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Review Article

A Review on Green Synthesis of Nanoparticles: Synthesis, Characterization, Applications and Antimicrobial Activities

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ABSTRACT

Nanoparticles have attracted considerable attention in recent years. As per ASTM and ISO standards, nanoparticles are particles of sizes ranging from 1 to 100nm with one or more dimensions. The nanoparticles show enhanced properties such as strength, high reactivity, sensitivity, surface area, stability, etc because of their small size. The nanoparticles are synthesized by various methods for commercial and research uses that are classified into three main types namely physical, chemical and mechanical processes that has seen a vast improvement over time. Green synthesis of nanoparticle is a novel way to synthesis nanoparticles by using biological sources. It is gaining attention due to its cost effective, ecofriendly and large scale production possibilities. Among all organisms plants seem to be the best candidates for biosynthesis of silver nanoparticles and they are suitable for large-scale biosynthesis. More than 100 different biological sources for synthesizing AgNPs are reported in the past decade by various authors. Metallic nanoparticles that have immense applications in industries are of different types, namely, gold, silver, alloy, magnetic, titanium, etc. Among all organisms plants seem to be the best candidates for biosynthesis of silver nanoparticles and they are suitable for large scale biosynthesis. Nanoparticles produced by plants are more stable and the rate of synthesis is faster than in the case of microorganisms. This review focuses on the green synthesis of silver nanoparticles using various plant sources. This review paper based on the study of synthesis of silver nanoparticles using the plants extracts (*Cissusquadrangularis*, *Adhatodavastica*, *Gymnemasyvestre* and *Tinosporacordifolia*) and characterized by UV- Vis spectroscopy, XRD, FTIR. Study of antimicrobial activity has been done in this paper.

Introduction

Nanotechnology has gained huge attention over time. The fundamental component of nanotechnology is the nanoparticles. Nanoparticles are particles between 1 and 100 nanometers in size and are made up of carbon, metal, metal oxides or organic matter (Hasan, 2015). Nanoparticles can be easily synthesized using various methods by various approaches available for the synthesis of silver nanoparticles include electrochemical (Yin *et al.*, 2003) chemical (Sun *et al.*, 2002), radiation (Dimitrijevic *et al.*, 2001), photochemical methods (Callegari *et al.*, 2003) and biological techniques (Naiket *et al.*, 2002). Most of the chemical methods used for the synthesis of nanoparticles involve the use of hazardous and toxic chemicals that create biological risks and not ecofriendly.

But the plant mediated synthesis of metal nanoparticles is receiving lots of attention due to its speedy synthesis, simplicity and eco- friendliness than the chemical methods (Sastri *et al.*, 2003). For biosynthesis method, plants are selected because they contain ascorbic acids, citric acid, reductases, flavonoids, etc (Pandey *et al.*, 2012).

A lot of literature has been reported till to date on biological synthesis of silver nanoparticles using microorganisms including bacteria, fungi and plants; because of their antioxidant or reducing properties typically responsible for the reduction of metal compounds in their respective nanoparticles. Silver is one of the basic elements, has preservative properties and medicinal value so it is well known from ancient time. Silver has better contact with microorganisms because of their extremely large surface area. In the field of nanotechnology, silver nanoparticles have gained most interests because of their various applications such as good conductivity, electronic components, food industries as food additives (Gupta, 1998) and preservatives, food packaging systems (Kumar *et al.*, 2005), Biomedical applications like wound healing (Tian *et al.*, 2007), antiseptic sprays and topical creams or ointments, textile fabrics (David *et al.*, 2010), plastic industry, environmental-friendly antimicrobial nanopaint (Kumar *et al.*, 2008), chemical stability, catalytic properties, biolabelling, cosmetic products and most important antibacterial (Loket *et al.*, 2006), antiviral,

antifungal (Ahmad *et al.*, 2003) in addition to anti-inflammatory activities (Klaus *et al.*, 2001).

Several plants have been used for the synthesis of silver nanoparticles. Silver nanoparticles have important applications in many fields. Silver nanoparticles are being used in numerous technologies and incorporated into a wide array of consumer products that take advantage of their desirable optical, conductive, and antibacterial properties. In this work, I will use four plants like *Cissusquadrangularis*, *Adhatodavasica*, *Gymnemasylvestre* and *Tinosporacordifolia* for biosynthesis of silver nanoparticles and then antimicrobial study of silver nanoparticles will be done. These nanoparticles will be used for the formation or production of drugs by using several microorganisms which would collect from hospital.

The objective set for this study will be used to prepare silver nanoparticles in a simple, cost effective and eco-friendly way unlike chemical procedures. We will use the extract of *Cissusquadrangularis*, *Adhatodavasica*, *Gymnemasylvestre* and *Tinosporacordifolia* as a reducing and capping agent. We will successfully characterize the biologically synthesized Ag-nanoparticles.

Synthesis of Nanoparticles

Bottom-up method: In bottom-up or constructive method, nanoparticles can be synthesized using chemical and biological methods by build-up of material from atoms to new nuclei or clusters which forms nanoparticles.

Sol-gel method: The sol-gel technique for synthesis of nanomaterials is a wet-chemical technique. The sol means in a liquid phase colloidal solution of solids are suspended and the gel means in a solvent solid macromolecules are submerged. Most of the nanoparticles are synthesized by this method because of its simplicity. In this technique, Chlorides and metal oxides are typically used precursors in this process (Ramesh, 2013). By shaking, sonication or stirring the precursor is then dispersed in a host liquid. After that nanoparticles are recovered by various methods like filtration, sedimentation and centrifugation and moisture is further removed by drying (Mann *et al.*, 1997).

Chemical Vapor Deposition (CVD): In chemical vapor deposition (CVD) method a thin film of gaseous reactants deposited on a substrate. In this process a chemical reaction occurs when a heated substrate comes in contact with combined gas (Bhaviripudi *et al.*, 2007), a thin film of product on substrate surface is recovered. High purity, hardness, uniformity, strong nanoparticles are some advantages of CVD. Disadvantage of this method are requirement of special equipment and gaseous by-products are highly toxic.

Spinning: In this method spinning disc reactor is used for the synthesis of nanoparticles. It contains a rotating disc inside a chamber by this temperature can be controlled. Nitrogen or other inert gases are filled in the reactor or chamber to remove oxygen from inside and avoid chemical reactions (Tai *et al.*, 2007). The disc is rotated at different speed where the liquid i.e. precursor and water is pumped in. by spinning the atoms or molecules fused together so they precipitated, collected and dried (Mohammadi *et al.*, 2014).

Pyrolysis: For large scale production of nanoparticles in industries, pyrolysis is most commonly used process. In this technique, flame is used for burning a precursor. As a

precursor, either vapor or liquid is used. At the high pressure, vapor or liquid is fed into furnace through a small hole where it burn (Kammler *et al.*, 2001). To recover nanoparticles, by product gases are classified. This technique is simple, cost effective, efficient and high yielding process.

Biosynthesis: This technique for the synthesis of nanoparticles, is green and eco-friendly technology (Hulakoti and Taranath, 2014). For the synthesis of metallic nanoparticles like silver, gold, iron, cadmium, titanium, zirconium and palladium both prokaryotes and eukaryotes are used like bacteria, fungi, actinomycetes and algae (Mann, 2001). Plant extracts also used. The biosynthesized nanoparticles have unique and enhanced properties have many applications.

Top-down method: In top-down or destructive method, bulk materials are reduced or break down into fine particles or nanoparticles.

Mechanical milling: This technique is most extensively used for the production of nanoparticles. For milling and post annealing method, this method is used during synthesis of nanoparticles where in an inert atmosphere, different elements are milled (Yadav *et al.*, 2012).

Laser ablation or LASiS: LASiS means laser ablation synthesis in solution, in this method, submerged metals irradiated by a laser beam which condenses a plasma plume. This method is very reliable for synthesis of metal based nanoparticles (Amendola, and Meneghetti, 2009).

Sputtering: In this method a thin layer of nanoparticles are deposited on a surface by ejecting particles from it by colliding with ions (Shah and Gavrin, 2006). The shape and size determined by Temperature, thickness of layer, substrate type etc. (Lugscheider *et al.*, 1998).

Nanolithography: In this method, nanometric scale structures which are one dimension in size of 1-100 nm are fabricated (Pimpin and Srituravanich, 2012). Electron beam, optical, multiphoton, nanoimprint etc. are various lithographic techniques (Hulteen *et al.*, 1999).

Green synthesis: this is a biological method, a new branch of nanotechnology and named as nanobiotechnology (Zakiret *al.*, 2014). Green synthesis of nanoparticles is a bottom-up approach (Mohanpuria *et al.*, 2008). Biological organisms such as microorganism (bacteria, fungi, algae, yeasts), plant extracts and biomass are used in this method, is a best alternative method than physical and chemical method for synthesis of nanoparticles. Several biological materials are also reported for the synthesis of silver nanoparticles such as egg white (Lu *et al.*, 2012), milk (Lee *et al.*, 2013), coconut water (Elumalai *et al.*, 2014), honey (Philip, 2010), lichen (Din *et al.*, 2015) etc. This method is simple, spontaneous, ecofriendly, non-toxic and inexpensive. For bioremediation methods, bio-inspired nanoparticles can be used because these nanoparticles clean the surface area by redox reactions by penetrating the contaminants.

Biosynthesis of nanoparticles using bacteria: Metal nanoparticles are synthesized by microbial cells has emerged as a novel approach (Gericke and Pinches, 2006). Microorganisms have the ability to synthesize inorganic nanoparticles like gold, silver, cadmium sulphide, magnesium etc. *Pseudomonas stutzeri* AG259 is the first bacteria which is

used to synthesizing silver nanoparticles, that was isolated from silver mine (Haefeliet al., 1984). Some microorganisms can grow easily in metal ion concentrations because of their resistance to that metal. These mechanisms involves efflux system, biosorption, precipitation alteration of solubility and toxicity via reduction or oxidation of metals, lack of specific metal transport systems (Husseinyet al., 2006). The main mechanism behind silver biosynthesis is the presence of nitrate reductase enzyme which convert nitrate to nitrite (Vaidyanathanet al., 2010). A common bacterial strain, *Lactobacillus* present in curd, synthesizes both gold and silver nanoparticles of well- defined morphology under standard conditions. *Klebsiella pneumonia*, *Escherichia coli* and *Enterobacter cloacae* strains (Nair and Pradeep, 2002) are reported to synthesize the metallic silver nanoparticles (Shahverdi et al., 2007). *Lactobacillus* sp. A09 could reduce silver ions in the dried cells of *Bacillus megaterium* D01, where bioreduction process is non-enzymatic (Fu et al., 2000).

Biosynthesis of nanoparticles using fungi: Fungi can secrete larger amount of proteins which directly translate to higher amount of nanoparticles and they have high binding capacity with metal ions so fungi can synthesize more nanoparticles than the bacteria ((Mohanpuria et al., 2008). On solid substrate fermentation, they are easy to grow and cultivate. By using *Fusarium oxysporum*, extremely stable silver hydrosols are synthesized where the particles were stabilized by the proteins excreted through the fungus (Ahmed et al., 2003). Silver particles of 5-25 nm range are extracellularly biosynthesized by using *Aspergillus fumigates* (Bhainsa, KC and D'Souza, 2006). *Phaenerochaete chrysosporium* commonly known as White rot fungus is used for the synthesis of silver nanoparticles (Vigneshwaran et al., 2006). By using a novel fungi *Hormoconis resinae*, 20-80 nm of silver nanoparticles are synthesized (Varshney et al., 2009).

Biosynthesis of nanoparticles using algae: In the plant kingdom, algae is a diverse group being explored for their

application in nanotechnology. *Chlorella vulgaris* used to synthesize nanoparticles at room temperature. *Caulerpa racemosa* (Kathiraven, 2014), *Sargassum polycystum* (Kanimozhi et al., 2015) and *Chaetomorpha* (Kannan, 2013) like marine alga were also used to synthesize silver nanoparticles.

Biosynthesis of nanoparticles using plants : Silver nanoparticles are synthesized by plant extracts are mainly used because they are easily available, ecofriendly protocol, safe, nontoxic, spontaneous, economic, large scale production, single step or quicker than microbes in biosynthesis technique ((Huang et al., 2007). Plants are used for the biosynthesis of nanoparticles due to phytochemicals which involved flavonoids, quinones, terpenoids, ketones, carboxylic acids, aldehydes and amides these are responsible for immediate reduction of ions (Prabhu et al., 2012). Various plants have been currently investigated for the synthesis of nanoparticles such as *Azadirachta indica* leaf (Shankar et al., 2004), *Aloe vera* leaf (Chandran et al., 2006), *Pelargonium graueolens* leaf (Shankar et al., 2003), *Alfalfa* sprouts (Gardea-Torresdey et al., 2003), *Saccharum officinarum* (Leela and vivekanandan, 2008), *Carica papaya* callus (Mude et al., 2009), *Eclipta* leaf (Jha et al., 2009), *Carica papaya* callus (Mude et al., 2009), *Syzygium cumini* leaf (Kumar et al., 2010), *Glycine max* (soybean) leaf (Vivekanandan et al., 2009), *Allium cepa* (Saxena et al., 2010), *Alternanthera sessilis* (Niraimathiet al., 2013), *Dillenia indica* fruit (Singh et al., 2013), *Morinda citrifolia* (Suman et al., 2013) etc.

For green synthesis of different nanoparticles, several plants and their parts are already reported such as root, stem, leaf, fruit, bark, pulp, a secretory substance like latex and in vitro raised calli. Green synthesis is advanced technique than the physical and chemical techniques of biosynthesis because there is no need to use temperature, high pressure, toxic chemicals, and time limit.

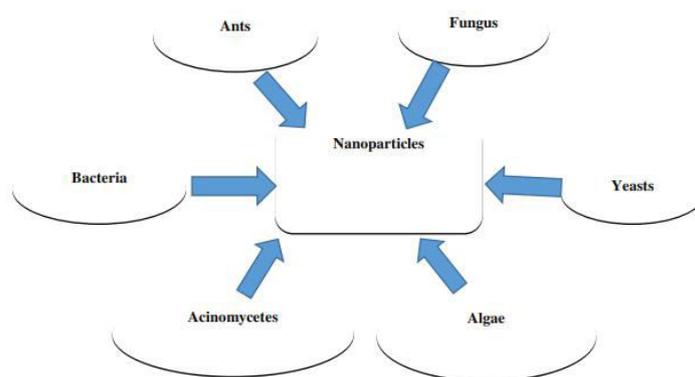


Fig 1: Different routes for green synthesis of nanoparticles

Biological Samples

Cissus quadrangularis

Classification:

Kingdom: Plantae

Subkingdom: Tracheobionta

Division: Magnoliophyta

Class: Magnoliopsida

Order: Vitales

Family: Vitaceae

Genus: *Cissus*

Species: *Cissus quadrangularis* L.

Characteristics: *Cissus quadrangularis* is a perennial plant of the grape family. It is also known as veld grape, asthisamharaka, hadjod, devil's backbone, pirandai etc. It is found to be native to Bangladesh, India, and Sri Lanka but it is also found in Africa, Arabia and Southeast Asia. It has been used as a medicinal plant since the ancient past. *Cissus* has been used in various Ayurvedic classical medicines to heal broken bones, injured ligaments and tendons. This plant is used in several medicines for the treatment of uterus disorder, gastric ulcer and rheumatic pain (Anjaria et al., 1997).

Adhatodavastica**Classification**

Kingdom: Plantae
 Subkingdom: Tracheobionta
 Division: Magnoliophyta
 Class: Magnoliopsida
 Order: Lamiales
 Family: *Acanthaceae*
 Genus: *Adhatoda*
 Species: *Vasica*

Characteristics: *Adhatodavastica*, commonly known in English as Malabar nut, adulsa, adhatoda, vasa or vasaka (Aslamet *al.*, 2013) is an important medicinal plant belongs to the family *Acanthaceae*. It is a large shrub grows crowded along waste land, roadsides etc. Leaves are simple, acute, opposite, ovate-lanceolate and shiney. Flowers are white in capsule shape. The plants parts have the properties like abortifacient (Wakhloo *et al.*, 1979), anticancer (Kulakarni, 1998), antitussive (Dhuley, 1996), antimicrobials (Doshiet *al.*, 1983). Various compounds of this plant had been used to treat cancer.

Gymnemasylvestre**Classification**

Kingdom: Plantae
 Subkingdom: Tracheobionta
 Division: Magnoliophyta
 Class: Magnoliopsida
 Order: Gentianales
 Family: *Asclepiadaceae*
 Genus: *Gymnema*
 Species: *Sylvestre*

Characteristics: *Gymnemasylvestre* are cultivated as medicinal plants, popularly known as gurmar and Madhunashini. It is perennial, woody climber plant found in tropical and subtropical region (Keshavamurthy *et al.*, 1990). *Gymnemasylvestre* can also found in Banda, Western Ghats, western and northern India (Kritikaret *al.*, 1998). The plant description is that its flowers are small, yellow in color, located in axillary and lateral umbel in cymes.

Tinosporacordifolia**Classification:**

Kingdom: Plantae
 Subkingdom: Tracheobionta
 Division: Magnoliophyta
 Class: Magnoliopsida
 Order: Ranunculales
 Family: *Menispermaceae*
 Genus: *Tinospora*
 Species: *cordifolia*

Characteristics: *Tinosporacordifolia*, commonly known as heart leaved moonseed, guduchi and giloy, is an herbaceous vine which is found in India, Myanmar and Shri Lanka. Guduchi is considered to be one of the most divine herbs. The plant extract have antilipoxygenase activity (Mohan *et al.*, 2017).

Characterization of Silver Nanoparticles

Size, shape and quantity of particles of silver nanoparticles are examined by characterization techniques. Various techniques are used for this purpose including UV-visible spectroscopy, Scanning Electron Microscopy (SEM), Fourier Transmission

Infrared Spectroscopy (FTIR), X-Ray Diffraction (XRD), and Dynamic Light Scattering (DLS).

UV-Vis Spectroscopy: The optical properties of a solution are determined by Absorbance spectroscopy. A light beam is passed through the sample solution and the amount of absorbed light is measured. At each different wavelength, the absorbance is measured. By using Beer-Lamberts Law, the concentration of the solution is measured by absorbance. This technique is limited to sample identification but is very useful for quantitative measurements.

Fourier Transmission infrared spectroscopy: This technique is chemical analytical technique, which measures wavelength (wave number of light) v/s infrared intensity. By this technique, possible bio molecules and also bonding interaction between themselves is analyzed. When an infrared light interacts with matter, chemical bonds will shows stretch, contract and bend form. In nanoparticle synthesis, FTIR data measures interaction between Ag salts and protein molecules, which accurate for the reduction of silver ions to silver nanoparticles.

X-Ray Diffraction: X-ray diffraction is now a common technique for studying crystal structures and atomic spacing. This technique is used to study the phase composition of a sample, structure of crystals, orientation and texture. The principle of XRD is that the X-rays are passed through a material and the pattern produced give information of size and shape of the unit cell. The atoms are crystal in structure arranged in a periodic array and thus can diffracted light at different angle. When X-ray passing through a crystal it produces a diffraction pattern, that diffraction gives the information about the atomic arrangement within the crystals.

Antimicrobial activity of silver nanoparticles

Silver and its salts exhibit strong antibacterial activity, this property was evaluated for the Ag-nanoparticles prepared. Several groups had already studied the antimicrobial activity of chemically synthesized silver nanoparticles. The conventional cup-plate method was used to determine the antibacterial activity of biologically prepared Ag-nanoparticles is. It's a screening test generally done for verifying antimicrobial activity of samples.

Significance of this review: Future investigations should be preparation of silver nanoparticles (AgNps) which aim at overcoming these kinds of challenges and would be useful in designing effective drug delivery agent, treating and diagnosing fatal diseases besides ensuring higher efficacy and safety. We will prove the effective antibacterial property of these nanoparticles; hence we can think of its medicinal usage. Due to the highest conductive properties, we can implement these Silver nanoparticles in advanced portable gadgets. We can specifically use these nanoparticles in the production of clothing, leather items and coatings because it can protect these items from the attack of harmful microbes. Nanoparticles may be used in agriculture and food production in the form of Nano sensors for monitoring crop growth and pest control by early identification of plant diseases.

Applications

For biomedical applications such as gene and drug delivery, cancer treatment, biosensors and diagnostic tools, nanoparticles are used, has been extensively studied throughout the past decade. Very recently, nanoparticles have gained significance

in the field of biomedicine. Plants and plant extracts can be effectively used in the synthesis of metals. Size and shape of nanoparticles is easily understood with the use of plant extracts. The nanoparticles extracted from plants are used in many applications for human benefits.

Conclusion

The biosynthesis of nanoparticles is of great interest such as economic prospects, biocompatibility, cost effectiveness and wide range of applications such as nanomedicine, catalysis medicine, pharmaceutical application, food industries, agriculture and biomedical industries. In scientific world, it is a new and emerging area of research, where day by day developments are noted for giving bright future for this field. Due to these unique properties, silver nanoparticles will have the role in many of the nanotechnology based processes.

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