidation; biosorption; bioaccumulation; extracellular complexation or precipitation of metals, and lack of specific metal transport systems (Bruins et al. 2000; Beveridge et al. 1997). The area of applications of these metal-microbe interactions is very vast in the field of biotechnology such as in bioremediation, biomineralization, bioleaching, and microbial corrosion (Prasad et al. 2018; Rajeshkumar 2016; Dhand et al. 2016). The microorganisms can be used in synthesis of metallic nanoparticles such as cadmium sulfide, gold, and silver. The use of nanoparticles in medical applications is limited due to chemical methods of synthesis that usually happen in the presence of toxic-reducing materials that may get attached with the surface of the prepared nanoparticles (Khatami et al. 2018a; Khan et al. 2012). Also, the physical and chemical methods of synthesis are expensive and require much energy (Dhanuskodi and Prabukumar 2018; Jana et al. 2001; Gontero et al. 2017; Dhanuskodi and Prabukumar 2017a; Jamdagni et al. 2018; Safaei et al. 2019; Torkzadeh-mahani et al. 2019; Khan et al. 2017b). This problem can be solved by synthesizing NPs using natural resources by which environment can be protected from toxic substances (Thg-Is et al. 2018; Singh et al. 2017; Ur et al. 2019; Javaid et al. 2018; Bharathi et al. 2018; Karthiga 2017; Goutam et al. 2017; Wongprecha et al. 2018; Stone 2002; Phull et al. 2016). Various natural biosources used to synthesize nanoparticles are shown in Fig. 5.1. Nanoparticles play a very important role in development of sustainable technology for the future. Plant extracts are used for the synthesis of nanoparticles that connects nanotechnology and plant biotechnology. Nanoparticles are formed by plant extracts by bioreduction of metal ions. Various metabolites present in plant extracts like sugars, terpenoids, polyphenols, alkaloids, phenolic acids, and proteins play a significant role in metal ion reduction into nanoparticles. If nanoparticles are synthesized by using chemical methods like citrate precursor, coprecipitation, autocombustion, etc. in which various chemicals are used, then it may be a serious issue for the environment because of their general toxicity and harmful fumes liberated during burning. Hence, to avoid this problem, green synthesis using biological route is a best option as the molecules of extracts derived from plant sources and many other sources is preferred over other chemical methods (Nithyaja et al. 2012; Kasyanenko et al. 2016; Lok et al. 2007; Dai et al. 2005; Verma et al. 2010; Bindhu and Umadevi 2015; Sonker et al. 2017; Marulasiddeshwara et al. 2017; Bonilla et al. 2017; Carmen et al. 2018; Nin 2008). Green synthesis of nanoparticles using various plant extracts are presented in Table 5.1. Nanoparticles synthesized by green approach have potential to fight against all types of cancer, neurodegenerative disorders, and other diseases. The bio-/green-synthesized nanomaterials have been efficiently controlling the various



Fig. 5.1 Various types of biosources used to synthesize nanoparticles

endemic diseases with less adverse effect. The trend of using natural products has increased lately, and the active biosource extracts are frequently used for discovering new drugs. Thus, green synthesis of nanoparticles using biological molecules derived from plants, bacteria, microorganisms, flowers, etc. in the form of extracts is found to be superior, economic, and environmental friendly over chemical methods.

From the past many years, a lot of studies have proven that the biosources play a significant role of a potential precursor for the synthesis of nanomaterials in environmental friendly ways. Various biosources are used for the synthesis of several greener nanoparticles such as cobalt, copper, silver, gold, palladium, platinum, zinc oxide, iron-based magnetite/hematite, etc. Recently, various biological systems that include plants and algae (Govindaraju and Khaleel 2008), diatoms (Scarano and Morelli 2002; Lengke et al. 2007), bacteria (Kowshik et al. 2002), yeasts (Rautaray et al. 2003), fungi (Anshup et al. 2005), and human cells have proved their ability to transform inorganic metal ions into metal nanoparticles by the reductive capacities of the proteins and metabolites present in these organisms (Jeevanandam et al. 2018). Synthesis/preparation of metallic/nonmetallic nanoparticles using biological entities has been of great interest due to their unusual optical (Kudelski et al. 2003) and chemical properties (Kumar et al. 2003). There are various varieties of plants like geranium (*Pelargonium graveolens*) (Shankar et al. 2003), leaf extracts of lemon-grass (*Cymbopogon flexuosus*) (Shankar et al. 2005), *Cinnamomum camphora* (Huang et al. 2007), neem (*Azadirachta indica*) (Shankar et al. 2004), aloe vera

Table 5.1 Green synthesis of nanoparticles using various plant extracts

Nanoparticle	Source of green synthesis	Name of source	Characterization	Size of the nanoparticles	Applications of nanoparticles	Reference
ZnO	Plant extract	<i>Coriandrum sativum</i>	XRD, SEM, FTIR, EDAX		Bimolecular detection, diagnostics, microelectronics, water remediation	Gnanasangeetha and Saralathambavani (2013)
Gold nanoparticles	Plant leaf extract	<i>Panax ginseng</i>			Therapeutic agent for cure of inflammation	Ahn et al. (2016)
ZnS	Plant extract	<i>Stevia rebaudiana</i>	XRD, EDAX, SEM, TEM, FTIR	8.35 nm	Capping and stabilizing agent	Alijani et al. (2018)
NiFe ₂ O ₄ nanorods	Plant extract	Rosemary extract	XRD, HRTEM, FESEM, XPS, FTIR, VSM	10–28 nm	Anticancer agent	Alijani et al. (2020)
Ni-cu-Zn ferrite	Plant extract	Aloe vera	SEM, TEM, XRD, FTIR, VSM	60 nm		Duong et al. (2008)
CoFe ₂ O ₄ and AgCoFe ₂ O ₄	Plant extract of flower and leaf	<i>Hibiscus rosa-sinensis</i>	XRD, SEM, FTIR, VSM	18.8 nm & 15.8 nm	Antimicrobial agent	Gholami et al. (2018)
CdO	Plant extract	<i>Andrographis paniculata</i>	XRD, FESEM, HRTEM, FTIR	22 nm	Antibacterial agent	Dhanuskodi and Prabhukumar (2017b)
Fe ₃ O ₄	Plant extract	Rosemary	UV-visible spectroscopy, XRD, TEM, FTIR	4 nm		Khatami et al. (2017)
Cu/Cu ₂ O	Plant extract	<i>Stachys lavandulifolia</i>	XRD, TEM, UV-visible spectroscopy, FTIR	80 nm	Antibacterial agent for <i>Pseudomonas aeruginosa</i>	Khatami et al. (2017)
Silver nanoparticles	Plant	Dried grass	UV-visible spectroscopy, XRD, TEM	15 nm	Anticancer, antifungal, and antibacterial agent	Khatami et al. (2018b)

(continued)

Table 5.1 (continued)

Nanoparticle	Source of green synthesis	Name of source	Characterization	Size of the nanoparticles	Applications of nanoparticles	Reference
CuFe ₂ O ₄ , NiFe ₂ O ₄ , ZnFe ₂ O ₄	Plant extract	Aloe vera	XRD, FTIR, SEM, TEM, VSM	15–70 nm		Laokul et al. (2011)
Gold and silver nanoparticles	Plant extract	<i>Panax ginseng</i> fresh leaves	XRD, FESEM, EDX, SAED		Drug delivery	Singh et al. (2016)
Ceria nanoparticles	Plant	Pullulan	XRD, TGA/DTG, FESEM, FTIR	12 nm	Biological and medical applications	Bagher et al. (2018)
CuFe ₂ O ₄	Plant extract	<i>Hibiscus rosa-sinensis</i>	XRD, FTIR, EDX, SAED, HRTEM, HRSEM, VSM	17.16 nm		Durka and Antony (2015)
CoFe ₂ O ₄	Plant extract	Aloe vera	XRD, EDX, SAED, TEM, UV-visible spectroscopy, VSM	14–26 nm		Manikandan et al. (2014)
CeO ₂	Plant extract	<i>Prosopis farcta</i>	PXRD, EDX, FESEM, TEM, UV-visible spectroscopy, Raman spectroscopy, FTIR	21–28 nm	Drug delivery	Miri and Sarani (2018)
Gold and silver nanoparticles	Plant extract	<i>Hibiscus rosa-sinensis</i>	XRD, FTIR, TEM, SAED, UV-visible spectroscopy			Philip (2010)
MFe ₂ O ₄ (M = Ni, Co, Mn, mg, Zn)	Plant extract	Aloe vera	XRD, SEM, TEM, SAED, VSM	8.2 nm 8.5 nm 15.9 nm 45.3 nm 17.9 nm		Phumying et al. (2013)
Silver nanoparticles	Plant extract	<i>Pseudomonas</i>	XRD, FESEM, DLS, FTIR	10–40 nm	Antibacterial and antimicrobial agent	Thg-Is et al. (2018)
Gold nanoparticles	Plant extract	<i>Hibiscus rosa-sinensis</i>	FTIR, TEM, UV-visible spectroscopy, FTIR	16–30 nm	Medical applications	Yasmin et al. (2014)

Hydroxyapatite (HAp) nanoparticles	Plant extract	Aloe vera	XRD, FTIR, TEM, Raman spectroscopy	43–171 nm	–	Klinkaewnarong et al. (2010)
Gold nanotriangles and silver nanoparticles	Plant extract	Aloe vera	UV-visible, TEM, EDAX, AFM, FTIR	15.2 ± 4.2 nm	Cancer hyperthermia, optical coating	Chandran et al. (2006)
Silver nanoparticles	Plant extract	<i>Zingiber officinale</i>	UV-visible, TEM, DLS, AFM	6–20 nm	Drug delivery and gene delivery	Kumar et al. (2012)

(Chandran et al. 2006), and tamarind (*Tamarindus indica*) (Ankamwar et al. 2005b) that are used for efficient and rapid extracellular synthesis of gold and silver nanoparticles. Also, fruit extracts of *Embllica officinalis* (Ankamwar et al. 2005a) have been effectively used for synthesizing gold nanoparticles. Biomasses of wheat (*Triticum aestivum*) and oat (*Avena sativa*), alfalfa (*Medicago sativa*) (Gardeatorresdey et al. 2003), native and chemically modified hop biomass (Lo et al. 2005), and remnant water collected from soaked Bengal gram bean (*Cicer arietinum*) (Ghule et al. 2006) have also been used for various nanoparticle synthesis. Also, gold nanoparticles have been synthesized from alfalfa (*Medicago sativa*), *Chilopsis linearis*, and Sesbania seedlings, and silver and Ag-Au-Cu alloy nanoparticles have been synthesized from alfalfa (*Medicago sativa*) (Gardeatorresdey et al. 2002) sprouts and *Brassica juncea* germinating seeds. There are various advantages of synthesis of nanoparticles using greener approach, for example, green techniques eliminate the use of expensive chemicals, consume less energy, and generate environmentally benign products and by-products. Green nanobiotechnology is a promising alternate route for synthesis of biocompatible stable nanoparticles (Narayanan and Sakthivel 2011).

5.2 Nanoparticle Synthesis

Nanoparticles can be synthesized by two different ways called “bottom-up” approach and the “top-down” approach. The two methods can be differentiated in the sense that bottom-up approach is meant for creating nanoparticles by grouping atoms and molecules. This grouping happens in a clear and managed way which results in an increase in the functionality of the structure of such materials. The top-down approach is clear reduction or breaking down of systems in their current state by making existing technologies more efficient. This causes reduction in the size into nanoscale aspects.

The bottom-up approach is more advantageous than the top-down approach because it has a better chance of producing nanostructures having less defects, more homogenous chemical composition, and better short- and long-range ordering. In bottom-up synthesis technique, the nanoparticles are synthesized onto the substrate by stacking atoms onto each other, which gives rise to crystal planes. Then, the crystal planes further stack onto each other, resulting in the synthesis of the nanostructures. A bottom-up approach can thus be viewed as a synthesis approach where the building blocks are added onto the substrate to form the nanostructures. In top-down synthesis approach, the nanostructures are synthesized by removing out crystal planes which are present on the substrate. A top-down approach can thus be viewed as an approach where the building blocks are removed from the substrate to form the nanostructure. Green synthesis of nanoparticles using other biosources are presented in Table 5.2.

There are various techniques of top-down approach for the nanoparticle synthesis. These techniques include chemical etching, laser ablation, mechanical milling/ball milling, sputtering, electroexplosion, etc. The bottom-up approach includes

Table 5.2 Green synthesis of nanoparticles using other biosources such as flower, fruit, fungi, etc.

Nanoparticle	Source of green synthesis	Name of source	Characterization	Size of the nanoparticles	Applications of nanoparticles	Reference
Silver nanoparticles	Fungus	<i>Aspergillus fumigatus</i>	XRD, TEM, UV-visible	5–25 nm		Bhainsa and Souza (2006)
Gold nanoparticles	Biosynthesis	Glucose and starch solution	UV-visible spectroscopy, TEM, AFM, electrochemistry		Biological and medical application	Engelbrekt et al. (2009)
Silver nanoparticles	Microorganism	<i>Neurospora intermedia</i>	UV-visible spectroscopy, DLS, SDS-PAGE, SEM			Hamedti et al. (2016)
Silver nanoparticles	Fruit extract	Longan fruit peel	UV-visible spectroscopy, XRD, EDX, HRTEM, FTIR	21 nm	Anticancer agent against breast cancer	Khan et al. (2018a)
Silver nanoparticles	American cockroach wings	<i>Periplaneta americana</i>	UV-visible spectroscopy, XRD, TEM	50 nm	Anti-insect agent on <i>Aphis gossypii</i>	Khatami et al. (2019)
ZnFe ₂ O ₄	Wood apple juice	<i>Limonia acidissima</i>	XRD, FTIR, SEM, EDAX, TEM, UV-DRS	20 nm	Degradation of MB dye and antibacterial agent	Naik et al. (2019)
Gold and silver nanoparticles	Fruit extract	<i>Chaenomeles sinensis</i>	XRD, FESEM, UV-visible spectroscopy, DPPH	20–40 nm & 5–20 nm	Antimicrobial and anticancer agent	Oh et al. (2017)
CeO ₂	Flower extract	<i>Hibiscus sabdariffa</i>	XRD, EDs, HRTEM, Raman spectroscopy, FTIR, XPS	3.9 nm		Thovhogi et al. (2015)
ZnO nanoparticles	Biosource	Gelatin	XRD, FESEM, TGA/DTA, AFM	–	Cosmetic and medicinal applications, optical and electrical devices	Darroudi et al. (2013)
Gold nanowires	Biosource	Sugar beet pulp	UV-visible, TEM, FTIR, EDS	–	Capping agent	Castro et al. (2011)
Gold and silver nanoparticles	Fruit extract	<i>Tanacetum vulgare</i>	UV-visible, XRD, EDX, FTIR	11 nm 16 nm	Biotechnological and biomedical applications	Prabha et al. (2010)

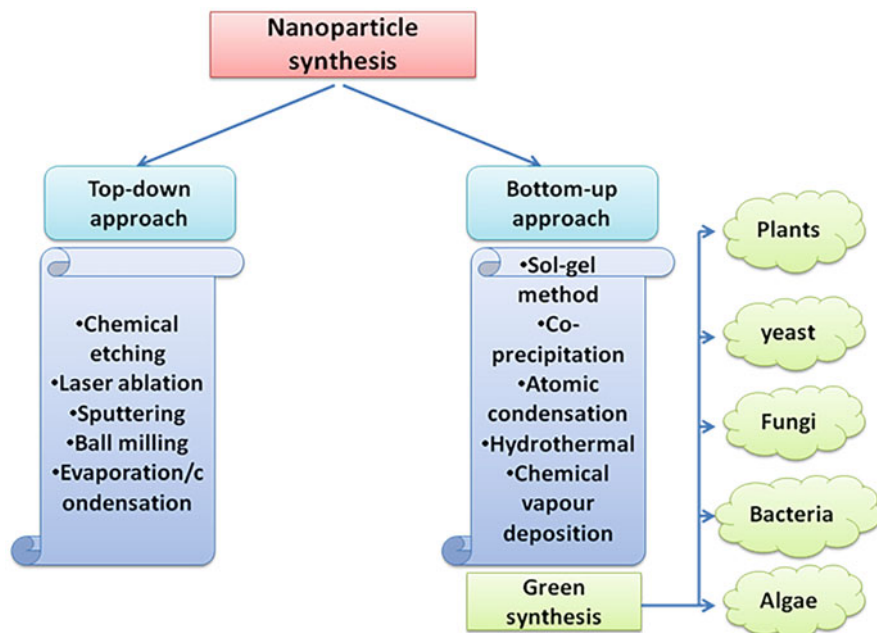


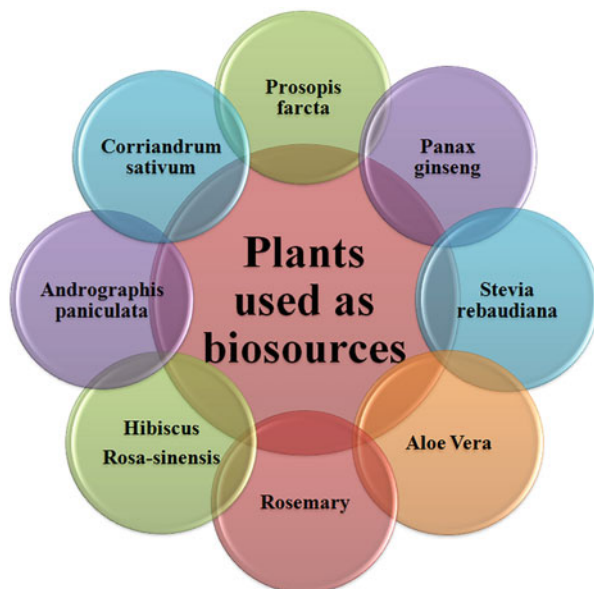
Fig. 5.2 Methods of nanoparticle synthesis

chemical vapor deposition, sol-gel processes, laser pyrolysis, spray pyrolysis, atomic/molecular condensation, and aerosol processes. The green synthesis of nanoparticles is also a bottom-up approach. Figure 5.2 shows various methods of nanoparticle synthesis. The green synthesis of nanoparticles is a cost-effective and environmental friendly method to synthesize nanoparticles in place of various chemical and physical methods. Green synthesis connects nanotechnology with nature. The synthesis happens at ambient temperature, neutral pH, and low cost and in environmentally friendly way. Synthesis of nanoparticles using plants is the best green synthesis technique as plants are nature's "chemical factories." They are cost-efficient and require low maintenance.

5.3 Green Synthesis Using Plant Extract

From the many past years, many chemical and physical methods have been used by researchers to synthesize nanomaterials that include electrochemical technique, chemical reduction, and photochemical reduction (Chen et al. 2001). It is verified from the previous studies that experimental conditions, kinetic interaction between metals and reducer agents, and stabilizer agent behavior influence the size, morphology, stability, and chemical and physical properties of the synthesized nanoparticles (Knoll and Keilmann 1999; Sengupta et al. 2005). Therefore, it is a great challenge to find a method to control nanoparticles' properties (Wiley and Sun 2007).

Fig. 5.3 Various plants used to synthesize nanoparticles



Nanoparticle synthesis using plants is one of the environmental friendly, affordable, nontoxic, and clean method (Dwivedi and Gopal 2010; Khatami et al. 2015; Castro et al. 2011; Kumar et al. 2012; Darroudi et al. 2013; Ponnuchamy and Jacob 2016; Prabha et al. 2010). Various plants can be used as natural sources for nanoparticle synthesis as shown in Fig. 5.3. Green chemistry gains its inspiration from nature through plants, yeast, fungi, and bacteria. In the nanoscience research, the integration of green chemistry principles is a key issue (Philip 2010). Figure 5.4 shows the green synthesis of nanoparticles by using different plants. The nanostructured magnetic ferrites can be prepared by using plant extracts from leaves, flowers, roots, or seeds in place of several chemical pathways using benign reagents and hence reduce the risk of hazardous substances (Manikandan et al. 2014; Phumying et al. 2013; Laokul et al. 2011). Figure 5.5 shows various plants' digital photographs whose extracts can be used for green synthesis of nanoparticles. A variety of metabolites that are released from the plants and also contained in plants such as carbohydrates, polysaccharides, phenols, amino acids, and vitamins. These metabolites can act as capping agents, reducing agents, and stabilizing and/or chelating agents for "capturing" the metal ions, and also they can also act as fuel. Size, shape, and morphology of the nanoparticles get influenced by the use of plant extracts during synthesis. Nanoparticles with high dispersity, high stability, and narrow size distribution can be produced by these metabolites (Laokul et al. 2011). Nowadays, metal oxides and mixed oxide nanoparticles can be obtained in bulk scale from many plant extracts like *Aloe vera* leaves, ginger roots, and *Hibiscus rosa-sinensis* flowers/leaves (Phumying et al. 2013; Laokul et al. 2011). Miri et al. (Miri et al. 2018) synthesized gold nanoparticles by using *Prosopis farcta* extract and studied its application for the decay of colon cancer cells.

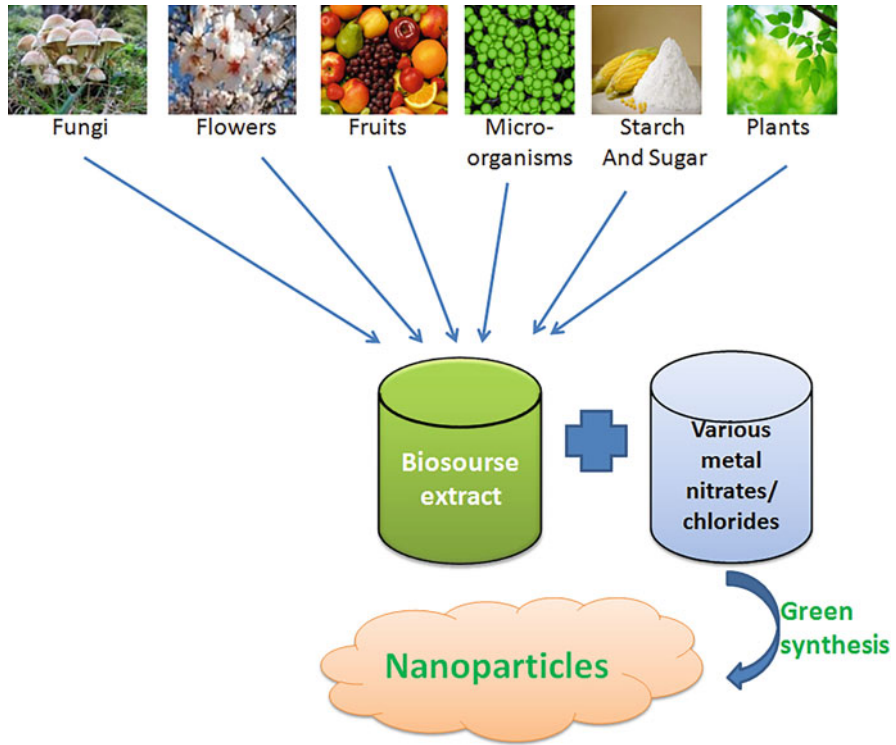


Fig. 5.4 Green synthesis of nanoparticles

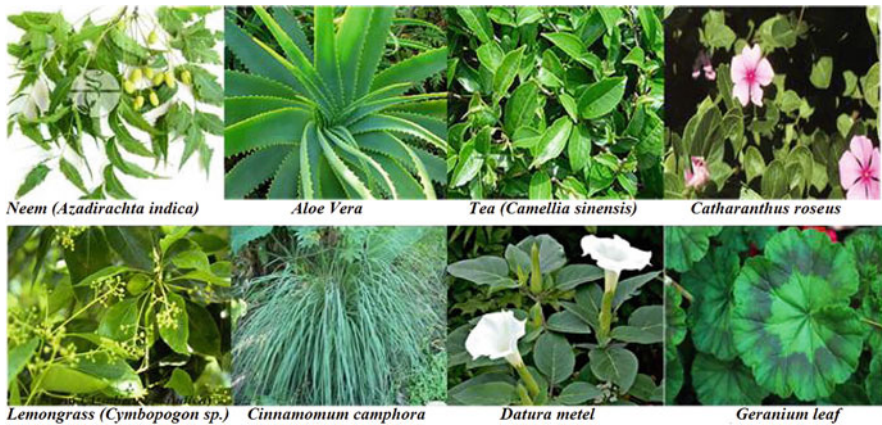


Fig. 5.5 Digital images of various plants used for green synthesis of nanoparticles (Reproduced by permission from Ref. Mittal et al. (2013), License No. 4830671099554, Copyright 2013, Elsevier)

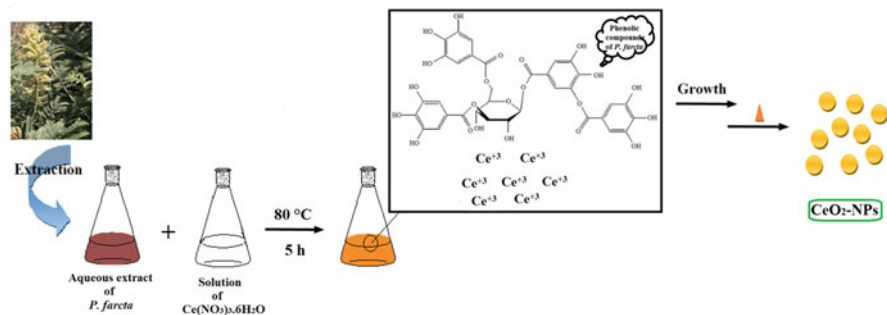


Fig. 5.6 Synthesis of CeO₂ NPs by using an aqueous extract of *P. farcta* (Reproduced by permission from Ref. Miri and Sarani (2018), License No. 4830660768290, Copyright 2018, Elsevier)

The various compounds present in the *Prosopis* are quercetin (flavonoids), tryptamine, apigenin 5-hydroxytryptamine (alkaloids), 1-arabinose, and lectin (Jannet 2005; Gulalp and Karcioğlu 2008). This plant named *Prosopis farcta* contains various medicinal properties to cure many diseases like gastric ulcers, fetus abortion, dysentery, arthritis, larynx inflammation, heart pains, and asthma, and it grows in the area of the Middle East (Al-Qura 2008). It contains phenolic compounds including tannins, vicenin-2, caffeic acid derivative, and apigenin c-glycoside (Jannet 2005) that can be helpful in the reduction process of Au⁺ to Au⁰. For the preparation of extract of *Prosopis farcta*, 5 mg of leaf powder was added to 50 mL of water, and prepared mixture was shaken at 150 rpm for 4 h, and resultant mixture was filtered to get a brown solution. This solution was then kept at 4 °C. To synthesize the gold nanoparticles, 5 mL of leaf extract was increased to 50 mL by addition of gold chloride solution, and that final mixture was then shaken at 150 rpm at 25 °C for 30 min. The prepared gold nanoparticles were found to have a very good potential for the decaying of cancer cells. Also, CeO₂ nanoparticles were synthesized using the extracts of *P. farcta* (Miri and Sarani 2018). The detailed synthesis process is shown in Fig. 5.6. The synthesized CeO₂ nanoparticles have found useful for application in decay of cancer cells. Figure 5.7 shows the TEM images and morphology of HT-29 cancer cells before and after treatment of nanoparticles.

Andrographis paniculata is a herbaceous plant having a height of 30–110 cm and is grown in moist shady areas. It can be found in South Asia, China, and Europe. This plant is effectively used to cure body illness like body heat, common cold, and upper respiratory tract infections including sinusitis and fever and is useful to dispel toxins from the body. Karthik et al. (Dhanuskodi and Prabukumar 2017b) synthesized CdO nanoparticles using *Andrographis paniculata* extract. For preparing the extract of *Andrographis paniculata*, 10 g leaves were washed using double-distilled water at 303 K and then immersed in water to extract the green-colored dye. Thereafter it was heated for 30 min at 393 K and filtered to remove residual solids. Then, 0.5 M of cadmium acetate was added in 100 mL of *A. paniculata* solution. The precipitate from the solution was washed with ethanol and double-distilled water

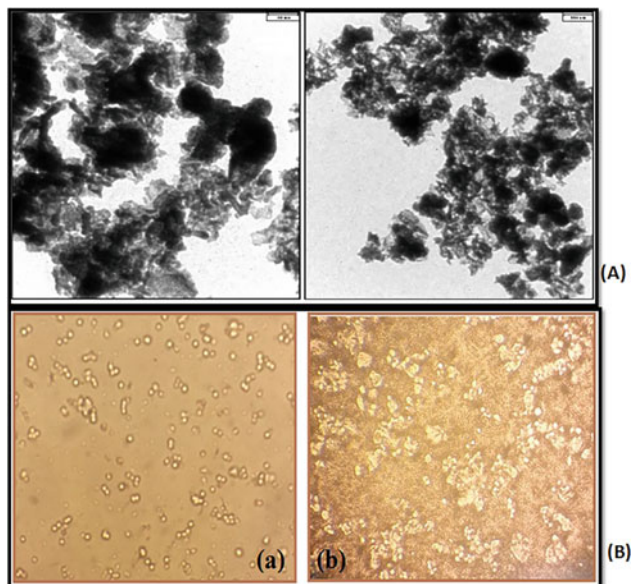


Fig. 5.7 (A) TEM images of synthesized CeO₂ NPs at 400 °C and (B) Morphology of HT-29 cells (a) before treatment and (b) after treatment with 200 µg/ml synthesized CeO₂ NPs (Reproduced by permission from Ref. Miri and Sarani (2018), License No. 4830660768290, Copyright 2018, Elsevier)

after stirring for 1 h and then dried at 303 K. After that, the resultant was sintered at 673 K for 4 h, and finally brown CdO powder was obtained. The antibacterial activity of green-mediated CdO nanoparticles was carried out against *Escherichia coli*, *Staphylococcus aureus*, *Aeromonas hydrophila*, *Vibrio cholerae*, and *Rhodococcus rhodochrous*. From the antibacterial studies, CdO nanoparticles exhibit an antibacterial activity that can be helpful in the food packaging industries.

5.3.1 Synthesis of Nanoparticles Using *Hibiscus Rosa-Sinensis*

Hibiscus rosa-sinensis is found throughout India, and known as shoe flower plant or Chinese hibiscus. It is an evergreen woody, showy shrub and glabrous plant found in India. Hibiscus is a medicinal herb that plays a role in many medical applications as it can be used as a native cure for hypertension, liver disorder, and pyrexia (Chen et al. 2003). It also prohibits adipogenesis and is used against the problem of dandruff, thereby promoting hair growth. Hibiscus plant extract can be used in the cure of diseases like diarrhea, fatigue and skin problems, gonorrhoea, and menorrhagia and as a medicine against diabetes (Venkatesh et al. 2008; Sachdewa and Khemani 2003). Figure 5.8 depicts the digital photograph of hibiscus leaf and TEM images of the synthesized gold nanoparticles using hibiscus leaf extract. The ingredients in the hibiscus leaf extract are proteins, vitamin C, organic acids,

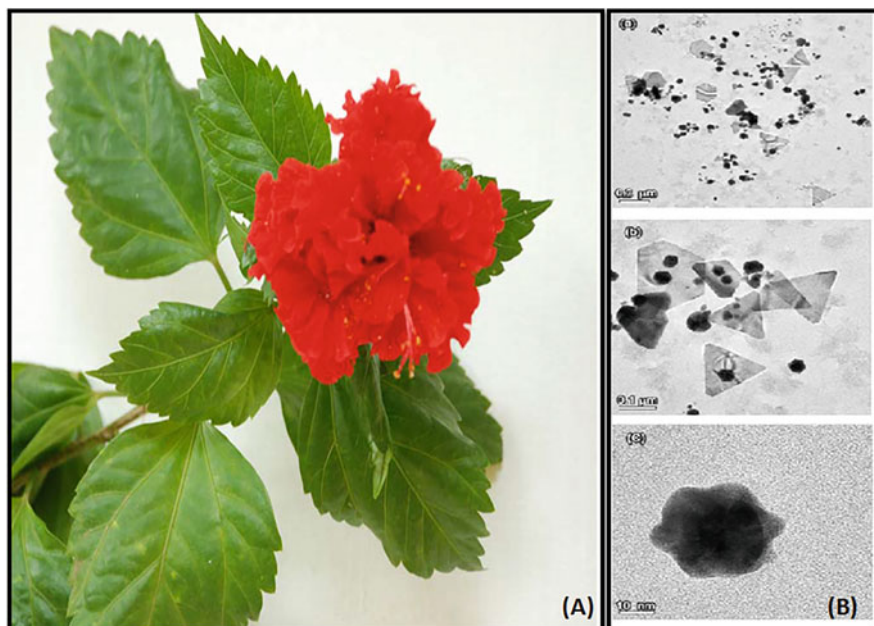


Fig. 5.8 (A) Digital image of the *Hibiscus* leaf (B) TEM images of gold nanoparticles (a) and (b) under different magnification (c) single multibranched gold nanoparticle. (Reproduced by permission from Ref. Philip (2010), License No. 4830661263292, Copyright 2010, Elsevier)

flavonoids, and anthocyanins. It is evident from the literature that *Hibiscus rosa-sinensis* flower/leaf extracts were often used to obtain Au and Ag nanoparticles (Bhainsa and Souza 2006; Muzammil 2013), ZnO, and CeO₂ nanoparticles (Yasmin et al. 2014; Thovhogi et al. 2015). The first study on the synthesis of spinel copper ferrite (CuFe₂O₄) using hibiscus flower extract was done by the Manikandan et al. (Durka and Antony 2015). Many diseases can be cured by *Hibiscus rosa-sinensis*. Its antibacterial activity is known for more than 50 years (Atwan and Saiwan 2010; Missoum 2018; Mak et al. 2013). The organic and phenolic acids such as citric, malic, succinic, lactic, gallic, hibiscus, and homogentisic acids form the chemical composition of this plant. Also, flavonoids such as luteolin, quercetin, and gossypetin and their glycosides are also present. The bright color of the flowers is due to anthocyanins. The chemical composition varies with the species, origin, age, and color. The antioxidant and antimicrobial activities are due to total phenolic compounds and flavonoids (Alaga et al. 2014). Gingasu et al. (Gingasu et al. 2016) synthesized CoFe₂O₄ and AgCoFe₂O₄ nanoparticles using *Hibiscus rosa-sinensis* flower and leaf extract and studied its potential for the antimicrobial actions. For the synthesis of CoFe₂O₄ nanoparticles, firstly 5 g of dried flowers were taken in 100 mL distilled water under continuous stirring. The mixture was then boiled for 15 min. The obtained bright red extract was cooled to room temperature and filtered to get the hibiscus flower extract. To obtain the hibiscus leaf extract, 5 g of fresh leaves

were taken and placed in 100 mL of distilled water after cutting under continuous stirring followed by boiling the mixture for 45 min till the formation of yellow-colored solution with pH = 6. The obtained solution was then cooled at room temperature and filtered. The cobalt ferrites were prepared using the self-combustion process. For this, the metal nitrates were added to the extract of *Hibiscus rosa-sinensis* flower under constant slow stirring. The obtained gel was put on a heater at 250–300 °C. Initially, the melting of gel took place followed by spontaneous decomposition by self-ignition, leaving behind voluminous foam. This was then annealed at 800 °C for 1 h to improve the degree of crystallization of cobalt ferrite. After that for the wet fertilization reaction, the metal nitrates in a proper ratio were added under stirring to the aqueous extract of *Hibiscus rosa-sinensis* leaf. The pH of the solution was raised to 10 by adding NH₄OH followed by separation of a dark brown precipitate. The suspension became magnetic after 4 h when maintained at 80 °C. A thermal treatment at 800 °C for 1 h led to the formation of a well-crystallized cobalt ferrite. For the synthesis of Ag-Co nanoferrite particles, the abovementioned self-combustion process was performed. The prepared ferrite nanoparticles showed good potential for the antimicrobial and antifungal activities. Philip et al. (Philip 2010) synthesized gold and silver nanoparticles using *Hibiscus rosa-sinensis* plant extract. For collecting the hibiscus leaf extract, firstly the leaf of the plant was washed many times using deionized water and cut followed by stirring at 300 K with 200 mL deionized water for 1 min. After that it was filtered to get the required extract. This filtrate acts as a reducing agent and stabilizer. In it 5 mL of hibiscus extract is added stirring vigorously for 1 min. Slow reduction happens and got completed in 1.5 h shown by the change in color to light violet of the solution. For the synthesis of silver nanoparticles, 20 mL of the hibiscus extract is added to a vigorously stirred 25 mL aqueous solution of AgNO₃ continuously stirring for 1 min. The pH of the solution was adjusted to 6.8 using NaOH. Reduction takes place rapidly, and it was indicated by golden yellow color of the solution. These colloids are best able for 4 months.

5.3.2 Synthesis of Nanoparticles Using *Stevia Rebaudiana* Bertoni

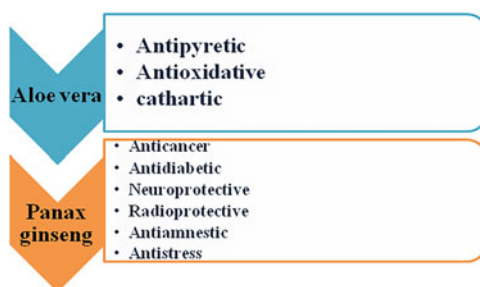
Stevia rebaudiana Bertoni is a herbaceous plant and sweet steviol glycosides can be produced (Karaköse et al. 2015). Until 2013, 34 sweet steviol glycosides had been discovered in leaf extracts (stevioside; steviolbioside (trace); rebaudiosides A, B (trace), C, D (trace), and E (trace); and dulcoside A) including 8 isomers and glycosylated forms of oxidized steviol derivatives (Vashist et al. 2017). These glycosides contain different glucose units (Melis et al. 2009). The gold, ZnS, Ag, and CdS nanoparticles can be synthesized by using glucose and biodegradable materials (Dhanuskodi and Prabukumar 2017b; Engelbrekt et al. 2009; Karthik et al. 2018b; Salari et al. 2018). This can help in reducing the cytotoxicity of the nanoparticles for their bioanalysis applications in the quantitation tags, signal transducers, encoded substrates, functional tags, and drug-gene delivery (Dhanuskodi and Prabukumar 2018). Because of its natural sweetness and

therapeutic effects of diterpene steviol glycosides, this plant is considered highly economical and scientific. This compound was found to be 250–300 times sweeter than sucrose (Karthik et al. 2018a). Its applications are found in the fields of biomedicine (Das 2008), food industry, and biotechnology (Karak et al. 2011). Alijani et al. (Alijani et al. 2018) synthesized zinc sulfide nanoparticles using extract of *Stevia rebaudiana*. For the synthesis of ZnS nanoparticles, the aqueous crude extract of *Stevia* and Na_2S were used as sources of glucose and sulfur, respectively. For the preparation of *Stevia rebaudiana* leaf extract, 15 g of dried leaf powder was added to 105 mL of deionized water at room temperature. After that, the flasks were shaken with a rotation rate of 90 rpm for 48 h at a temperature of 37 °C. Then, centrifugation was done at 4500 rpm to separate the resultant mixture for a period of 10 min. Thereafter, 100 mL of 1 M $\text{Zn}(\text{NO}_3)_2$ solution was added dropwise to 100 mL of 1 M Na_2S stirring continuously. Then, the white-colored solution was stirred for 16 h followed by adding 100 mL of this resultant crude extract dropwise to the solution and achieved light green-colored solution. This was then incubated at 70 °C for 6 h. Then, centrifugation was done to separate the resultant mixture, and finally the obtained light green-colored product was dried at 50 °C for 4 h. The particle size of the synthesized nanoparticles ranged from 1 to 40 nm. FTIR study confirmed the role of the prepared nanomaterial as capping and stabilizing agents.

5.3.3 Synthesis of Nanoparticles Using *Aloe vera* Extracts

Recently, biosynthesis has emerged as an alternative synthesis technique to prepare nanocrystalline inorganic materials. The metal and semiconductor nanoparticles are synthesized using fungi, actinomycetes, and plant extracts. *Aloe vera* is a native plant in Thailand and several other countries and contains 99.5% water content in the leaves. The rest is solid material containing over 75 different ingredients including vitamins, minerals, enzymes, sugars, anthraquinones or phenolic compounds, lignins, saponins, sterols, amino acids, and salicylic acids. Cosmetic industries use *aloe vera* gel as a hydrating ingredient in liquids, creams, sun lotions, lip balms, healing ointments, etc. Also, the gel is used in pharmacology for wound healing and anti-inflammatory and burn treatment. Properties of *Aloe vera* and *Panax ginseng* are shown in Fig. 5.9. Phumying et al. (Phumying et al. 2013) synthesized magnetic

Fig. 5.9 Various properties of *Aloe vera* and *Panax ginseng*



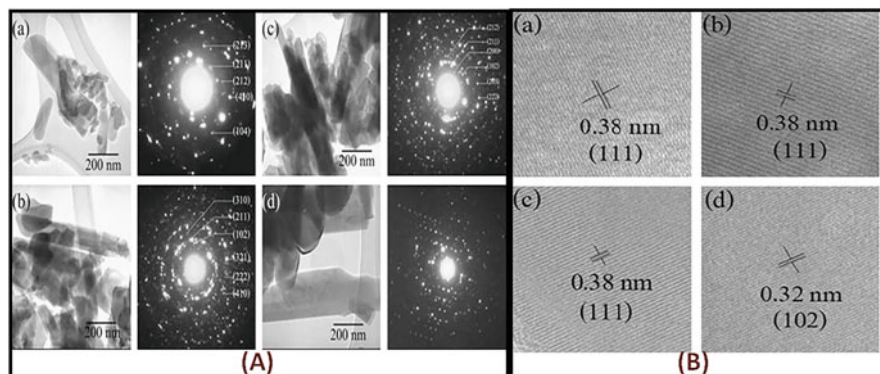


Fig. 5.10 (A) TEM images with corresponding selected area electron diffraction (SAED) patterns of HAp powders calcined in air for 2 h at (a) 500 °C, (b) 600 °C, (c) 700 °C, and (d) 800 °C. (B) HRTEM images of HAp powders calcined in air for 2 h at (a) 500 °C, (b) 600 °C, (c) 700 °C, and (d) 800 °C. (Reproduced by permission from Ref. Klinkaewnarong et al. (2010), License No. 4830681177416, Copyright 2010, Elsevier)

ferrites by using aloe vera extract. Aloe vera-extracted solution was prepared from 35 g *Aloe vera* leaves after cutting and boiling in 100 ml of deionized water. The resulting extract was used as an aloe vera extract. In the preparation of MFe_2O_4 ($M = Ni, Co, Mn, Mg, Zn$) samples, all the starting materials were first dissolved in 50 ml aloe vera extract solution and stirred for 1 h at room temperature. The solution was sealed in a Teflon-lined autoclave of 100 mL capacity and heated at 200 °C for 2 h. It was then gradually cooled to room temperature, filtered and washed with deionized water and ethanol several times. The resultant was then dried in air at 85 °C. The hydrothermal synthesis using aloe vera extract is a relatively new method to produce precursors of nanocrystalline spinel ferrite powders at a low temperature and in short time. Synthesis of nanomaterials using aloe vera extract is a simple, efficient, and green method (Chandran et al. 2006; Phumying et al. 2013; Laokul et al. 2011; Klinkaewnarong et al. 2010). Figure 5.10 shows TEM and HRTEM images of the HAp nanoparticles synthesized using aloe vera gel (Klinkaewnarong et al. 2010). There are many advantages of using aloe vera extract solution for the synthesis of nanomaterials, for example, aloe vera plant extract is an environment friendly, nonpolluting solvent system, an eco-friendly reducing agent, and a non-hazardous agent for the stabilization of the nanostructures (Visinescu et al. 2011; Varma 2012). Aloe vera plant contains water ranging from 97.5% to 99.5% of fresh matter. Also, the components of this plant are water, fat-soluble vitamins, minerals, enzymes, polysaccharides, phenolic compounds, and organic acids. About 60% of the left out solid is made up of polysaccharides (Boudreau et al. 2017). The metal oxides can be prepared with aloe vera extract by using it as a bio-reducing agent due to the long-chain polysaccharides present in the Aloe vera plant extract that affords the homogeneous distribution of metal oxides. In one such study, Manikandan et al. (Manikandan et al. 2014) synthesized ferrite nanoparticles using aloe vera extract.

For the preparation of extract solution, a 5 g portion of thoroughly washed *Aloe vera* leaves was finely cut, and the obtained gel was added in 10 ml of deionized water, clear solution was then obtained after stirring for 30 min. For preparing CoFe_2O_4 samples, firstly ferric nitrate (10 mmol) and cobalt nitrate (5 mmol) were dissolved in the prepared extract solution under vigorous stirring at room temperature for 1 h to obtain a clear transparent solution. *Aloe vera* plant extract solution acts both as a reducing and gelling agent for the synthesis of mixed metal oxides. Then, the precursor mixture of metal nitrates in *Aloe vera*-extracted solution was put in a domestic microwave oven and exposed to the microwave energy in a 2.45 GHz multimode cavity at 850 W for 10 min. Initially, boiling of the precursor mixture took place followed by evaporation and then decomposition with the evolution of gases. After reaching the point of spontaneous combustion, it vaporized and instantly became a solid. The obtained solid was then washed with ethanol and dried at 70 °C for 1 h. Therefore, *aloe vera* plant-extracted microwave combustion synthesis can be used as an eco-friendly method to produce precursors for nanocrystalline spinel ferrite powders with low temperature, energy consuming, and short time. Also Laokul et al. (Laokul et al. 2011) synthesized copper ferrite, nickel ferrite, and zinc ferrite nanoparticles using *aloe vera* extract, wherein, the modified sol-gel method was used to synthesize MFe_2O_4 using various appropriate metal nitrates such as $\text{Ni}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$, $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$, and *aloe vera* extract solution as starting materials. For this, firstly 250 mL *aloe vera* plant extract solution was dissolved with 50 g of nitrate with stirring on a hot plate for 60 min at room temperature. After that, the temperature was increased to 90 °C so that a dried solid precursor was obtained. Finally, the calcination was done at different temperatures in the range of 600–900 °C for 2 h in a furnace with heating rate of 5 °C/min. This is a simple method with the use of cheap precursors of *Aloe vera* plant extract that gives very high-yield nanomaterials having a well crystalline structure and acceptable magnetic properties.

5.3.4 Nanoparticle Synthesis Using *Panax Ginseng*

The meaning of ginseng is the essence of man, and it is a very important pharmacological herbal medicinal plant. *P. ginseng* was found in China over 5000 years ago, and since that time, its roots are used as a very important medicinal herb in traditional Chinese medicine (Radad et al. 2006). This plant which resembles the “human body” is a very slow growing perennial herb. The roots of the plant grow from the third year, and an increase in its diameter can be seen in the fourth year. By the sixth year, the length of the plant reaches to 7–10 cm and breadth 3 cm (Immer 1996). The components of the *P. ginseng* plant include ginsenosides, sugar residues, flavonoids, proteins, etc. having ginsenoside as the major pharmacological ingredient (Kim et al. 2014). It has been proposed that the pharmacological efficacies of ginseng plants and their products are due to the ginsenosides. However, ginsenosides are found mainly in roots of the plant, but leaves also contain a large amount of it and can be used

easily as compared to roots (Wang et al. 2006). Gold and silver nanoparticles can be synthesized using this plant. Gold nanoparticles of anti-inflammatory nature were synthesized using *Panax ginseng* leaves (Ahn et al. 2016). The experiment was performed with decreased availability of inflammatory mediators in macrophages. The synthesized gold nanoparticles were found to have a potential application for therapeutic application for inflammatory diseases by blocking of NF- κ B via p38 MAPK.

Singh et al. (Singh et al. 2016) synthesized gold and silver nanoparticles using *Panax ginseng* fresh leaves. Although *P. ginseng* roots, leaves, and other products have the capability of preparing the extract, leaves are found to have very rapid, facile, stable, economical source and result in nanoparticles of high pharmacological importance and effect. Hence, gold and silver nanoparticles synthesized using *P. ginseng* leaves are used in in vitro trial for biocompatibility, anticancer, and anti-inflammatory efficacies. The characterization done of the synthesized silver and gold nanoparticles was in terms of biological active groups, surface charge, and temperature stability, and further applied for antioxidant efficacy, cell viability on normal cells lines, anticancer efficacy on lung cancer and skin cancer cell lines (melanoma cancer), and anti-inflammation effect on RAW 264.7. Hence, the green synthesis using *P. ginseng* is an advantageous and cost-effective method for the development of herbal medicinal plant-mediated, low-cost, and safe nano-drug carriers in targeted drug delivery systems, cancer diagnostic, photothermal therapy, biosensing, and medical imaging. The use of such plants that have medical application with therapeutic importance can create a new platform for effective and green nanoparticle synthesis that have many applications on medical platform.

5.4 Green Synthesis of Nanoparticles Using Flower Extracts

5.4.1 Synthesis of Nanoparticles Using *Hibiscus sabdariffa*

Hibiscus sabdariffa (*H. sabdariffa*) is a shrub that belongs to the family Malvaceae with red flowers in the form of calyces. From the study of the composition of this plant, it is evident that various phenolic compounds are present in this plant that include various organic and phenolic acids like citric acid, hydroxycitric acid, and hibiscus acid (Mahadevan and Kamboj 2009). Also this plant contains flavonoids such as quercetin, luteolin, or gossypetin and their glycosides. The red color in the flowers is due to anthocyanins that are present in high amount. The anthocyanins present in flowers contain cyanidin-3-glucoside, delphinidin-3-glucoside, cyanidin-3-sambubioside, and delphinidin-3-sambubioside (Alaga et al. 2014; Qourzal et al. 2005; Pacôme et al. 2014; Guardiola and Mach 2014; Patel 2013; Borrás-linares et al. 2015). Thovhogi et al. (Thovhogi et al. 2015) synthesized CeO₂ nanoparticles using *Hibiscus sabdariffa* flower extract. Firstly, the washing of the dried red flowers was done to remove dust. To synthesize the required nanoparticles, 10.0 g of clean *H. sabdariffa* flowers was weighed in a beaker, and 400 ml distilled water was added to it and left for 2 h at room temperature. Filtering of the obtained solution was done

twice to remove residual solids followed by adding 2.0 g of $\text{Ce}(\text{NO}_3)_3 \cdot 6\text{H}_2\text{O}$ in 100 mL of the prepared solution. The solution was mixed homogeneously and heated for ~2 h. The precipitate formed is presumably CeO_x and $\text{Ce}(\text{OH})_x$ mixture. Then, after the centrifugation at ~10,000 rpm, it was then dried in oven at ~100 °C. Then, the resultant product was annealed at ~500 °C for 2 h using a high-temperature tubular furnace. The nanoparticles formed are spherical in shape having a diameter of 3.9 nm.

5.4.2 Synthesis of Nanoparticles Using *Stachys Lavandulifolia*

Stachys lavandulifolia flower extract was used for the synthesis of copper and copper oxide nanomaterials (Khatami et al. 2017). In that research, copper chloride was used as a precursor of copper ions, and the extract as the other reagent. The pH of the extract of *S. lavandulifolia* flowers was adjusted to alkali for the synthesis of nanomaterials. Various characterizations were performed like transmission electron microscopy (TEM), X-ray diffraction (XRD), UV-visible spectroscopy, and Fourier-transform infrared spectroscopy (FTIR) for the characterization of the nanoparticles. Finally, agar well diffusion method was used to study the antibacterial activity of the nanoparticles against *Pseudomonas aeruginosa*. For the synthesis, firstly, 5.0 g of *S. lavandulifolia* flowers was added in deionized water, and then the sepals were transferred into an Erlenmeyer flask having 100 mL hot deionized water and boiled for 20 min. Then, the resultant was filtered using a filter paper, and this extract was used to synthesize copper and copper oxide nanomaterials. Then, 50 mL of copper chloride solution was added dropwise to 25 ml herbal extract with rapid stirring at 50 °C. The pH of the solution was adjusted to 10 by adding NaOH solution. Then, centrifugation was done to separate the precipitate, and washing was done with water and ethanol. The resultant was then dried at ambient temperature. The samples were kept at room temperature. The nanomaterials using the *S. lavandulifolia* can be synthesized using no additional surfactants, polymers, or chemical reagents at room temperature and pressure. This is an attempt to further develop the green synthesis approach of nanoparticles (Table 5.3).

5.4.3 Synthesis of Nanoparticles Using Rosemary Extract

Rosmarinus officinalis, a renewable biological resource, has high antioxidants (Genena et al. 2008) and is used as a safe food flavoring because of its favorable taste and aroma (Nieto et al. 2018). Many researchers have used rosemary extract for the synthesis of nanomaterials. Khatami et al. (2017) synthesize Fe_3O_4 nanoparticles using rosemary extract. *R. officinalis* leaves were obtained from the local market of Kerman city. Firstly, 10 g of leaves was taken and washed with double-distilled deionized water and dried at air temperature (28 °C). Then, the leaves were powdered with mortar and added to the Erlenmeyer containing 1000 mL of sterile double-distilled deionized water. This final mixture was heated at 70–80 °C for

Table 5.3 Various biosources used for green synthesis

Plant	Native place	Ingredients	Properties	Synthesized nanoparticles	References
<i>Panax ginseng</i>	China	Ginsenosides, sugar residues, flavonoids, proteins	Anti-inflammatory	Gold and silver nanoparticles	Attele et al. (1999); Meyer (2012)
Aloe vera	Thailand and several other countries	99.5% of water, vitamins, minerals, enzymes, sugars, anthraquinones or phenolic compounds, lignin, saponins, sterols, amino acids, and salicylic acid	Used for wound healing, anti-inflammatory, burn treatment, and cosmetic industry	Nanoferrites	Manikandan et al. (2014); Boudreau et al. (2017)
<i>Hibiscus rosa-sinensis</i>	India and China	Proteins, vitamin C, organic acids, flavonoids, and anthocyanins	Used to cure hypertension, liver disorder, pyrexia, dandruff, diarrhea, fatigue, skin problems, and gonorrhoea	Various ferrites, gold nanoparticles, silver nanoparticles	Muzammil (2013); Durka and Antony (2015); Missoum (2018); Mak et al. (2013)
<i>Andrographis paniculata</i>	South Asia, China, and Europe		Cure gastric ulcers, fetus abortion, dysentery, arthritis, larynx inflammation, heart pains, and asthma	CdO nanoparticles	Dhanuskodi and Prabukumar (2017b)
<i>Prosopis farcta</i>	Grows in the Middle East	Quercetin (flavonoids), tryptamine, apigenin 5-hydroxytryptamine (alkaloids), L-arabinose, and lectin	Cure gastric ulcers, fetus abortion, dysentery, arthritis, larynx inflammation, heart pains, and asthma	Gold nanoparticles	Miri et al. (2018); Jannet (2005); Gulalp and Karcioglu (2008)

30 min (Khanna-chopra and Semwal 2011). Then, centrifugation was done for 5 min, and the supernatant was collected for further processing. These all procedures were performed inside the laminar airflow cabinet. Therefore, a sterile condition was maintained during the experiments. After that, the ferric (III) chloride hexahydrate stock solution (0.1 M) was prepared by addition of 1000 mL of sterile double-distilled deionized water to 27.03 g of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$. For the preparation of iron oxide (Fe_3O_4) nanoparticles (NPs), 10 mL of filtered *R. officinalis* extract was mixed to a 1 mM FeCl_3 solution with constant stirring at room temperature. After some time, light yellow color was changed to black denoting the synthesis of nanoparticles (Groiss et al. 2016). In this report, nanoparticles were synthesized using a green method and were mainly spherical and homogeneous having an average size of about 4 nm. The green method of synthesis used was an eco-friendly, simple, and inexpensive method. Alijani et al. (Alijani et al. 2020) synthesized nickel ferrite nanoparticles using rosemary extract. NiFe_2O_4 nanoparticles were synthesized by green and sustainable process using natural plant extract. Firstly, distilled water containing sodium hypochlorite was used to wash rosemary young leaves, and then the surface moisture was removed by placing at room temperature. For the extract of rosemary leaves, 200 g of rosemary healthy leaves was added to 1000 mL of deionized water and heated at 80 °C for 1 hour. After that the resultant was filtered with filter paper. For synthesis of nickel ferrite nanoparticles, 0.5 g of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ and 0.1 g of $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ were dissolved in 30 mL of rosemary extract at 70 °C under vigorous stirring. The pH of the solution was maintained at 7.4. Finally, nanoparticles were washed with ethanol and deionized water three times each and then dried in the oven at 60 °C for 12 h. The NiFe_2O_4 nanorod particles were synthesized using greener and cost-effective methodologies using rosemary extract. Metallic nanomaterials have their application in biomedicine and environment. This technique helps the industries' application faster to the end products. Various characterizations were performed using HRTEM, XRD, FeSEM, XPS, VSM, and FTIR. Also, NiFe_2O_4 nanoparticles had a cytotoxicity effect on MCF-7 cell survival which suggests that NiFe_2O_4 nanoparticles can be used as a new class of anticancer agent in designing novel cancer therapy research. These prepared nickel ferrite nanorod particles can be used to increase the level of public health. Also, the nickel ferrite nanoparticles using rosemary extract can be used as medical sensor or antibacterial application.

5.5 Green Synthesis of Nanoparticles Using Fruit Extracts

Among all the methods of synthesis of nanoparticles, biosynthesis comes out to be a good, eco-friendly method to synthesize nanomaterials as in this process, nontoxic reducing and capping agents are used. These capping agents get adsorbed on the surface of nanoparticles. The fruit extracts can be used for the green synthesis of nanomaterials (Isaac et al. 2013). *Solanum torvum* fruit extract is also used for the synthesis of gold and silver nanoparticles (Ramamurthy et al. 2013). The prepared nanoparticles showed good antibacterial and antioxidant properties as shown by

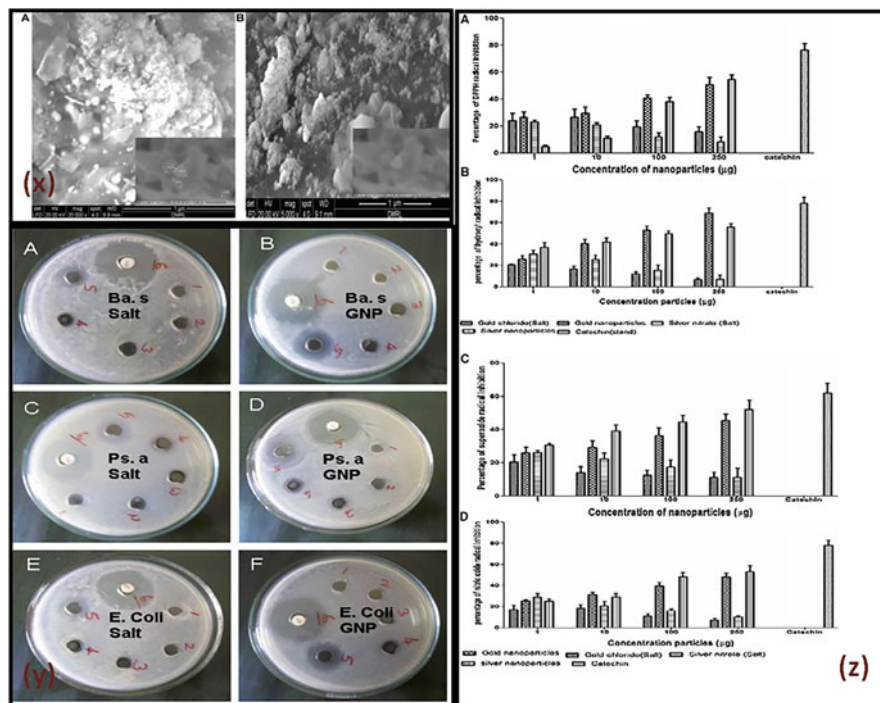


Fig. 5.11 (X) SEM photographs of *S. torvum*-synthesized gold nanoparticles and SEM photographs of *S. torvum*-synthesized silver nanoparticles. (Y) Antioxidant properties of gold and silver nanoparticles and their respective salts. (A) DPPH antioxidant assay of gold and silver nanoparticles; (B) hydroxyl radical scavenging assay of gold and silver nanoparticles; (C) superoxide radical scavenging assay of gold and silver nanoparticles; (D) nitric oxide radical scavenging activity of gold and silver nanoparticles. Catechin was used as positive control. (Z) Antimicrobial activity of gold nanoparticles against (A and B) *Bacillus subtilis*; (C and D) *Pseudomonas aeruginosa* and (E and F) *E. coli* (Reproduced by permission from Ref. Ramamurthy et al. (2013), License No. 4830671457452, Copyright 2013, Elsevier)

Fig. 5.11. Also, tansy is a fruit that is considered a cure for intestinal worms, rheumatism, digestive problems, fevers, etc. This fruit extract was used for the synthesis of silver and gold nanoparticles (Prabha et al. 2010). Figure 5.12 shows the digital image of the tansy fruit and TEM images of the synthesized nanoparticles. The *Limonia acidissima* is a fleshy fruit, and its components include large amounts of carbohydrate (18.1 g), protein (7.1 g), fat (3.7 g), iron (6 mg), and vitamin C (3 mg). These components act as a reducing agent and metal nitrates as oxidizers that are useful for microwave process. Naik et al. (Naik et al. 2019) used *Limonia acidissima* juice for the synthesis of zinc ferrite nanoparticles and tested their application as a photocatalyst and antibacterial agent. The zinc nitrates and ferric nitrates with a ratio of 1:2 in 5 mL of *Limonia acidissima* juice were used as starting materials for the synthesis of zinc ferrite nanoparticles. The *L. acidissima* juice acts

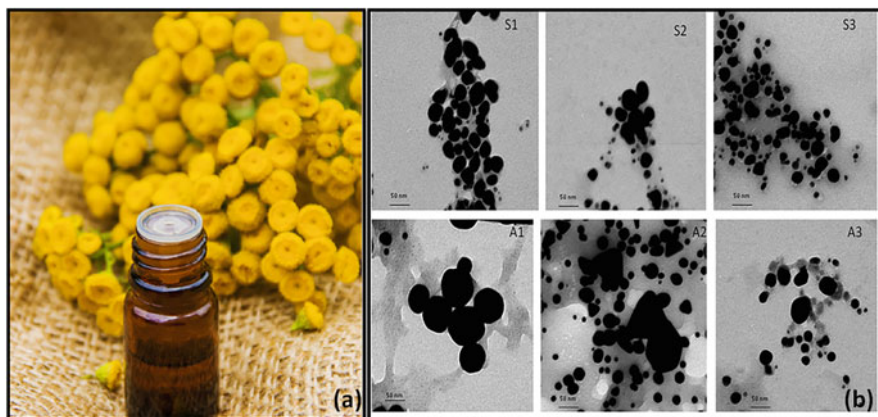


Fig. 5.12 (a) Digital image of the tansy fruit extract. (b) TEM images of silver nanoparticles. (Reproduced by permission from Ref. Prabha et al. (2010), License No. 4830680693395, Copyright 2010, Elsevier)

as a reducing agent. The whole prepared mixture was taken in 45 mL deionized water. Then, the prepared mixture was stirred for 60 min to get the homogeneous solution. The microwave irradiation was done in a domestic microwave oven (2.54 GHz at 900 W) for 15 min. Then, calcination was done at 600 °C for 4 h. Further, the prepared nanoparticles are used for structural, optical, morphological, magnetic, photocatalytic (Evans blue and methylene blue), and antibacterial (foodborne pathogens) studies. Figure 5.13 shows the SEM, TEM, and EDAX spectra of the synthesized nanoparticles. The synthesized zinc ferrites showed significant photocatalytic activity for Evans blue and methylene blue dyes. The prepared ferrites are ferromagnetic in nature as seen in VSM study. Also, the prepared ZnFe_2O_4 nanoparticles show an effective antibacterial activity against foodborne pathogens. The antibacterial activity is shown in Fig. 5.14. The microwave-assisted green synthesis of ZnFe_2O_4 nanoparticles are the suitable materials for wastewater treatment and biomedical applications.

5.5.1 Synthesis of Nanoparticles Using Longan Fruit

Longan (*Euphoria longana Lam.*) fruit is a crop in Thailand and used through whole of Asia. The components of this fruit are “Gallic acid, corilagin and ellagic acid” (Rangkadilok et al. 2005). All these compounds are polyphenols, and are good agents to synthesize AgNPs as well as to stabilize them. Khan et al. (Khan et al. 2018a) synthesized silver nanoparticles using the fruit peel extract of *Longan* fruit. *Longan* fruits were firstly hand peeled, dried in shade and powdered using grinder. Then, 20 g of ground peel was soaked in 160 mL of distilled water at 30 °C for 24 h, filtered and centrifuged at 5000 rpm for 10 min at 4 °C to remove the remaining peel debris. The supernatant was then carefully removed after centrifugation and used for

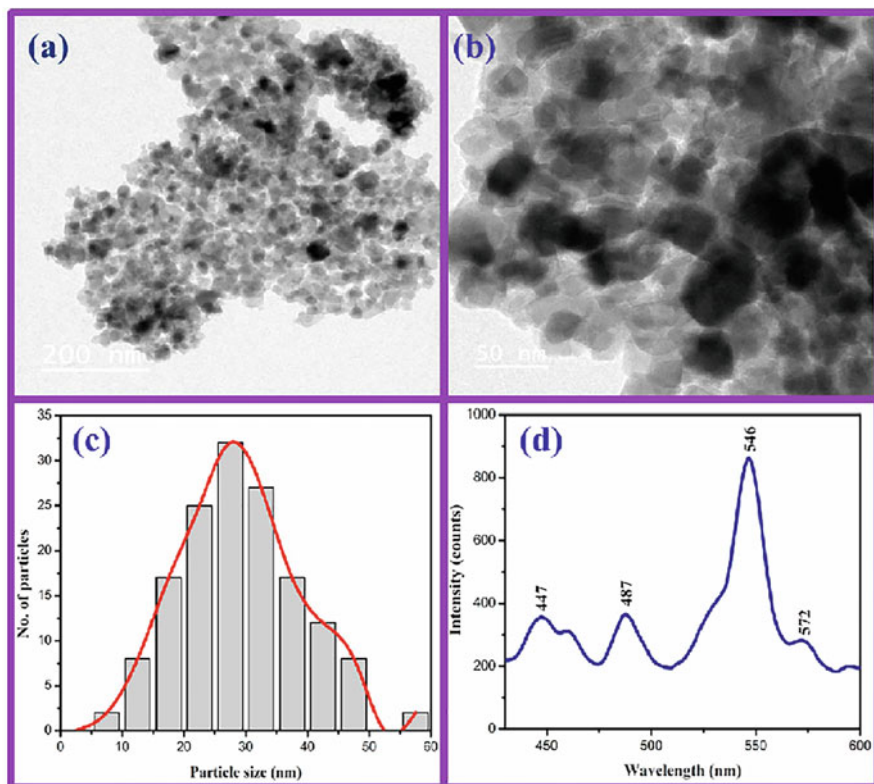


Fig. 5.13 (a) SEM, (b, c) TEM images, (c) typical histogram of the particles' diameter distribution and (d) EDAX spectra of microwave-assisted ZnFe_2O_4 nanoparticles. (Reproduced by permission from Ref. Naik et al. (2019), License No. 4830660251110, Copyright 2019, Elsevier)

the synthesis and stabilization of AgNPs. For the synthesis of AgNPs, 10 mL of 3 mM aqueous solution of AgNO_3 was added to 10 mL aqueous extract of *Longan* fruit peel in a 100 mL beaker and stirred at 300 rpm (30 °C). The color change from brownish to dark black in 55 min showed the formation of nanoparticles. These prepared silver nanoparticles were found to be very good anticancer agents in the treatment of breast cancer.

5.5.2 Synthesis of Nanoparticles Using *C. Sinensis*

C. sinensis (Thouin) Koehne, commonly known as Chinese quince or “Guang Pi Mu Gua,” is found in Korea, China, and Japan. This fruit is commonly used in traditional Chinese medicine and Korean traditional medicine to cure inflammation, vitalize digestion, and reduce cholesterol and sugar levels (Zhang et al. 2009). Also, this fruit is used to cure and alleviate rheumatoid arthritis, cough, common cold, and diarrhea

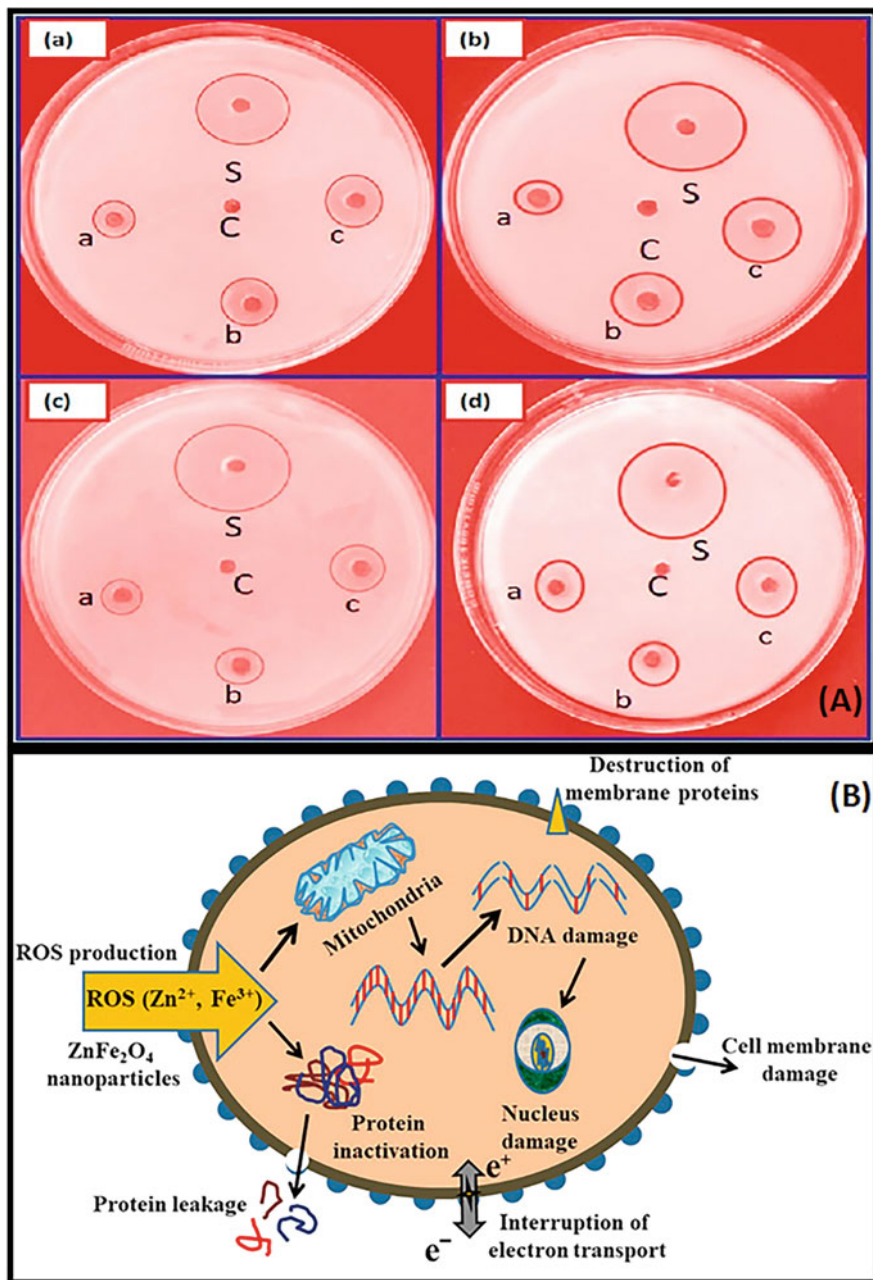


Fig. 5.14 (A) Antibacterial activity of microwave-assisted green synthesis of ZnFe₂O₄ nanoparticles against pathogenic bacterial strains such as (a) *Staphylococcus aureus*, (b) *Escherichia coli*, (c) *Pseudomonas desmolyticum*, and (d) *Klebsiella aerogenes* (S = standard antibiotic; C = control; a, b, and c are the different concentrations of nanoparticles: 50, 100, and 150 µg/µL). (B) Antibacterial activity mechanism of microwave-assisted green synthesis of ZnFe₂O₄ nanoparticles. (Reproduced by permission from Ref. Naik et al. (2019), License No. 4830660251110, Copyright 2019, Elsevier)

(Sawai et al. 2008). The compounds present in this fruit are pentacyclic triterpene acids, flavonoids, lignans, and simple phenolic compounds. Some of these compounds isolated from the extract of *C. sinensis* have the properties of antioxidant, antitussive, antifatulent, antipruritic, and diuretic activities (Ku et al. 2003). Oh et al. 2017) synthesized gold and silver nanoparticles using the fruit extract of *C. sinensis*. For the synthesis, the dried fruits of *C. sinensis* (10 g) were powdered using grinder and then dispersed in 100 ml distilled water. After that the suspension was autoclaved for 1 hr. at 100 °C to obtain an aqueous extract the resultant solution was filtered to remove solid waste and concentrated to 70% using distilled water. Then, H₂SO₄ (1 mM) was added into this diluted solution in room temperature for 10 s. Similarly, AgNO₃ (1 mM) was mixed with the diluted extract at 80 °C for 65 min to initiate metal ion reduction. In each reaction, a change in color was observed that indicated the formation of nanoparticles. The synthesized gold and silver nanoparticles were centrifuged and washed with sterile water at least thrice (to remove water-soluble biomolecules) and finally washed with 80% MeOH. The washed nanoparticles were then air-dried. The synthesized silver and gold nanoparticles were found to be effective antimicrobial agents against pathogenic *Staphylococcus aureus* and *Escherichia coli*. Also these nanoparticles have the ability to inhibit the proliferation of breast cancer cells.

5.6 Greener Synthesis Using some Other Biosources

The synthesis of nanoparticles using the green approach is of very much importance when they are used in the biomedical applications such as anticancer (Miri and Sarani 2018; Khan et al. 2018b). Many plants and even enzymes are used by researchers for the synthesis of nanoparticles (Miri et al. 2018; Darroudi et al. 2014; Muhammad et al. 2017; Nadagouda et al. 2014; Hebbalalu et al. 2013; Kou and Varma 2012; Virkutyte and Varma 2011; Nadagouda and Varma 2006). The reports are available that show the role of a wide variety of phytochemical compounds like terpenoids, tannins, glucose, amino acids, proteins, phenols, and alkaloids in the synthesis and stabilization of nanoparticles (Engelbrekt et al. 2009; Mirzaei and Darroudi 2017; Mittal et al. 2013; Huang et al. 2011).

5.6.1 Synthesis of Nanoparticles Using American Cockroaches

American cockroaches are found to be one of the most widespread insects, and besides that they have a role in medicines. In some parts of Asia, for example, in the southwestern part of China, the diseases such as hepatitis, trauma, stomach ulcers, burns, and heart disease are cured by using the American cockroach extract (Wang et al. 2011; Patterson and Slater 2002). Khatami et al. (2019) synthesized silver nanoparticles by using *Periplaneta americana* wings' extract. Chitin-rich, *Periplaneta americana* (American cockroach) wings' extract has been studied as a novel biomaterial to synthesize silver NPs, and cockroach ball was used to

synthesize water-soluble silver NPs in a size range less than 50 nm and was examined for the insecticidal applications. For this, the American cockroach wings were sterilized in 70% ethanol for 10 s, and after that washing is done using deionized water and left in air. Then, 10 cc of deionized water having 1 drop of acetic acid is added to the wings after breaking them into pieces. Then, it was heated for 1 h at 60 °C and kept in the dark. After that the solution was filtered, and centrifuged at 2500 rpm for 20 min. Then, the extract was strained using Whatman filter paper No. 40. For preparing 0.1 M solution of silver nitrate, 50 ml distilled water was added to 0.899 g of silver nitrate powder. Thus, 1 mM of the solution made was then added to 10 mL of the extract of the wings. The results show a significant fatal effect of silver NPs at a concentration of 100 µg/mL on *Aphis gossypii*. However, concentrations of 1–40 µg/mL did not significantly effect on the mortality of immature aphids. It is clear from all results that silver nanoparticles have the lethal effect of green-synthesized silver NPs on *A. gossypii*, in vitro.

5.6.2 Synthesis of Nanoparticles Using Fungi

Since many years, various biological sources have been used for the nanoparticle synthesis. These biosources include bacteria, fungi, plants, etc. and are effective alternate sources for nanoparticle synthesis (Ramamurthy et al. 2013; Wei et al. 2012; Rajasekharreddy and Usha 2010; Gou et al. 2015; John et al. 2020; Rai and Yadav 2013; Kora and Arunachalam 2013). Among these, fungi are the more efficient candidates for synthesis of nanoparticles because of high protein secretion capacity, higher productivity, and easy handling in large-scale production. In addition, extracellular biosynthesis using fungi can also make downstream processing much easier than when employing bacteria (Honary et al. 2013; Musarrat et al. 2010). Various types of fungi were used by researchers like *Fusarium oxysporum* (Ahmad et al. 2003; Masumeh et al. 2012), *Cladosporium cladosporioides* (Balaji et al. 2009), *Penicillium* sp. (Kumar et al. 2010; Ranjan and Nilotpala 2011), and *Aspergillus flavus* (Vigneshwaran et al. 2007) to synthesize nanoparticles. Hamedi et al. (Hamedi et al. 2016) synthesized silver nanoparticles using *N. intermedia*. For the biosynthesis of the nanoparticles, *N. intermedia* fungus was grown aerobically in culture media containing PDB medium supplemented with 0.5% yeast extract. HCL was used to adjust the final pH of the medium to 5.8. After 72 h of fermentation, the culture broth was separated using centrifugation for 20 min at 6000 rpm. For the production of silver nanoparticles, the resultant supernatant and 2 mM silver nitrate solution were mixed together with volume ratios of 1:1. Finally, the mixtures were put in incubators at 28 °C and agitated at 200 rpm to reach the nanoparticle formation. All reactions were performed in the presence of light. After that filtration was done to separate the fungal mycelia, and washing of the resultant mycelia was done three times with sterilized distilled water. Then, 10 g of harvested mycelia was submerged in a 100 ml sterilized distilled water and incubated on an orbital shaker operating at 200 rpm and 4 °C for 72 h. This suspension was filtered, and finally, the obtained filtrate (50 ml) was mixed with 50 ml of a 2 mM aqueous silver nitrate. The

reaction mixtures were incubated at 200 rpm, 28 °C until the maximum absorbance at λ_{max} was attained. These experiments were conducted in triplicate under light conditions. After the cultivation period, the mycelial mass was separated from the culture broth by sterile filter paper, and washing of the mycelia was done three times with sterile distilled water. Subsequently, 10 g of harvested mycelia was submerged in 100 ml of a 1 mM aqueous silver nitrate. Finally, the reaction mixtures were incubated until reaching a maximum absorbance at λ_{max} in a shaker that operated at 200 rpm and at 28 °C.

Bhainsa et al. (Bhainsa and Souza 2006) synthesized silver nanoparticles using *Aspergillus fumigatus*. *A. fumigatus* (NCIM 902) was obtained from the National Chemical Laboratory, Pune, India, and maintained on potato dextrose agar slants. The fungus was grown aerobically in a liquid medium containing (g/l) KH_2PO_4 , 7.0; K_2HPO_4 , 2.0; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.1; $(\text{NH}_4)_2\text{SO}_4$, 1.0; yeast extract, 0.6; and glucose, 10.0 to prepare biomass for biosynthesis studies. The flasks were inoculated, incubated on orbital shaker at 25 °C, and agitated at 150 rpm. The biomass was sieved using a plastic sieve and then harvested after 72 h of growth. After that, extensive washing with distilled water was done to remove any medium component from the biomass. Typically 20 g of biomass (fresh weight) was brought in contact with 200 ml of Milli-Q deionized water for 72 h at 25 °C in an Erlenmeyer flask and agitated. After the incubation, the cell filtrate was obtained by passing it through the Whatman filter paper. For the synthesis of silver nanoparticles, AgNO_3 , 1 mM final concentration was mixed with 50 ml of cell filtrate in a 250 ml Erlenmeyer flask and agitated at 25 °C in dark conditions. A sample of 1 ml was taken at different time intervals, and the UV-visible spectrophotometer was used to measure the absorbance at a resolution of 1 nm. After 72 h of incubation, the cell filtrates containing nanoparticles were characterized using transmission electron microscopy (TEM). Balaji et al. (Balaji et al. 2009) synthesized silver nanoparticles from *Cladosporium cladosporioides* fungus. The TEM images of the synthesized nanoparticles are shown in Fig. 5.15.

5.6.3 Synthesis of Nanoparticles from Glucose and Starch Solution

To solve the problem of cellular toxicity, so that the nanoparticles can be used in biomedical applications, the synthesis of nanoparticles using starch and glucose is a good option. Engelbrekt et al. (Engelbrekt et al. 2009) synthesized the gold nanoparticles using glucose and starch solution. However, gold nanoparticles may be synthesized using other biosources such as lemongrass plant, tea, seaweed, human cells, fungi, microorganisms, protein, but in that cases sometimes it is very difficult to control chemical composition and purity. Hence, glucose and starch were used as the reducing and stabilizing agents, respectively. For the synthesis of Au nanoparticles, 0.5–2.0 mM of AuCl_4 , 10–30 mM glucose, and 0.6–1% (w/w) starch were mixed in 20–100 mL of 10–30 mM buffer. It was observed that the solution immediately changed to a strong red color at room temperature in MES buffer. In all other buffers, pronounced colors were visible only after the solutions had been

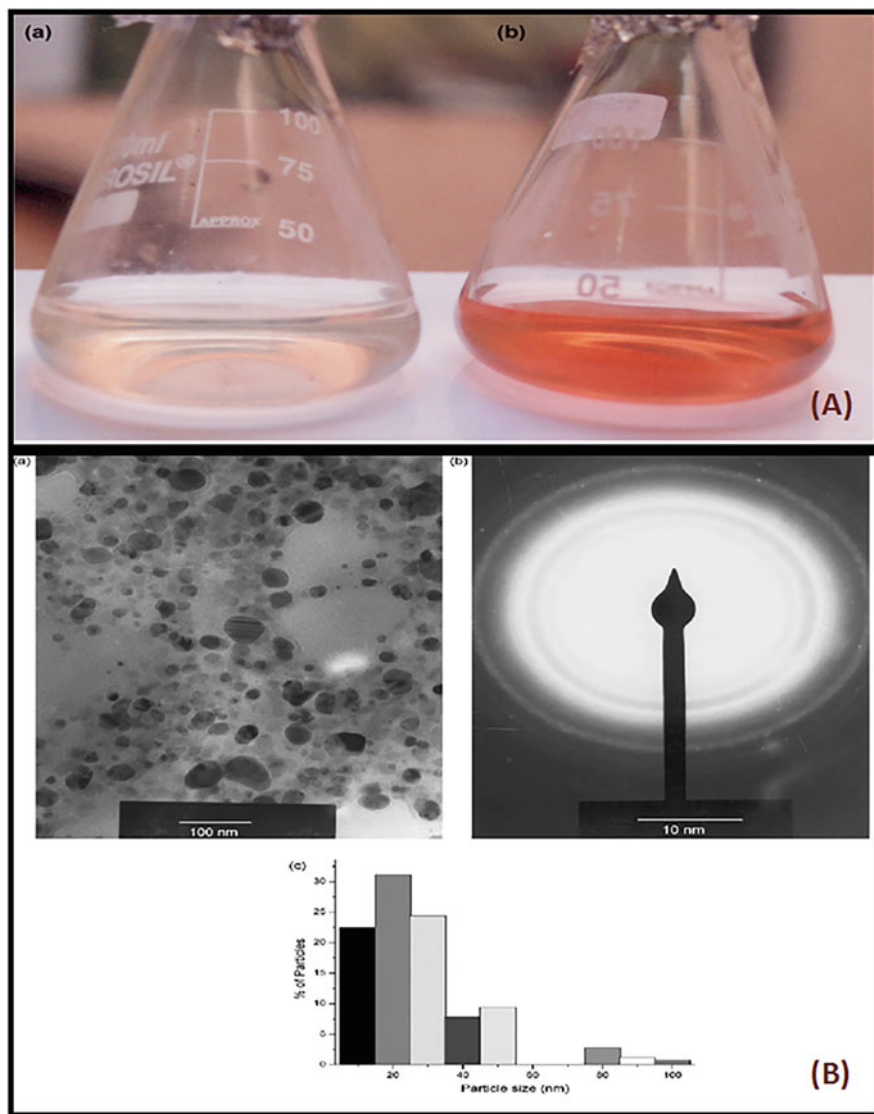


Fig. 5.15 (A) Picture of conical flasks containing the extracellular filtrate of the *Cladosporium cladosporioides* in aqueous solution of AgNO_3 at the beginning of the reaction (flask 1) and after 1 day of reaction (flask 2), (B) (a) TEM images of AgNP. (b) ED pattern images of AgNP. (c) Particle size distribution histogram of AgNP determined from TEM. (Reproduced by permission from Ref. Balaji et al. (2009), License No. 4830680282082, Copyright 2019, Elsevier)

heated at 80–94 °C for 1–2 h. Starch is a key component, although of substantially variable abundance in a variety of vegetable nutrients. The equivalent of 3 g of dry matter, i.e., 13.3 g fresh potato, 20.6 g fresh carrot, or 22.9 g fresh onion, all peeled

and were heated at 95 °C in 200 mL of Millipore water for 3 h. These all contained different amounts of starch. After cooling to room temperature, the liquid solutions were centrifuged to remove insoluble parts, and the supernatant is used for further synthesis. That research work has potential both for larger-scale production of stable and variable-size AuNPs and for biological applications of the AuNPs. Due to starch coating, the layers around the AuNPs may further invoke new properties of the AuNPs, and new strategies for new nanostructures. The prepared nanomaterials may be used in bioelectrochemistry.

5.7 Conclusion

Nature has its own manners of producing miniaturized functional materials. Increasing awareness of green chemistry and the benefit of synthesis of nanoparticles using biosources such as plant extracts, fruits extracts, flower extracts, fungi, bacteria, microorganism, starch and sugar, etc. can be ascribed to the fact that it is ecofriendly, is low in cost, and provides maximum protection to human health. Green-synthesized nanoparticles have remarkable significance in the field of nanotechnology. The synthesized nanoparticles using green technology produce nanostructures that have potential applications in medical field. This study will help researchers to know about novel nanostructures using green technology.

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