A REVIEW ON GREEN SYNTHESIS AND CHARACTERIZATION OF SILVER NANOPARTICLES AND THEIR APPLICATIONS: A GREEN NANOWORLD.

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ABSTRACT

Nanoparticles are the spearheads of the rapidly expanding field of nanotechnology. An array of physical and chemical methods is used for the synthesis of nanoparticles. The development of immaculate protocols for the synthesis of highly monodisperse nanoparticles of various sizes, geometries and chemical composition is one of the most challenging obstructions in the field of nanotechnology. The use of toxic chemicals and non-polar solvents in synthesis leads to the inability to use nanoparticles in clinical fields. Therefore, development of clean, non-toxic, biocompatible and eco-friendly method for synthesis of nanoparticles deserves recognition. Silver nanoparticles are of interest because of the unique properties (e.g., size and shape depending optical, electrical and magnetic properties) which can be incorporated into antimicrobial applications, biosensor materials, composite fibers, cryogenic superconducting materials, cosmetic products, and electronic components. Several physical and chemical methods have been used for synthesizing and stabilizing silver nanoparticles. The present review explores the immense plant diversity to be employed towards rapid and single step protocol preparatory method with green principles over the conventional ones and describes the antimicrobial activities of silver nanoparticles.

KEYWORDS: size and shape depending optical, electrical and magnetic properties, silver nanoparticles, antimicrobial etc.
INTRODUCTION
Nanotechnology can be defined as the understanding of phenomena and manipulation of matter of matter at the atomic, molecular or macromolecular levels at dimensions between approximately 1–100 nanometer. Due to its small size, it produces structures, devices, and systems with at least one novel/superior characteristic property. These properties may differ in important ways from the properties of bulk materials and single atoms and molecules. Currently, there are two main approaches for the synthesis of nanomaterials and fabrication of nanostructure: “top down” and “bottom up” approaches.

Top-down approach is a physical method and also refers to microfabrication method where tools are used to cut, mill and shape materials into the desired shape and order. Integrated circuits is an example. It include preparation by lithographic techniques, etching, grinding in a ball mill, sputtering, etc. However, the most acceptable and effective approach for nanoparticle preparation is the “bottom up” approach, where devices are “grown” from smaller building blocks such as atoms or molecules by self assembly. In this way, it is possible to control the size and shape of the nanoparticle depending on the subsequent application through variation in precursor concentrations, reaction conditions (temperature, pH, etc.), functionalizing the nanoparticle surface, using templates, etc. Thus, it produces products with high precision accuracy. Colloidal dispersion is an example.
Figure 1: Top-down and Bottom-up synthesis approaches. Reprinted from Sci. Tot. Environ., Vol. 400 (1-3), Ju-Nam, Y. and Lead, J.R., Manufactured nanoparticles: An overview of their chemistry, interactions and potential environmental implications, pp396-414, Copyright 2008 with permission from Elsevier.

Figure 2: Different approaches of synthesis of silver nanoparticles.

Green Chemistry of the Synthesis of Nanomaterials
The growing demand in the chemical industries is due to the ample benefits of nanomaterials and preparation of nanomaterials include chemicals such as solvents, raw materials, reagents, and template materials. Such chemicals have engendered the noxious intermediates and products. To diminish the undesirable products, the concept of green chemistry was brought in the chemical industries\textsuperscript{[1]} The advantages of green synthesis of nanoparticles over the physical and chemical methods are:

a) Clean and eco-friendly approach, as toxic chemicals are not used;

b) the active biological component itself act as reducing and capping agent, therefore reduction the overall cost of synthesis process;
c) can be used at large scale production of nanoparticles;
d) external experimental conditions like high energy and high pressure are not required, leads to energy saving process.

**Elemental Silver and Silver Nanoaprticles**

Elemental or metallic silver (Ag) is a chemical element which is malleable and ductile transition metal with a white metallic luster appearance. Of all metals, silver has $^{[2,3]}$

- highest electrical conductivity (higher than copper that is currently used in many electrical applications)
- thermal conductivity
- lowest contact resistance
- high optical reflectivity $^{[4]}$

Silver is stable in pure air and water. The presence of ozone or hydrogen sulfide or sulfur in the air or water may result in silver tarnishing $^{[5]}$ due to the formation of silver sulfide. Silver can be present in four different oxidation states: Ag$^0$, Ag$^{2+}$, Ag$^{3+}$. The former two are the most abundant ones, the latter are unstable in the aquatic environment. $^{[6]}$ Silver has many isotopes with $^{107}$Ag being the most common. Although acute toxicity of silver in the environment is dependent on the availability of free silver ions, investigations have shown that these concentrations of Ag$^+$ ions are too low to lead toxicity. $^{[7]}$ Metallic silver appears to pose minimal risk to health, whereas soluble silver compounds are more readily absorbed and have the potential to produce adverse effects. $^{[8]}$ The reduction in the size of silver to nanosized silver increases its ability to control bacteria and fungi. Due to the large surface area of nanomaterials leads to increased contact with bacteria and fungi which increases its effectivity. Nanosilver, when in contact with bacteria and fungus, adversely affects the cellular metabolism of the electron transfer systems and the transport of substrate in the microbial cell membrane. Bacteria and fungi causes itchiness, infection, odor, sores, the use of nanosilver repress the proliferation of bacteria and fungi. Nanosilver have been widely used due to its antibacterial microbial activity for the development of products containing silver include food contact materials (such as cups, bowls and cutting boards), odor-resistant textiles, electronics and household appliances, cosmetics and personal care products, medical devices, water disinfectants, room sprays, children’s toys, infant products and ‘health’ supplements.
Properties of Silver Nanoparticles

Surface effects and quantum effects are two factors which differentiate nanomaterials from bulk.\[^{9}\]\ Due to these effects there significant change in chemical, mechanical, optical, electrical and magnetic properties of materials. Therefore nanosilver has unique optical and physical properties that are not present in bulk silver, and which are very useful in medical applications. Mainly the properties of silver nanoparticles are antibacterial, antifungal\[^{10}\]\ Antiviral\[^{11}\]\, anti-inflammatory\[^{12}\]\ etc.

Antibacterial properties

Nanosilver kill the gram- positive and gram- negative bacteria very effectively, so it can called as killing agent\[^{13,14,15}\]\, including antibiotic-resistant strains.\[^{16}\]\ Gram-negative bacteria are the bacteria which retain the colour of the stain even after washing with alcohol or acetone and include genera such as \textit{Acinetobacter}, \textit{Escherichia}, \textit{Pseudomonas}, \textit{Salmonella}, and \textit{Vibrio}. \textit{Acinetobacter} species are associated with nosocomial infections, i.e., infections that are the result of treatment in a hospital or a healthcare service unit, but secondary to the patient’s original condition. Gram-positive bacteria are those which lose the colour of the stain after wash with alcohol or acetone and include many well-known genera such as \textit{Bacillus}, \textit{Clostridium}, \textit{Enterococcus}, \textit{Listeria}, \textit{Staphylococcus} and \textit{Streptococcus}. Antibiotic-resistant bacteria are the bacteria that are not controlled or killed by antibiotics which include strains such as methicillin-resistant and vancomycin-resistant \textit{Staphylococcus aureus} and \textit{Enterococcus faecium}. To enhance the antibacterial activity of various antibiotics\[^{17}\]\, penicillin G, amoxicillin, erythromycin, clindamycin and vancomycin against \textit{Staphylococcus aureus} and \textit{Escherichia coli}. silver nanoparticles (diameter 5-32 nm, average diameter 22.5 nm) play very prominent role. Size-dependent (diameter 1-450 nm) The antimicrobial activity of silver nanoparticles depends on their size\[^{18}\]\ and Gram-positive bacteria. Small nanoparticles with a large surface area to volume ratio provide a more efficient means for antibacterial activity even at very low concentration. Also the antimicrobial activity of silver nanoparticles depend upon the concentration and shape.\[^{19}\]\ The different shapes silver nanoparticles of (spherical, rod-shaped, truncated triangular nanoplates) have been developed by synthetic routes. Due to their large surface area to volume ratios, truncated triangular silver nanoplates display the strongest antibacterial activity.
ACTION OF SILVER NANOPARTICLES ON MICROBES
Silver nanoparticles are able to physically interact with the cell surface of various bacteria. This is particularly in the case of Gram- negative bacteria where numerous studies have been observed the adhesion and accumulation of AgNPs to the bacteria surface. Silver nanoparticles have the ability to anchor to the bacterial cell wall and subsequently penetrate it, thereby causing structural changes in the cell membrane which renders bacteria more permeable. AgNPs accumulate on the membrane cell creates gaps in the integrity of the bilayer which predisposes it to a permeability increase and finally bacterial cell death. There have been electron spin resonance spectroscopy studies that suggested that there is formation of free radicals by the silver nanoparticles when in contact with the bacteria and these free radicals have the ability to damage the cell membrane and make it porous which can ultimately lead to cell death.\(^{[20]}\) It is likely that a combined effect between the activity of the nanoparticles and the free ions contributes in different ways to produce a strong antibacterial activity of broad spectrum. It has also been proposed that there can be release of silver ions by the nanoparticles\(^{[21]}\) and these silver ions can interact with the thiol groups of many vital enzymes and inactivate them.\(^{[22]}\) The bacterial cells in contact with silver take in silver ions, which inhibit several functions in the cell and damage the cells. Then, there is the generation of reactive oxygen species (ROS), forming free radicals with a powerful bactericidal action, which are produced possibly through the inhibition of a respiratory enzyme by silver ions and attack the cell itself. Silver ions have the tendency to enter the microbial body causing damage to its structure. As a consequence, ribosomes may be denatured with inhibition of protein synthesis, as well as translation and transcription can be blocked by the binding with the genetic material of the bacterial cell. Protein synthesis has been shown to be altered by treatment with AgNPs and proteomic data have shown an accumulation of immature precursors of membrane proteins resulting in destabilization of the composition of the outer membrane. Silver is a soft acid, and there is a natural tendency of an acid to react with a base, in this case, a soft acid to react with a soft base. The cells are majorly made up of sulfur and phosphorus which are soft bases. The action of these nanoparticles on the cell can cause the reaction to take place and subsequently lead to cell death. Another fact is that the DNA has sulfur and phosphorus as its major components; the nanoparticles can act on these soft bases and destroy the DNA which would definitely lead to cell death.\(^{[23]}\)
Figure 3: Various modes of action of silver nanoparticles on bacteria

Applications of Silver Nanoparticles

Scientific Applications
Due to the surface Plasmon resonance (SPR)\textsuperscript{[24,25]} and surface enhanced raman scattering (SERS) properties of silver nanoparticles, they have many applications such as sensing applications including detection of DNA sequences\textsuperscript{[26]}, laser desorption/ionization mass spectrometry of peptides\textsuperscript{[27]}, colorimetric sensors for Histidine\textsuperscript{[28]}, enhanced IR absorption spectroscopy, biolabeling and optical imaging of cancer\textsuperscript{[29]}, biosensors for detection of herbicides\textsuperscript{[30]} and glucose sensors for medical diagnostics\textsuperscript{[31]}

Medical Applications
Nanosilver is used for coating such as incorporated in wound dressings, diabetic socks, scaffolds, sterilization materials in hospitals, medical textiles etc. One website claims that “the number of people using colloidal silver as a dietary supplement on a daily basis is measured in the millions”.


<table>
<thead>
<tr>
<th>Medical domains</th>
<th>Examples</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Anesthesiology</strong></td>
<td>Coating of breathing mask and endotracheal tube for mechanical ventilatory support</td>
<td>Patent -</td>
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<tr>
<td><strong>Dentistry</strong></td>
<td>Silver-loaded SiO₂ nanocomposite resin filler as additive in polymerizable dental materials</td>
<td>[32]</td>
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<tr>
<td><strong>Diagnostics</strong></td>
<td>Nanosilver pyramids for enhanced biodetection</td>
<td>[33]</td>
</tr>
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<td></td>
<td>Ultrasensitive and ultrafast platform for clinical assays for diagnosis of myocardial infarction</td>
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<tr>
<td></td>
<td>Fluorescence-based RNA sensing</td>
<td></td>
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<tr>
<td></td>
<td>Magnetic core/shell Fe₃O₄/Au/Ag nanoparticles with tunable plasmonic properties</td>
<td></td>
</tr>
<tr>
<td><strong>Drug delivery</strong></td>
<td>Remote laser light-induced opening of microcapsules</td>
<td>[34]</td>
</tr>
<tr>
<td><strong>Eye care</strong></td>
<td>Coating of contact lens</td>
<td>[35]</td>
</tr>
<tr>
<td><strong>Imaging</strong></td>
<td>Silver dendrimer nanocomposite for cell labeling</td>
<td>[36]</td>
</tr>
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<td></td>
<td>Fluorescent core-shell Ag@SiO₂ nanoballs for cellular imaging Molecular imaging of cancer cells</td>
<td></td>
</tr>
<tr>
<td><strong>Neurosurgery</strong></td>
<td>Coating of catheter for cerebrospinal fluid drainage</td>
<td>[37]</td>
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<td><strong>Orthopedics</strong></td>
<td>Additive in bone cement</td>
<td>[38]</td>
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<td></td>
<td>Implantable material using clay-layers with starch-stabilized silver nanoparticles Coating of intramedullary nail for long bone fractures Coating of implant for joint replacement Orthopedic stockings</td>
<td></td>
</tr>
<tr>
<td><strong>Patient care</strong></td>
<td>Superabsorbent hydrogel for incontinence material</td>
<td>[39]</td>
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<tr>
<td><strong>Pharmaceutics</strong></td>
<td>Treatment of dermatitis</td>
<td>[40]</td>
</tr>
<tr>
<td></td>
<td>Inhibition of HIV-1 replication Treatment of ulcerative colitis Treatment of acne</td>
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</table>

**Industrial Applications**

**Catalysis**

The silver nanomaterials and silver nanocomposites are used as catalyst in many such as CO oxidation, benzene oxidation to phenol, photodegradation of gaseous acetaldehyde and the reduction of the p-nitrophenol to p-aminophenol. To catalyze the reduction of dyes by sodium borohydride (NaBH₄), silver nanoparticles immobilized on silica spheres etc.
Electronics
Nanosilver has high electrical and thermal conductivity along with the enhanced optical properties leads to various applications in electronics. In nanoelectronics, silver nanowires are used as nanoconnectors and nanoelectrodes. Other applications include), optoelectronics, nanoelectronics (such as single-electron transistors and electrical connectors), data storage devices, the preparation of active waveguides in optical devices high density recording devices, intercalation materials for batteries, making micro-interconnects in integrated circuits (IC) and integral capacitors etc.

Plant Extract Mediated Green Synthesis of Silver Nanoparticles
There is growing imposition of silver nanoparticles in the field of medicine, optics, biotechnology, microbiology, environmental remediation and material science has lead to increasing demand in chemical industry. For the production of silver nanoparticles, various reducing agents are reported such as H₂ gas, sodium borohydride, hydrazine, ethanol, ethylene glycols, Tollen’s reagent, ascorbic acid and aliphatic amines. Depending on the strength of the reducing agents the particle size can be controlled. Hence umpteen number of toxic chemicals have been utilized to blend the silver nanoparticles as a reducing and stabilizing agent and poly (ethylene glycol) block copolymers are used to reduce the Ag⁺ ions into Ag nanoparticles in aqueous or non-aqueous solution. Although these methods may successfully manufacture the well defined nanoparticles but they are quiet expensive and dangerous to environment. Thus, the concept of green chemistry was introduced to nanoparticles synthesis with the elimination or decline tradition of toxic chemicals. In previous study, plants and herbs antioxidant are present as a phytochemical constituents in seeds, stems, fruits and in leaves. The plant-based phytochemicals in the synthesis of nanoparticles creates a meaningful symbiosis between natural/plant science and nanotechnology. This connection provides a green approach to nanotechnology, referred to as “green nanotechnology” or Green Synthesis. The major foredeal of using plant extracts for silver nanoparticle preparation is that they are easily available, safe and nontoxic in most cases, have a broad variety of metabolites that can aid in the reduction of silver ions and quicker than microbes in the synthesis. The main mechanism considered for the route is plant-assisted reduction due to phytochemicals. The main phytochemicals involved are terpenoids, flavones, ketones, aldehydes, amides and carboxylic acids. Flavones, organic acids and quinones are water-soluble phytochemicals that are responsible for the instant reduction of the ions and formation of nanoparticles. Studies have revealed that xerophytes
contain emodin, an anthraquinone that undergoes tautomerization, leading to the formation of the silver nanoparticles.

Mechanism of Plant Mediated Synthesis of Silver nanoparticles
In plants the abundant compounds are Ascorbic acid (C6H8O6) and polyphenols. The acid has functions in photosynthesis as an enzyme cofactor (including synthesis of ethylene, gibberellins and anthocyanins) and in the control of cell growth. Also in plants the widely chemicals are polyphenol or hydroxyphenol are found which are used as a reducing agents for one pot synthesis of silver nanoparticles nature, polyphenol is one of the most important chemicals in many reductive biological reactions widely found in plants and animals.

Figure 4: Ascorbic acid reduction mechanism of gold and silver ions to obtain Ag° and Au° nanoparticles.

Figure 5: Mechanism of Plant mediated synthesis of Silver nanoparticles
### Table 2: Plants extract mediated synthesis of silver nanoparticles

<table>
<thead>
<tr>
<th>Plants</th>
<th>Size (nm)</th>
<th>Plants’s part</th>
<th>Shape</th>
<th>References</th>
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<td>Rhizome</td>
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<td>25</td>
<td>Whole plant</td>
<td>Spherical</td>
<td>[54]</td>
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<td>Tea extract</td>
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<td>Leaves</td>
<td>Spherical</td>
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<td>Spherical</td>
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<td>22</td>
<td>Inflorescence</td>
<td>Spherical</td>
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<td>Abutilon indicum</td>
<td>7–17</td>
<td>Leaves</td>
<td>Spherical</td>
<td>[58]</td>
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<td>Seeds</td>
<td>Spherical</td>
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<td>8–40</td>
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<td>Spherical &amp; fcc</td>
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<td>Portulaca oleracea</td>
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<td>Leaves</td>
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<td>Musa paradisiacal</td>
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<td>Leaves</td>
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<td>Eclipta prostrate</td>
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<td>Triangles, pentagons, hexagons</td>
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<td>Spherical, triangular</td>
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<td>Citrus sinensis</td>
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<td>Eucalyptus hybrid</td>
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<td>Vitis vinifera</td>
<td>30–40</td>
<td>Fruit</td>
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<td>[92]</td>
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Table 3: Antimicrobial activities of silver nanoparticles synthesized using plant extracts.

<table>
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<tr>
<th>Biological entity</th>
<th>Test microorganisms</th>
<th>Method</th>
<th>References</th>
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<tr>
<td>Alternanthera dentate</td>
<td>Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia and Enterococcus faecalis</td>
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<tr>
<td>Boerhaavia diffusa</td>
<td>Aeromonas hydrophila, Pseudomonas fluorescens and Flavobacterium branchiophilum</td>
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<td>Tea</td>
<td>E. coli</td>
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<tr>
<td>Tribulus terrestris</td>
<td>Streptococcus pyogens, Pseudomonas aeruginosa, Escherichia coli, Bacillus subtilis and Staphylococcus aureus</td>
<td>Kirby-Bauer</td>
<td>[96]</td>
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<tr>
<td>Coccos nucifera</td>
<td>Klebsiella pneumoniae, Bacillus subtilis, Pseudomonas aeruginosa and Salmonella paratyphi</td>
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</tr>
<tr>
<td>Aloe vera</td>
<td>E. coli</td>
<td>Standard plate count</td>
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<tr>
<td>Solanus torvum</td>
<td>P. aeruginosa, S. aureus, A. flavus and Aspergillus niger</td>
<td>Disc diffusion</td>
<td>[99]</td>
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<td>Trianthema decandra</td>
<td>E. coli and P. aeruginosa</td>
<td>Disc diffusion</td>
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<td>Argimone mexicana</td>
<td>Escherichia coli; Pseudomonas aeruginosa; Aspergillus flavus</td>
<td>Disc diffusion for bacteria and food poisoning for fungi</td>
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<td>S. typhi, E. coli, S. aureus and B. subtillus</td>
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<td>[102]</td>
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<td>Cymbopogan citratus</td>
<td>P. aeruginosa, P. mirabilis, E. coli, Shigella flexaneri, S. somenei and Klebsiella pneumonia</td>
<td>Disc diffusion</td>
<td>[103]</td>
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</table>

**Literature Review**

Green Synthesis of Silver nanoparticles using plant extract is a very simple and cost-effective way that satisfies the demand of research community and eliminates the possibility of environment hazards simultaneously. Till date several groups had reported the synthesis of silver nanoparticles using plant extract which are listed in Table 2. such as bioreduction of silver ions to yield metal nanoparticles with the help of living plants, geranium, Neem leaf, Aloevera plant extracts, Emblicaofficinalis (amla, Indian Gooseberry. The green synthesis of silver nps using Capsicum annuum leaf extract has been reported. These biogenic silver nanoparticles show high antimicrobial activity against gram-positive and gram-negative bacteria. Table 3.

In a recent report, these nanoparticles have been synthesized on irradiation using an aqueous mixture of Ficuscarica leaf extract. The silver nanoparticles were formed after three hour of incubation at 37°C using aqueous solution of 5mM silver nitrate, Cymbopogan citratus (DC) stafp (commonly known as lemon grass) a native aromatic herb from India and also
cultivated in other tropical and subtropical countries showed strong antibacterial effect against P. aeruginosa, P. mirabilis, E. coli, Shigella flexneri, S. Somenei and Klebsiella pneumonia.\textsuperscript{[105]} Many plants such as Pelargonium graveolens\textsuperscript{[106]}, Medicagosativa\textsuperscript{[107]}, Azadirachta indica\textsuperscript{[108]}, Lemongrass\textsuperscript{[109]}, Aloevera\textsuperscript{[110]}, Cinnamomum Camphora\textsuperscript{[111]}, Emblica officinalis\textsuperscript{[112]}, Capsicum annuum\textsuperscript{[113]}, Diospyros kaki\textsuperscript{[114]}, Caricapapaya\textsuperscript{[115]}, Coriandrum sp.\textsuperscript{[116]}, Boswellia ovalfoliolata\textsuperscript{[117]}, Tridax procumbens, Jatropha curcas, Solanum melongena, Datura metel, Citrus aurantium\textsuperscript{[118]} and many weeds\textsuperscript{[129,120]} have shown the potential of reducing silver nitrate to give formation of AgNPs.

The bio-synthesis of silver nanoparticles from a silver nitrate solution using two medicinal plant extract namely \textbf{Ocimum tenuiflorum}(Ag NP1) and \textbf{Catharanthus roseus}(Ag NP2) has been reported by \textbf{Dulen Saikia}.\textsuperscript{[121]} The plant leaf extracts were prepared by mixing 5 g of dried leaves in 100 ml of deionized water in an Erlenmeyer flask, boiled for 30 minutes at desired temperature and then filtered through a Whatman 42 no. filter paper. In a typical reaction, 9 mL of the leaf extracts was added to the desired amount of aqueous AgNO\textsubscript{3} solution (1×10\textsuperscript{-3} mol dm\textsuperscript{-3}, 2×10\textsuperscript{-3} mol dm\textsuperscript{-3}, 3×10\textsuperscript{-3} mol dm\textsuperscript{-3}, 4×10\textsuperscript{-3} mol dm\textsuperscript{-3} and 5×10\textsuperscript{-3} mol dm\textsuperscript{-3}AgNO\textsubscript{3}) and the reaction mixture was left at room temperature for the reduction process to take place. When the leaf extract was mixed in the aqueous solution of the silver ion complex, it started to change colour (within 30 minutes) from brown to reddish brown.
Figure 6: Colour change of AgNO$_3$ solution with the change in concentration of AgNO$_3$ solution (1mM to 5mM) containing Ocimum tenuiflorum leaf extract; (b) Colour change of AgNO$_3$ solution with the change of concentration of AgNO$_3$ solution (1mM to 5mM) containing Catharanthus roseus leaf extracts; (c) UV-Vis absorption spectra of AgNP1 using different concentrations of aqueous AgNO$_3$ solution; (1) 1×10$^{-3}$ mol dm$^{-3}$, (2) 2×10$^{-3}$ mol dm$^{-3}$, (3) 3×10$^{-3}$ mol dm$^{-3}$, (4) 4×10$^{-3}$ mol dm$^{-3}$, (5) 5×10$^{-3}$ mol dm$^{-3}$; (d) UV-Vis absorption spectra of AgNP2 using different concentrations of aqueous AgNO$_3$ solution; (1) 1×10$^{-3}$ mol dm$^{-3}$, (2) 2×10$^{-3}$ mol dm$^{-3}$, (3) 3×10$^{-3}$ mol dm$^{-3}$, (4) 4×10$^{-3}$ mol dm$^{-3}$, (5) 5×10$^{-3}$ mol dm$^{-3}$.

Figure 7: (a) XRD pattern of AgNP1 (b) XRD pattern of AgNP2
RESULTS

The reduction of the silver ions through leaf extracts leading to the formation of AgNPs of fairly well-defined dimensions is being demonstrated in UV-Vis, XRD analysis. The XRD pattern reveals the face-centred crystal structure of AgNPs. The average grain size obtained for AgNP1 is 29 nm and for AgNP2 is 19 nm.

In another study, Sunlight-induced rapid and efficient biogenic synthesis of silver nanoparticles using aqueous leaf extract of Ocimum sanctum Linn. With enhanced antibacterial activity has been reported by Goutam Brahmachari et.al.[122] In this study the O.sanctum leaf extracts of varying concentrations (10%, 7%, 5% and 3%) transferred (5mL each) into four different 100-mL conical flasks containing 45mL of 10–30M silver nitrate solution leads to final volumes of 50mL each. The resulting solutions were kept under direct sunlight; gradual colour change was then noticed as an indication of silver nanoparticle formation (Figure 9) and confirmed by UV-Vis spectrophotometer studies at a regular interval of time. The nanoparticles were characterized with the help of UV-visible spectrophotometer and transmission electron microscopy (TEM). The prepared silver nanoparticles exhibited considerable antibacterial activity.
Figure 9: Optical image. (A) Gradual colour change for the formation of AgNPs by 7% of O. sanctum leaf extract at different time intervals. (B) AgNPs formation with O. sanctum leaf extract at different concentrations (10%, 7%, 5% and 3%) measured at 60 min.

Figure 10: (a) UV-visible spectra for different concentrations of O. sanctum Linn. leaf extract (PLE) with 10–3M AgNO3 measured at 60 min. (b) UV-visible spectra of 7% aqueous leaf extract (PLE) of three different plants (Curve A) Ocimum sanctum Linn. (Curve B) Citrus limon L (Curve C) Justicia adhatoda L with 10–3 M AgNO3 measured at 60 min.
Figure 11: (a) TEM image of biosynthesized silver nanoparticles using O. sanctum leaf extract at 100 nm scale (b) Effect of the treatment of actively growing cells. With AgNPs (dispersed in the aqueous leaf extract) formed by 7% leaf extracts of O. Sanctum on growth pattern of Staphylococcus aureus [(●—●) for untreated and (○—○) for treated] and Pseudomonas aeruginosa [(▲—▲) for untreated and (Δ—Δ) for treated]. All values are means of three sets of experimental data.

In one more study, the Green Synthesis of Silver nanoparticles by Mulberry Leaves Extract has been reported by Akl M. Awwad, Nidà M. Salem\textsuperscript{[123]} by utilizing the reduced property of mulberry leaves extract and silver nitrate to synthesize a silver nanoparticles (Ag NPs) at room temperature and characterized by using UV-visible absorption spectroscopy, scanning electron microscopy (SEM) and X-ray diffraction (XRD). Further, silver nanoparticles showed effective antibacterial activity toward Staphylococcus aureus and Shigella sp.
Figure 12: (a) Effect of contact time on AgNPs synthesised by mulberry leaves extract (b) FT-IR spectra of Mulberry leaves powder.

Figure 13: (a) XRD pattern of AgNPS synthesized by mulberry leaves extract (b) SEM image of silver nanoparticles.
DISCUSSION

It was found that the average size of silver nanoparticles was 20 ± 2.8 nm that was calculated by using Debye-Scherer equation. The presence of structural peaks in XRD patterns and average crystalline size around 20 nm clearly illustrates the crystalline nature of AgNPs.

The SEM image of silver nanoparticles, showed cubical and relatively uniform shape of nanoparticle formation with diameter range 20-40 nm. The larger silver particles may be due to the aggregation of the smaller ones.

In this study, Banerjee et al.\textsuperscript{124}, proposed a Leaf extract mediated green synthesis of silver nanoparticles from widely available Indian plants: Musa balbisiana (banana), Azadirachta indica (neem) and Ocimum tenuiflorum (black tulsi) and their synthesis, characterization, antimicrobial property and toxicity analysis. This study investigates an efficient and sustainable route of AgNP preparation from 1 mM aqueous AgNO3 using leaf extracts of three Indian plants. The Ag NPs were characterized by UV-visible spectrophotometer, particle size analyzer (DLS), scanning electron microscopy (SEM), transmission electron microscopy (TEM) and energy-dispersive spectroscopy (EDS), Fourier transform infrared spectrometer (FTIR) to determine the nature of the capping agents in each of these leaf extracts. Ag NPs showed significantly higher antimicrobial activities against Escherichia coli (E. coli) and Bacillus sp. in comparison to both AgNO3 and raw plant extracts. Additionally,
a toxicity evaluation of these Ag NPs containing solutions was carried out on seeds of Moong Bean (Vigna radiata) and Chickpea (Cicer arietinum). Results depicts the treatment of seeds with Ag NPs solutions exhibited better rates of germination and oxidative stress enzyme activity (nearing control levels), though detailed mechanism of uptake and translocation are yet to be analyzed.

Figure 15: UV–Vis absorption spectrum of silver nanoparticles. From (A) banana, (B) neem and (C) Tulsi leaf extracts.

Figure 16: Graphs obtained from FTIR analysis of AgNPs. Curve: (A)- Banana, (B)-Neem and (C)- Tulsi leaf extracts respectively.
Figure 17: SEM images of silver nanoparticles formed by the reaction of 1 mM silver nitrate and 5% leaf extract of (A) banana. (B) neem and (C) tulsi leaves respectively.

Figure 18: TEM images of silver nanoparticles formed by the reaction of 1 mM silver nitrate and 5% leaf extract of (A) banana. (B) neem and (C) tulsi leaves respectively.
Table 4: Showing zone of inhibitions found in Bacillus and E. coli cultures

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Bacteria</th>
<th>Zone of inhibition (in mm)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>By leaf extract</td>
</tr>
<tr>
<td>Banana (leaf)</td>
<td>+ (Bacillus)</td>
<td>6 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>− (E. coli)</td>
<td>6 ± 0.005</td>
</tr>
<tr>
<td>Neem (leaf)</td>
<td>+</td>
<td>8 ± 0.013</td>
</tr>
<tr>
<td></td>
<td>−</td>
<td>8 ± 0.015</td>
</tr>
<tr>
<td>Tulsi (leaf)</td>
<td>+</td>
<td>8 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>−</td>
<td>8 ± 0.02</td>
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</table>

Cultures were treated with crude leaf extracts, AgNO₃ and AgNPs. Results were expressed as Mean ± SD.

CONCLUSION
As metal nanoparticles seems to fascinate for the future diverse industry due to their enrich chemical, electrical and physical properties. Metal nanoparticles are synthesized predominantly by wet chemical methods, where the chemical used are toxic and flammable. So there is need of eco friendly nanoparticles synthesis approach. Green nanotechnology accomplish this need by synthesize the metal nanoparticles without using any toxic chemical as a reducing agent. Plant extract mediated biological synthesis of nanoparticles is known as Green Synthesis or Green Nanotechnology and these nanoparticles are known as biogenic nanoparticles. Green synthesis provides benefits over chemical and physical method as it is cost effective, eco friendly, more stable and there is no need any high energy, pressure, temperature and toxic chemicals.

REFERENCES


34. Weisbarth, R.E., Gabriel, M.M., George, M., Rappon, J., Miller, M., Chalmers. R., Winterton, L. Creating antimicrobial surfaces and materials for contact lenses and lens cases, Eye and Contact Lens, 2007; 33: 426-429.
37. Alt et al., 2004 Podsiadlo et al., 2005 Alt et al., 2006 Chen et al., 2006 Pohle et al., 2007.
38. Lee et al., 2007.
39. Bhol et al., 2004; Bhol & Scheckter, 2005 Elechiguerra et al., 2005; Sun et al., 2005 Bhol & Scheckter.


