Environmental Sustainability Using Green Technologies

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Green Synthesis of Ecofriendly Nanoparticles and Their Medical Applications

Publication details
https://www.routledgehandbooks.com/doi/10.1201/9781315364339-4

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How to cite :- Sahadevan Renganathan, George Philomin Doss Geoprincy, Patchaimuthu Kalainila. 21 Sep 2016, *Green Synthesis of Ecofriendly Nanoparticles and Their Medical Applications from:* Environmental Sustainability Using Green Technologies CRC Press
Accessed on: 21 Jan 2022
https://www.routledgehandbooks.com/doi/10.1201/9781315364339-4

CHAPTER 3

Green Synthesis of Ecofriendly Nanoparticles and Their Medical Applications

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Abstract

Silver nanoparticles are nontoxic to humans but most effective against bacteria, fungi, and viruses, even at low concentrations. Moreover, several salts of silver and their derivatives are commercially manufactured as antimicrobial agents. Microbes may produce resistance against antibiotics by producing repeated mutations to protect themselves from further attack. This is why microbes that are initially sensitive to certain chemotherapeutic agents eventually become resistant upon subsequent dosages of antibiotics. But, with respect to the activity of silver nanoparticles, there exists a complexity for microorganisms to offer resistance. This is because the metal nanoparticles attack a broad range of targets, which makes it difficult for the organism to develop mutations. Thus silver nanoparticles can be used a potential antimicrobial agent. The silver nanoparticles, synthesized using the leaf extracts of *Lucas aspera*, exhibit significant antibacterial and antifungal activity. *Lucas aspera*, commonly known as thumbai, is found to be rich in phytochemical constituents, which offers pharmacological effects. The medicinally active compounds present in the leaf extract reduce the silver ions to form

silver nanoparticles, which can be further used for therapeutic applications.

3.1 INTRODUCTION

Our environment is undergoing great damage, and a large amount of hazardous and unwanted chemicals, gases, and substances are released due to rapid industrialization and urbanization. So, we need to learn about the secrets that are present in nature and its products that will lead to the growth of advancements in the synthesis processes of nanoparticles. Nanoparticle application is highly suitable for biological materials because of their choice properties. Nanotechnology is one of the interesting areas of research in the modern field of material science and biological science (Kaviya et al. 2008).

Most chemical methods used for the synthesis of nanoparticles are too expensive and also involved with the use of toxic and hazardous chemicals that are responsible for various biological risks. Nevertheless, in the case of biological methods, nanoparticle synthesis using plant extracts is the most adopted method, because it is ecofriendly, the green production of nanoparticles, it can act as a source of several metabolites, it is much safer to handle, and easily available (Ankamwar et al. 2005).

Also, most of the antibiotics are less sensitive toward many organisms. Hence, many researchers started to seek a new potential antimicrobial agent (Kim et al. 2007) with this respect, and silver and silver-based compounds have a strong bactericidal and fungicidal activity (Spadaro et al. 1974; Zhao and Stevens 1998). Nanoparticles that have a larger surface-area-to-volume ratio tend to possess higher antimicrobial activity (Lok et al. 2007). Also, silver has a lower tendency to stimulate microbial resistance than many other antimicrobial agents (Silver et al. 2007). Based on these properties, green synthesis of silver nanoparticles has been investigated. Several experiments have been performed on the synthesis of silver nanoparticles using medicinal plants such as *Zea mays, Saccharum officinarum*, *Helianthus annus, Sorghum bicolour*, and *Oryza sativa*. Silver nanoparticles using a methanolic extract of *Eucalyptus hybrida* have also been reported (Jeong et al. 2005).

The development of green processes of nanoparticles is evolving into an important branch of nanotechnology. The research on synthesized nanomaterials and their characterization is an emerging field of nanotechnology from the past two decades due to their huge application in the fields of physics, chemistry, biology, and medicine.

3.2 NANOPARTICLES

Nanoparticles are a group of atoms or molecules in the size range of 1 to 100 nm. The use of nanoparticles is gaining ground in the present century, as they possess defined optical, chemical, and mechanical properties (Gong et al. 2007). The metallic nanoparticles are most promising; they have good antibacterial properties due to their large surface-area-to-volume ratio, which is of much interest to researchers due to the growing microbial resistance against metal ions, antibiotics, and the development of resistant strains. Different types of nanomaterials, including zinc, copper, silver, titanium (Retchkiman-Schabe et al. 2006), magnesium, gold (Gu et al. 2003), alginate (Ahmad et al. 2005), and silver, have been researched, but silver nanoparticles have proven to be most effective, as they have good antimicrobial efficacy against microorganisms such as viruses, bacteria, and other eukaryotic microorganisms.

Silver nanoparticles show effective antimicrobial property compared to other nanoparticles due to their extremely large surface area, which provides better contact with microorganisms. The nanoparticles get attached to the cell membrane and also penetrate inside the bacteria. The bacterial membrane contains sulfur-containing proteins, and the silver nanoparticles interact with these proteins in the cell as well as with the phosphorus-containing compounds like DNA. The nanoparticles preferably attack the respiratory chain and cell division finally leading to cell death. The nanoparticles release silver ions in the bacterial cells, which enhance their bactericidal activity (Feng et al. 2000).

Nanoparticles smaller than 10 nm interact with bacteria and produce electronic effects, which improve the reactivity of nanoparticles. Thus, it is proven that the bactericidal effect of silver nanoparticles is size dependent (Morones et al. 2005; Raimondi et al. 2005). Sastry et al. (2003) reported the biosynthesis of nanoparticles using plant leaf extracts and their potential application. Researchers have also studied bioreduction of chloraurate ions and silver ions by extracts of geranium (Shankar et al. 2003) and neem leaf (Shankar et al. 2004a).

Biological methods of nanoparticle synthesis using microorganisms, enzymes, fungus, and plants have been suggested as possible ecofriendly alternatives to chemical and physical methods. Sometimes the synthesis of nanoparticles from plants can prove advantageous over other biological

TABLE 3.1 Sources Utilized for Synthesis of Nanoparticles

Source	**Type of Nanoparticle**	**Reference**
Catharanthus roseus	Silver	Ponarulselvam et al. 2012
S. tricobatum, S. cumini, C. asiatica	Silver	Logeswari et al. 2015
Capparis zylanica leaf	Copper	Saranyaadevi et al. 2014
Polyalthia longifolia leaf	Silver	Kaviya et al. 2008
Emblics officinalis fruit	Silver, gold	Ankamwar et al. 2005
Geranium leaf	Silver	Shankar et al. 2003
Azadirachta indica leaf	Silver, gold	Shankar et al. 2004a
Dioscorea bulbifera	Silver	Sougata et al. 2012
Papaya fruit	Silver	Jain et al. 2009
Vitex negundo	Silver	Mohzen et al. 2011

methods by eliminating the elaborate process of maintaining microbial cultures. There are various sources utilized for synthesis of nanoparticles (see Table 3.1).

3.3 TYPES OF NANOPARTICLES

Many nanoparticles are synthesized including as silver, copper, iron, gold, zinc, platinum, and palladium. A few of these are discussed next. These nanoparticles have been utilized for biomedical applications.

3.3.1 Silver Nanoparticles

Nanoparticles have been known to be used for numerous physical, chemical, biological, and pharmaceutical applications. Silver nanoparticles (Ag NPs) are used as antimicrobial agents. It is a well-known fact that silver ions and silver-based compounds are highly toxic to microorganisms (Slawson et al. 1992; Zhao and Stevens 1998). This aspect of silver makes it an excellent choice for multiple roles in the medical field. Generally, silver is used in the nitrate form to induce antimicrobial effect, but with the use of Ag NPs, there is a large surface area available for the microbe to be exposed. Thus Ag NPs find use in many antibacterial applications.

3.3.2 Copper Nanoparticles

Copper nanoparticles (Cu NPs) are very attractive due to their heat transfer properties, such as high thermal conductivity. Cu NPs have a low production cost, high surface-area-to-volume ratio, antibacterial potency, optical properties, catalytic activity, and magnetic properties as compared to precision metals such as gold, silver, or palladium (Borkow 2010; Borkow et al. 2009). Copper nanoparticles have great interest due to their catalytic, optical, mechanical, and electrical properties. Copper is an excellent alternative material for noble metals such as Au and Ag, as it is highly conductive and much more economical (Athanassiou et al. 2006). Copper plays an important role in electronic circuits because of its excellent electrical conductivity. Copper nanoparticles are highly inexpensive and their properties can be controlled depending on the synthesis method (Kantam et al. 2007).

3.3.3 Gold Nanoparticles

Elemental gold has many unique properties that have attracted interest in the biomedical field. It has been used for many decorative, ceremonial, colorful, and religious artifacts. Properties of gold nanoparticles are different from gold's bulk form, because bulk gold is yellow solid and it is inert in nature, whereas gold nanoparticles are wine red in color in solution and are reported to be antioxidant. Gold nanoparticles also exhibit different shapes such as spherical, suboctahedral, octahedral, decahedral, icosahedral multiple twined, multiple twined, irregular shape, tetrahedral, nanotriangles (Shankar et al. 2004b), nanoprisms, hexagonal platelets, and nanorods. Gold nanoparticles are widely used in biomedical science including tissue or tumor imaging, drug delivery, photothermal therapy, and immune chromatographic identification of pathogens in clinical specimens due to the surface plasmon resonance (SPR) (Chithrani et al. 2010).

3.3.4 Iron Nanoparticles

The synthesis of superparamagnetic nanoparticles has been intensively developed not only for fundamental scientific interest but also for many technological applications such as magnetic storage media, biosensing applications, and medical applications, such as targeted drug delivery. Superparamagnetic iron oxide nanoparticles with appropriate surface chemistry can be used for numerous in vivo applications (Gupta and Gupta 2005), such as MRI contrast enhancement, tissue repair, immunoassay, detoxification of biological fluids, hyperthermia, drug delivery, and cell separation. All of these biomedical applications require that the nanoparticles have high magnetization values, a size smaller than 100 nm, and a narrow particle size distribution. These applications also need particular surface coating of the magnetic particles, which has to be nontoxic and biocompatible and must also allow for a targetable delivery. Such magnetic nanoparticles can bind to drugs, proteins, enzymes, antibodies, or nucleotides, and can be directed to an organ, tissue, or tumor using an external magnetic field (Petri-Fink et al. 2005).

The most common methods including coprecipitation, thermal decomposition, hydrothermal synthesis, microemulsion, sonochemical synthesis, and sonochemical synthetic route can all be directed to the synthesis of high-quality iron oxide NPs. In addition, these NPs can also be prepared by other methods such as electrochemical synthesis, laser pyrolysis techniques, and microorganism or bacterial synthesis (Roh et al. 2006).

3.3.5 Zinc Oxide Nanoparticles

Zinc oxide is an inorganic compound with the molecular formula ZnO. It appears as a white powder and is nearly insoluble in water. The powder ZnO is widely used as an additive in numerous materials and products including ceramics, glass, cement, rubber (e.g., car tires), lubricants, paints, ointments, adhesives, plastics, foods (source of Zn nutrient), batteries, ferrites, and fire retardants.

ZnO semiconductor has several unique properties such as good transparency, high electron mobility, wide band gap, and strong room temperature luminescence. These properties account for its applications in transparent electrodes, in liquid crystal display, and in energy-saving or heat-protecting windows and other electronic applications. The wide band gap and large excitonic binding energy have made zinc oxide important for both scientific and industrial applications (Wang et al. 2004).

3.3.6 Cadmium Sulfide Nanoparticles

The wet chemical method is used for the fabrication of materials, typically a metal oxide starting either from a chemical solution or colloidal particles to produce an integrated network particle. Typical precursors are metal alkaloxide and metal chloride, which undergo hydrolysis in polycondensate reactions to form a colloid, a system composed of solid particles (size ranging from 1 nm to 100 nm) dispersed in a solvent.

Cadmium sulfide is a chemical compound with the formula CdS. Cadmium sulfide is yellow in color and is a semiconductor (2.42 eV). It exists in nature as two different minerals: green kite and hawleyite. Cadmium sulfide is a direct band gap semiconductor (gap 2.42 eV) and has many applications, for example, in light detectors. It forms thermally stable pigments ranging from deep red to yellow.

CdS nanoparticles have been extensively studied due to their potential applications, such as field effect transistors, light-emitting diodes, photo catalysis, and biological sensors. Many synthetic methods have been employed to prepare CdS nanoparticles including soft chemical reaction,

TABLE 3.2 Nanoparticles Utilized for Biomedical Applications

Source	Type of Nanoparticle	Application	Reference
Paederia foetida leaf	Silver	Antibacterial activity	Lavanya et al. 2013
Mimosa pudica leaf	Silver	Antibacterial activity	Akash Raj et al. 2014
Annona squamosa leaf	Silver	In vitro cytotoxicity effect on MCF-7 cell	Viveka et al. 2012
Erythrina indica lam root	Silver	Antimicrobial activity and cytotoxic activity	Rathi Sre et al. 2015
Carica papaya	Copper oxide	Photocatalytic dye degradation	Sankar et al. 2014
Commelina nudiflora L.	Gold	Antibacterial and antioxidant activity	Kuppusamy et al. 2015
Acalypha indica Linn.	Silver and gold	Cytotoxic effects against MDA-MB-231	Krishnaraj et al. 2014
Acorus calamus	Gold	Antibacterial and UV blocking	Ganesan et al. 2014
Ocimum sanctum leaf	Iron oxide	Spectroscopic and microscopic studies	Balamurughan et al. 2014
Rosa damascena petals	Silver	Anticancer activity	Venkatesan et al. 2014

solid state reaction, sol gel process, microwave heating, photo etching, and reverse micelle. There are various nanoparticles utilized for biomedical applications (Table 3.2).

3.4 METHODS AVAILABLE TO SYNTHESIZE SILVER NANOPARTICLES

Nanoparticles can be produced using many different techniques, typically classified as bottom-up or chemical methods, and top-down or physical methods (Bali et al. 2006). In the bottom-up approach, the structure of nanoparticles is constructed by atoms, molecules, or clusters. In the top-down approach, a bulk piece of a required material is reduced to nanosized dimensions using cutting, grinding, and etching techniques, i.e., nanomaterials are prepared from larger entities without atomic-level control. Chemical reduction, microemulsion (colloidal) techniques, sonochemical reduction, electrochemical, microwave-assisted, and hydrothermal syntheses are the main techniques for the synthesis of nanoparticles through the chemical approach. Biological or biosynthesis techniques are also considered as bottom-up or chemical processes. Physical methods for nanoparticles synthesis are laser (pulse) ablation, vacuum vapor deposition, pulsed wire discharge (PWD), and mechanical milling. A wide range of nanoparticles can be produced using physical methods with little modification for different metals, but the main disadvantages of these methods are the quality of the product, which is less as compared to nanoparticles produced by chemical methods. Usually these methods require costly vacuum systems or equipment to prepare nanoparticles. During the chemical synthesis of nanoparticles, the morphology and growth can be controlled by optimizing reaction conditions, such as concentration, temperature, surfactant precursor, capping/stabilizing agent, and the type of solvent (Chen et al. 2006). Using these optimum reaction conditions, a narrow size distribution can be achieved during chemical synthesis. These methods for the production of nanoparticles are appropriate for laboratory-scale synthesis but are not economical for a large-scale or commercial setup.

3.4.1 Chemical Methods

There are many methods are available to synthesize nanoparticles by chemical routes such as chemical reduction, microemulsion, sonochemical, electrochemical, and solvothermal decomposition. A few techniques are discussed next.

3.4.1.1 Chemical Reduction Method

The chemical reduction method is the simplest, easiest, and the most commonly used synthetic method for nanoparticles. In fact, the production of nanoparticles with good control of sizes and morphologies using chemical reduction methods can be achieved (Huang et al. 1997).

In the chemical reduction techniques, nanoparticles are reduced by a reducing agent such as sodium borohydride, hydrazine (N_2H_4), ascorbate, polyol, isopropyl alcohol with cetyl trimethyl ammonium bromide (CTAB) as well as glucose (Panigrahi et al. 2006).

3.4.1.2 Microemulsion Method

Microemulsion is a technique for the synthesis of nanoparticles in which two immiscible fluids such as water in oil (W/O) or oil in water (O/W) or water in supercritical carbon dioxide (W/Sc. CO_2) become a thermodynamically stable dispersion with the aid of a surfactant. An emulsion is a single phase of three components: water, oil, and a surfactant (Kapoor et al. 2002). Normally oil and water are immiscible but with the addition of a surfactant, the water and oil become miscible because the surfactant is able to bridge the interfacial tension between the two fluids (Kitchens and Roberts 2004).

Microemulsion consists of surfactant aggregates that are in the ranges of 1 nm to 100 nm. The location of the water, oil, and surfactant phases affects the geometry of aggregate. If the microemulsion is to be oil in water, the water is the bulk fluid and the presence of oil is in less quantity with small amounts of surfactant. Similarly, if it is water in oil, the oil is the bulk fluid and water is present in less quantity. The creation of oil in water and surfactant is called micelles, which is an aggregate formed to reduce free energy. The W/O microemulsion carries oil or organic solvent as bulk then the system is thermodynamically stable and called reverse micelles (Dadgostar 2008).

3.4.1.3 Sonochemical Method

Acoustic cavitation is a physical phenomenon that is responsible for sonochemical reaction. This method was initially proposed for the synthesis of iron nanoparticles. Nowadays, this method is used to synthesize different metals and metal oxides. The main advantages of the sonochemical method are its simplicity, operating conditions (ambient conditions), and easy control of the size of nanoparticles by using precursors with different concentrations in the solution (Suslick et al. 1996).

Sonochemical reactions of volatile organometallics have been exploited as a general approach to the synthesis of various nanophase materials by changing the reaction medium. There are many theories presented by different researchers that have been developed to explain the mechanism of breakup of the chemical bond under 20 KHz ultrasonic radiations. They have explained the sonochemistry process in these theories, i.e., how bubble creation, growth, and its collapse is formed in the liquid.

3.4.2 Physical Methods

3.4.2.1 Pulsed Laser Ablation/Deposition

The laser ablation method is a commonly used technique for the preparation of nanoparticles in colloidal form in a variety of solvents. Nanoparticles are prepared in colloidal form to avoid oxidation. The process of pulsed laser ablation takes place in a vacuum chamber and in the presence of some background/inert gas. In pulsed laser deposition, a laser beam is focused inside a vacuum chamber in which a high-power pulse strikes a target in the material creating plasma, which then is converted into a colloidal solution of nanoparticles. Mostly second harmonic generation (Nd:YAG) lasers are being used to prepare the nanoparticles. There are many factors that affect the final product, such as the number of pulses, pulsing time, type of laser, and type of solvent (Song et al. 2007).

3.4.2.2 Mechanical/Ball Milling Method

Milling is a solid-state processing technique for the synthesis of nanoparticles. This technique was first used by Benjamin for the production of superalloys. In the milling process, raw material of micron size is fed to undergo several changes. Different types of mechanical mills are available that are commonly used for the synthesis of nanoparticles. These mills are characterized according to their capacities and applications.

Mechanical mills that are commonly used for the synthesis of nanoparticles are vibratory, planetary, attritor, and uniball. It is very difficult to produce ultrafine particles, due to mechanical limitations and long length of time. However, simple operation, low cost of production of nanoparticles, and the possibility to produce large quantities are the main advantages of mechanical milling. The important factors affecting the quality of the final product are the type of mill, container, milling speed, time, atmosphere, temperature, size and size distribution of the grinding medium, process control agent, and weight ratio of ball to powder and extent of filling the vial (Suryanarayana 2001).

3.4.2.3 Pulsed Wire Discharge Method

Pulsed wire discharge (PWD) is a physical technique to prepare nanoparticles. Compared to the other previously mentioned methods, synthesis of metal nanoparticles by the PWD technique follows a completely different mechanism. In PWD, a metal wire is evaporated by a pulsed current to create a vapor, which is then cooled by using an ambient gas to form nanoparticles. Preparations of metal, nitride, and oxide nanoparticles by PWD have been reported. This method has a high-energy efficiency, high production rate, and a simple apparatus consisting of a vacuum chamber to be used for the nanoparticle preparation using a powder collection filter and a discharging circuit. This process is not used conventionally for common industrial purposes, because it is not only very expensive but also impossible to use explicitly for different metals. It is mainly useful for high electrical conductivity metals that are easily available in the thin wire form (Jiang and Yatsui 1998).

Muraia et al. (2007) found that copper nanoparticles covered with organic matter can also be successfully prepared by evaporation of copper wire in an oleic acid vapor/mist, with the thickness of the coating layer up to a few nano meters. They prepared nanoparticles of size 10 to 25 nm using the pulsed wire technique.

3.4.3 Biological Synthesis

There is a need for biosynthesis of nanoparticles design, as the physical and chemical processes are costly and hazardous. Therefore, in the search for cheaper pathways, scientists used microorganisms and plant extracts for synthesis for nanoparticles. Nature has planned various processes for the synthesis of nano and microlength-scaled inorganic materials, which have contributed to the development of relatively new and largely unexplored areas of research based on the biosynthesis of nanomaterials. Biosynthesis of nanoparticles is also considered to be a bottom-up technique, where the oxidation/reduction is the main reaction that occurs during the production. Metals are usually reduced into their respective nanoparticles because of the microbial enzymes or the plant phytochemicals with antioxidant or reducing properties.

In the biosynthesis of nanoparticles, three important parameters are (1) the choice of the solvent medium used, (2) the choice reducing agent, and (3) the choice of a nontoxic material for the stabilization of the nanoparticles. The use of bacteria, actinomycetes, organisms, plants, and fungi to synthesize nanoparticles is being practiced (Bali et al. 2006). The biosynthesis of nanoparticles involves easy preparation protocols and less toxicity, and includes a wide range of applications according to their morphology. The size of nanoparticles can be controlled using this technique but not in a complete manner. The field of nanobiotechnology needs more research focused on the mechanism of nanoparticles formation, which may lead to fine-tuning of the process; ultimately it is very important to the synthesis of nanoparticles with a strict control over the size and shape parameters.

Certain plants are known to accumulate higher concentrations of metals compared to others and such plants are termed as hyperaccumulators. *Brassica juncea* had better metal-accumulating ability and also integrated the metal as nanoparticles (Cioffi et al. 2005).

3.5 CHARACTERIZATION TECHNIQUES

3.5.1 Ultraviolet-Visible Spectrometry

Samples for ultraviolet-visible (UV-Vis) spectrophotometry are most often liquids and gases, and even solids can be measured. Samples are always placed in a cuvette. Cuvettes are typically rectangular in shape, generally with an internal width of 1 cm.

UV-Vis absorption spectra of the colloidal dispersions were recorded using the Ultro spec 2100 spectrophotometer. The distribution of the particle size was measured by the Zeta-Sizer system (Malvern Instruments). The biosynthesized silver nanoparticles from mangosteen leaf has a resolution of 1 nm between 300 and 700 nm and possess a scanning speed of 300 nm/min, which was determined by UV-Vis absorption. Synthesis of silver nanoparticles from plant extract shows that maximum absorbance occurs at 430 nm, which increases as a function of reaction time. There is no evidence of absorbance for the UV-Vis spectra range between 400 nm 800 nm for the pure *Solanum torvum* plant extract, but when the plant extract gets exposed to $AgNO_3$ solutions, maximum absorbance was found at 434 nm, due to the formation of nanoparticles. The width and frequency of the surface plasmon absorption depends on the size and shape of the metal nanoparticles as well as on the dielectric constant of the metal itself and the surrounding medium (Sastry et al. 1997).

3.5.2 Fourier Transform Infrared Spectroscopy

In Fourier transform infrared spectroscopy (FTIR), infrared radiation is passed through a sample. The infrared radiation is absorbed by the sample and some of it is passed through the sample or transmitted. Finally, the resulting spectrum represents the molecular absorption and transmission, creating a molecular fingerprint of the sample. Elemental analysis of the synthesized silver nanoparticles from *Lucas aspera* was studied by FTIR.

FTIR analysis is mainly for determining functional groups present in the compound. FTIR absorption spectra of *Dioscorea bulbifera* tuber extract show a strong peak at 3300 cm^{-1} representing the O-H bond. But after bioreduction, it is not seen in the extracts of *D. bulbifera*. The absorbance bands at 2931 cm^{-1}, 1625 cm^{-1}, 1404 cm^{-1}, and 1143 cm^{-11} are associated with respect to the stretch vibrations of alkyl C=C, conjugated C-C with a benzene ring, bending of C-O-H and C-O stretch in saturated tertiary or secondary highly symmetric alcohol in *D. bulbifera*. The presence of peaks at 3749 cm^{-1} and 1523 cm^{-1} indicate the NH_2 symmetric stretching and N-O bonds in nitro compounds (Sougata et al. 2012).

FTIR measurements for *Gliricidia sepium* shows the absorption peak at around 1020 cm^{-1} can be assigned as absorption peaks of -C-O-C- or C-O-. The absorption spectra at 1638 cm^{-1} result from stretching of vibration of -C=C-. The peak at around 1640 cm^{-1} indicates the amide I bonds of proteins. The bonds or functional groups such as -C-O-C-, C-O-, and -C=C- are derived from heterocyclic compounds. The amides I bond derived from the proteins are the capping ligands of the nanoparticles (Raut et al. 2009).

3.5.3 X-Ray Diffraction

For x-ray diffraction (XRD), the Collidge tube is arranged and a power of 1.2 kw is produced from 40 kv and 30 mA to produce the x-ray of wavelength 1.5406 Å. The prepared sample is mounted on the focusing circle. The x-rays are allowed to fall on the sample and the counter is initially set at an angle of 0°. The program is set to run such that the detector counter moves through the required angle at specific counts and scans the sample. The angle range is between 10° and 70°.

As the x-ray beam is diffracted by the sample and detected at different angles, the output peak is generated on the computer screen. The output is a graph with different peaks corresponding to different planes of the crystal and the graph drawn was between 2θ in the x-axis and intensity in the y-axis. The obtained peak is compared with the data in the Joint Committee on Powder Diffraction Standards (JCPDS) tool, which is a standard. From this even the particle size of the crystal was calculated using the formula

$$D = 0.9\lambda/\beta\cos\theta$$

Formation of silver nanoparticles from papaya fruit extract shows three intense peaks that range from 10° to 80°. The average size of the particles was measured as 15 nm (Devendra et al. 2009). The XRD patterns of Ag/*Vitex negundo* indicate the face-centered cubic structure of silver nanoparticles. The silver nanoparticles showed XRD peaks at 38.17°, 44.31°, 64.44°, 77.34°, and 81.33°, corresponding to the face-centered cubic planes (111, 200, 220, 311, and 222, respectively) of the silver crystals (Mohzen et al. 2011).

3.5.4 Scanning Electron Microscope

A scanning electron microscope (SEM) is a type of electron microscope that images a sample by scanning it with a high-energy beam of electrons in a raster scan pattern. The electrons interact with the atoms that make up the sample producing signals that contain information about the sample surface topography, composition, and other properties such as electrical conductivity. SEM is extensively used to study the detailed images of the sample taken for investigation.

Green synthesis of silver nanoparticles from *Cleome viscosa* was analyzed by SEM. SEM observations were established using the ZEISS EVO 40 EP electron microscope. SEM analysis shows that the silver nanoparticles are uniformly distributed on the surface. But it does not indicate that all the nanoparticles are bound to the surface. This may mean that the particles dispersed in the solution may also be deposited onto the surface (Bharathi et al. 2012).

3.6 BIOMEDICAL APPLICATIONS OF NANOPARTICLES

3.6.1 Antimicrobial Activity of Silver Nanoparticles

It is known that silver ions and nanoparticles are highly toxic and hazardous to microorganisms. Silver nanoparticles have many inhibitory and bactericidal effects and so their application is extended as an antibacterial agent. The antibacterial activity of silver nanoparticles is estimated by the zone of inhibition. Silver nanoparticles not only interact with the surface of the membrane but can also penetrate inside the microorganism. Recently, nanoparticles have gained significance in the field of biomedicine. The most distinguishing and significant property of nanoparticles is that they exhibit a large surface-area-to-volume ratio. When the surface area of the nanoparticles gets increased, their surface energy also increase, and hence their biological effectiveness increases (Srivastava et al. 2011). Smaller nanoparticles with a larger surface-area-to-volume ratio provide a more effective antibacterial activity even at a very low concentration. Silver nanoparticles of many different shapes (spherical, rod, truncated, triangular nanoplates) were developed by various synthetic routes. The triangular shape of silver nanoplates were found to show the strongest antibacterial activity. This property could be due to their larger surface-area-to-volume ratios and their crystallographic surface structures.

Silver nanoparticle is an effective and a fast-acting fungicide against a broad spectrum of common fungi including genera such as *Aspergillus, Candida,* and *Saccharomyces*. The standard well diffusion method was used to assay the antibacterial activity against human pathogenic bacteria such as *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus subtilis*, and *Klebsiella pneumonia* (Aditi et al. 2011). In vitro antibacterial activity of the prepared nanoparticles was studied using the Kirby-Bauer technique, which confirmed the recommended standards of the National Committee for Clinical Laboratory Standards (NCCLS), now known as the Clinical and Laboratory Standards Institute (CLSI). The agar well diffusion method was used to assess the antibacterial activity of synthesized Ag nanoparticles. The zone of inhibition produced by various antibiotics was compared with the inhibitory zone produced by silver nanoparticles (Geoprincy et al. 2011). The antibacterial assays were performed on human pathogenic bacteria like *Escherichia coli* and *Pseudomonas aeruginosa* by standard disc diffusion method. Luria Bertani (LB) broth/agar medium was used to cultivate bacteria. Basically, nanoparticle has antimicrobial (including antibacterial and antifungal) applications. The silver or gold nanoparticles that are produced extracellular from *Fusarium oxysporum* can be used in several materials like clothes. Such clothing is sterile and used in hospitals to prevent or to minimize infections with pathogenic bacteria like *Staphylococcus aureus*. The average zones of inhibition expressing a profound inhibitory effect was represented as 35 mm in *P. aeruginosa*, 30 mm in *K. pneumonia*, 36 mm in *S. aureus*, 40 mm in *S. typhi*, 38 mm in *S. epidermis*, and 34 mm in *E. coli* (Shirley et al. 2010).

For the concentration of 20 µg, 40 µg, 60 µg, and 80 µg of the nanoparticle, *Staphylococcus aureus* exhibited characteristic inhibitory zones of 14 mm, 16 mm, 18 mm, and 20 mm diameter, respectively, whereas *Enterococcus faecalis* exhibited inhibitory zones of 11 mm, 13 mm, 14 mm, and 17 mm diameter, respectively (Karthick et al. 2011). Nanoparticles were also used in controlled drug delivery, biological detection, optical filters, and sensor design.

3.6.1.1 Disk Diffusion Method

Four major pathogens—*B. cereus*, *B. subtilis*, *K. pneumonia*, and *V. cholerae*—were taken for analysis. Nutrient agar media were prepared and poured into Petri dish plates and swabbed with respective microbial inoculum. In each plate, four disks were fixed at equal distance. The first one was loaded with a concentration of 25 µg/ml (A) silver nanoparticle. The second disk was impregnated with a 50 µg/ml (B) concentration of silver nanoparticle. The third disk was impregnated with a 50 µg/ml (D) equimolar concentration of silver nanoparticle and rifamycin, and the fourth disk was loaded with a 50 µg/ml concentration of reference antibiotic rifamycin. Well C was maintained as blank without nanoparticle and rifamycin. All 16 plates were kept at 37°C for incubation overnight. The zone of inhibition was measured after 24 h of incubation.

3.6.1.2 Thin Layer Chromatography

Thin layer chromatography (TLC) is used to separate the individual compounds formulated in antibiotics (crude). The separation of the compound also depends on the type of solvent used. The maximum zone of inhibition (rifamycin) based on the disk diffusion method was used for TLC analysis. For that, a 10 mg/ml concentration of antibiotics in methanol was prepared.

From this solution, 4 µl of the sample was taken and spotted on the silica-coated TLC plates. Then it was kept in slanting position with the solvent to run under capillary pressure. Methanol and chloroform in the ratio of 3:7 was used as a solvent. The spots were then identified both in the UV light, far infrared light in the iodine chamber (Nabi et al. 2006).

The R_f values were calculated. The R_f value is defined as the distance traveled by a given component divided by distance traveled by the solvent front.

3.6.1.3 Bioautography Agar over Layer Method

The developed TLC plates were kept in the sterile Petri plate. The nutrient agar was prepared and poured in a thin layer to cover the entire Petri dish. Twenty-four-hour cultures of *B. cereus* were swabbed on it. The plates were then incubated at 37°C for 24 hours. The zone of inhibition obtained at varying separation points were observed. Similarly chromatogram was developed by loading a 1 µg concentration of nanoparticle impregnated with rifamycin (antibiotic). Thus the characteristic zone of inhibition was obtained by the agar over layer method (Pandey et al. 2004).

3.6.2 Biological Applications of Gold Nanoparticles

Gold nanoparticles have been widely used in many fields, such as chemical and biological sensors, electronics, dyes, conductive coatings, catalysis, fundamental research, and electron microscopy. Almost every chemical process involves catalysis. Nanoparticles can efficiently act as catalysts, since they have a large surface-to-volume-ratio and special binding sites. Gold nanoparticles exhibit high catalytic activity in the oxidation and reduction of hydrocarbons (Zhong and Mathew 2001). Nanoparticles exhibit different physical and chemical properties from their bulk solid materials. When gold nanoparticles are conjugated with saccharide and oligo deoxyribonucleic acid, the combination of organic functionality with dielectric properties of gold nanoparticles resulted in a new material, which can be used as a sensitive colorimeter for the detection of polynucleotides (Zhu et al. 2004).

Gold nanoparticles can also be used as biosensors due to their unique optical properties. Anti-EGFR (epidermal growth factor receptor) gold nanoparticles conjugated with gold nanoparticles can distinguish cancerous and noncancerous cells, which is proven by SPR (surface plasmon resonance) scattering and SPR absorption spectroscopy. Hence, it can be used in cancer diagnostics. Gold nanoparticles play an important role in drug and gene delivery. Gold nanoparticles when irradiated with light in water will create local heating and can be used in photothermal destruction of tumors. Also gold nanoparticles enhance the efficiency of photothermal therapy 20-fold (Guo and Wang 2007). The excellent biocompatibility and unique properties made gold nanoparticles attractive material for biosensors, chemosensors, and electrocatalysts. An electrochemical device with gold nanoparticles will provide new opportunity for gene diagnostics.

3.6.3 Silver Nanoparticles: Biological and Clinical Significance

In general, silver nanoparticles (Ag NPs) are nontoxic to humans, but most effective against bacteria, virus, and other eukaryotic microorganism at low concentrations (Krutyakov et al. 2008). Moreover, several salts of silver and their derivatives are commercially manufactured as antimicrobial agents (Kasthuri et al. 2009). These silver nanoparticles provide significant pharmaceutical, clinical, biological, and immunological applications. They are used to prevent infection, in (burn and traumatic) wound dressings, diabetic ulcers, coating of catheters, dental works, scaffold, and medical devices (Thomas et al. 2007). In addition, they are used as anticancer agents in the treatment of cancer, in the field of tissue engineering, and in drug delivery. The bactericidal properties of silver nanoparticles are due to the release of silver ions from the particles, which is highly effective for antimicrobial activity (Amarendra and Krishna 2010). Also, the potency of the antibacterial effects corresponds to the size of the nanoparticle. The smaller particles have higher antibacterial activities. With respect to the clinical applications of nanoparticles, microorganisms including diatoms, fungi, bacteria, and yeast-producing nanoparticles through biological synthesis were found to be more biocompatible (Guidelli et al. 2011).

3.7 CONCLUSION

It is concluded that plant-mediated synthesis of silver nanoparticles possesses potential antimicrobial applications. The characterization analysis proved that the particle, produced in nano dimensions, would be equally effective as that of antibiotics and other drugs in pharmaceutical applications. The use of silver nanoparticles in drug delivery systems might be the future thrust in the field of medicine. It was concluded that silver nanoparticles can serve as potential drugs with various clinical and pharmacological properties, thereby demonstrating enhanced characteristic anticancer activity, antiapoptotic activity, antioxidant activity, wound healing activity, antimicrobial activity, and in tissue engineering. Hence, it was demonstrated that as a novel therapeutic agent green synthesized silver nanoparticle will be useful in many biomedical applications.

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