

## **A REVIEW ON PLANT MEDIATED SYNTHESIS OF SILVER NANOPARTICLES AS A GREENER APPROACH**

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**P.A.S.R. Wickramarachchi <sup>1</sup>**

**Y.L. Paragodaarachchi <sup>2</sup>**

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### **Abstract**

Metal nanoparticles are used in all phases of science, including medical fields. They still seduce scientists to explore new dimensions for their respective value, which is usually attributed to their corresponding small sizes. Among the other metallic nanoparticles, silver nanoparticles (AgNPs) aroused a keen interest. The chemical methods used to synthesize AgNPs involve reducing and stabilizing agents, which subsequently become risks to the environment. For these reasons, the green synthesis of AgNPs is a significant area of interest. Among the variety of biological molecules used to synthesize AgNPs, the biological molecules obtained in the form of plant extracts are superior to others because of the ease of handling and the reduction of costs. Many plant parts have been used to synthesize AgNPs such as whole plants, leaves, seeds, bark etc. The synthesis of AgNPs by plant extracts is due to the presence of a large amount of organic chemicals such as carbohydrates, fats, proteins, enzymes and coenzymes, phenolic flavonoids, terpenoids, alkaloids, gum, etc., capable of donating an electron to reduce  $\text{Ag}^+$  ions to  $\text{Ag}^0$ . The active ingredient responsible for the reduction of  $\text{Ag}^+$  ions varies depending on the

extract used. The size and size distribution of AgNPs synthesized with plant extracts depend on plant extract concentration, silver nitrate concentration, pH of the medium, incubation time and temperature. These factors can be modified to refine the properties of AgNPs. Almost all of these conditions have an optimal value to obtain smaller size AgNPs with a narrow size distribution.

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**1. Department of Chemistry , University of Kelaniya.  
2. Postgraduate Institute of Science, University of Peradeniya.**

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## INTRODUCTION

Nanoscience is an interdisciplinary science that deals with knowledge of fundamental properties of nano-sized objects. For a particular object to be nano-sized, at least one dimension of that object should be in the nanoscale which is 1-100 nm. In this size range materials often develop chemical, biological and physical properties that differ from their bulk counterparts. This is mainly due to the increased surface area to volume ratio of these nanoparticles (El-nour, Al-warthan and Ammar, 2010)(Zhang *et al.*, 2016). Metal and semiconductor nanoparticles are of higher interest due to their applications in many fields such as electronics, catalysis, optics, environmental and biotechnology (Raveendran *et al.*, 2003)(Sharma, Yngard and Lin, 2009). Metallic nanoparticles show size and shape-dependent properties. For an example, the antibacterial properties of silver colloids depend on the size of the silver nanoparticles, smaller the particles higher the antibacterial activity whereas the catalytic activity depends on the size and shape among many other variables such as chemical-physical environment and size distribution (El-nour, Al-warthan and Ammar, 2010). The use of size-dependent properties dates back to 300 AD, according to records. There are a number of famous examples of ancient artefacts which were created using nanocomposites. Roman's [Lycurgus cup](#) (400 AD), which was made of glass containing gold-silver alloyed nanoparticles, changed color between green and red when light was shone through it. [Damascus steel swords](#) (between 300 AD and 1700 AD), which are known for their exceptionally sharp cutting edge, contain oriented nanoscale wire-and-tube-like structures. These ancient artefacts are still a challenge to modern day nanotechnologists (Nunes *et al.*, 2019).

During the last decade, the application of nano materials has extensively increased, so nanoparticle (NP) synthesis has become inevitable today.

An important aspect of NP synthesis deals with the development of experimental processes for the synthesis of nanoparticles (NPs) of different sizes, shapes and controlled dispersity. Synthesis of NPs has been extensively studied employing chemical and physical methods. The synthesis process is normally divided into two broad groups: the ‘top-down’ and the ‘bottom-up’ approaches. In the top-down approach, the size of bulk materials is reduced to nanoscale by employing mechanical, chemical or other forms of energy. The breakdown of large structures can be achieved by the use of mechanical grinding/ ball milling, thermal/ laser ablation and vaporization, followed by cooling (Ahmed *et al.*, 2016). The bottom-up approach involves synthesizing nanomaterials from atomic or molecular species via self-assembly or chemical reactions allowing the precursor particles to increase in size. Classical chemical synthetic methods use well-known reducing agents to reduce the metal ion in solution. Radiation chemical synthesis methods use ionizing radiation to generate solvated electron gas which initiate the reduction process (El-nour, Al-warthan and Ammar, 2010). However, physical methods need more energy and chemical methods are toxic. The biological methods of NP synthesis are an alternative method to overcome the drawbacks of conventional chemical and physical synthetic methods. Three main steps must be evaluated for green chemistry in chemical methods of synthesis of metallic nanoparticles. They are choice of solvent, choice of reducing agent and choice of a stabilizing agent, all of which should be environmentally friendly (El-nour, Al-warthan and Ammar, 2010)-(Sharma, Yngard and Lin, 2009). The pressure, temperature and pH of the medium should also be ambient. Most of the time, organic solvents are used to synthesise metallic nanoparticles due to the hydrophobicity of capping reagents. Capping reagents are used to passivate the nanoparticles. Many of the commonly

used capping reagents are polymers. Most of the chemicals used during the synthesis of metallic nanoparticles are not environmentally friendly. This is one of the reasons, like high costs and higher energy demands, for the growing interest in using biological synthetic methods (Ahmed *et al.*, 2016). NP synthesis using microorganism, enzyme and plant or plant extract has been suggested as a possible ecofriendly alternative to chemical and physical methods.

Due to the higher growth rates, cheap costs of culturing and effortless control and manipulation of growth conditions, bacteria are commonly used to synthesise nanoparticles. Some species of bacteria are known to have special procedures to circumvent the toxic effects of metals and heavy metals. Due to these properties, bacteria synthesise NPs using in-situ and ex-situ conditions. They make use of biochemical pathways and reducing agents present in bacteria such as proteins, enzymes etc. for the reduction and stabilization of NPs (Ren *et al.*, 2017).

Actinobacteria which are immobile, aerobic and mostly filamentous gram-positive bacteria can produce antibiotics which are secondary metabolites. They have the ability to detoxify heavy metals and therefore, are resistant to heavy metals. Heavy metal detoxification is conducted by the reduction of toxic metal ions or by precipitation of these ions. Therefore, NPs with different medicinal properties like antimicrobial, antioxidant etc. can be synthesized using these bacteria (Manivasagan *et al.*, 2016).

Fungi could digest food extracellularly into simple compounds that are easy to take up by the fungi. Therefore, they have a wide range of enzymes that are released to the external environment. This ability of fungi can be used to synthesize NPs. Fungi that are fast breeding and be easily cultured can be used to synthesize nanoparticles extracellularly or intracellularly. The incubation conditions and nature of the metallic ion

solution affect the nanoparticle size produced. Some molds cannot be used to synthesize nanoparticles due to their pathogenicity towards humans (Moghaddam *et al.*, 2015).

Algae are used for synthesis of NPs for their easy access and efficacy. Biomolecules like sugars, ketones, aldehydes, proteins, amines, amino acids, and phenols involve in the reduction and stabilization of NPs. Properties of the NPs depend on the concentration of the algal extract, metal salt, pH of the medium, temperature and incubation time. NP synthesis can be carried out using intracellular or extracellular pathways (Siddiqi and Husen, 2016).

Interest for using plants in synthesis of NPs grew due to the ability of plants to absorb heavy metals from the surrounding environment followed with bioaccumulation and detoxification. The processes involved in bioaccumulation and detoxification were aimed to be exploited for the synthesis of NPs. Extracts made with plant parts are used to reduce the metal ions in solution. Gold and silver nanoparticles are commonly synthesized using plant extracts (Iravani, 2011).

This review focuses on the use of plant extract in the synthesis of silver nanoparticles (AgNPs) as a green approach, their properties and applications.

### **SILVER NANOPARTICLES (AgNPs)**

AgNPs have become a product with significant interest due to numerous interesting properties like antibacterial, electrochemical, catalytic and optical properties (Zhang *et al.*, 2016)(Okafor *et al.*, 2016). Due to the presence of these properties AgNPs are incorporated into products to obtain enhanced activities. For example, AgNPs are incorporated into wound dressings to obtain antimicrobial properties (Ahmed *et al.*, 2016).

Currently, AgNPs are used to treat open wounds and chronic ulcers as an antimicrobial agent (He *et al.*, 2017). AgNPs are successfully used in cancer research as well (Ahmed *et al.*, 2016).

### ***Properties of AgNPs***

#### **Optical properties**

A proper knowledge of the properties of AgNPs is crucial to maximize the potential applications of AgNPs. AgNPs show surface plasmon resonance (SPR) in the UV-Visible region. Surface plasmon resonance arises due to the small size of the nanoparticles. Due to the SPR effect AgNPs absorb light in the range 400 – 530 nm depending on the size, shape and the local refractive index of the particles (*Silver Nanoparticles: Optical Properties – nanoComposix*, 2020). The changes in absorption or wavelength gives a measure of size, shape and interparticle properties (Sharma, Yngard and Lin, 2009). When the nanoparticles are exposed to sunlight, free electrons of the nanoparticles interact with photon energy, producing sub waves and conducting electrons in oscillating mode. These collective oscillations impart the surface plasmon resonance. Due to SPR effect, enhanced local heating effect, SPR-powered electron/hole generation, enhanced UV-Vis absorption, reduced electron/hole diffusion length, enhanced local electric effect and molecular polarization effect, quantum tunneling effect and high catalytic effect like properties are gained by the AgNPs (Chouhan 2019).

#### **Thermal properties**

Due to the thermodynamic size effect, AgNPs have low melting temperatures. Melting point is also size dependent for the nanoparticles.

The melting point of AgNPs reduces when the size of AgNPs decrease and thermal conductivity of AgNPs is also lower due to the presence of organic stabilizers (Syafiuddin *et al.*, 2017).

### **Catalytic properties**

Dyes such as methylene blue, yellow-12, 4-nitrophenol, Rose Bengal, eosin, and methyl orange have been effectively reduced using AgNPs (Syafiuddin *et al.*, 2017). When electrons get excited due to SPR, they relax back to the ground state, releasing energy. This released energy bring about a heating effect. This heating effect induces reaction of molecules that are absorbed on to the nanoparticles. AgNPs could also absorb UV light, and UV light bring about inter-band transitions between 4d and 5sp bands. Therefore, AgNPs can be considered as photocatalysts that utilize the full solar spectrum (Chen *et al.*, 2010).

### **Magnetic properties**

Magnetization data of AgNPs differ significantly from their bulk counterparts. Bulk silver is known to be paramagnetic while the nanoparticles contain ferromagnetic and paramagnetic phases. The core of the nanoparticles contains the paramagnetic components and the surface of the nanoparticles contains the ferromagnetic components (Cochrane *et al.*, 2017).

## **SYNTHESIS OF SILVER NANOPARTICLES**

### **Chemical approaches**

In chemical synthesis, silver salts in aqueous or organic solvent is reduced to gain a colloidal dispersion of AgNPs (Lee and Jun 2019). Silver nitrate is commonly used as the silver salt for chemical synthesis. Commonly used reducing agents are sodium borohydride, citrate, ascorbate and glucose (Syafiuddin *et al.*, 2017). When synthesizing AgNPs of specific shape and size, strength, and type of reducing agent



and stabilizing agent must be taken into consideration. Use of stabilizing agents is to prevent the aggregation of the nanoparticles (Lee and Jun 2019). Commonly used stabilization agents are poly(vinyl alcohol), poly(acrylamide), poly(ethylene glycol), poly(vinyl pyrrolidone), 2-mercaptobenzimidazole, polyalkylamine hydrochloride, sodium dodecyl sulfate, aerosol form of sodium bis(2-ethyl-hexyl)- sulfosuccinate, and cetyltrimethylammonium bromide (Bae *et al.*, 2011).

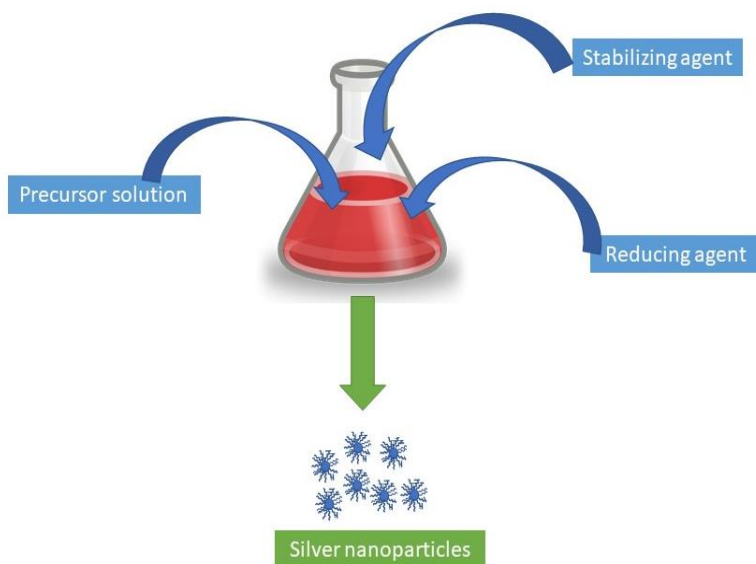


Fig: 01 Schematic illustration of chemical synthesis of AgNPs

Stabilization of nanoparticles is usually discussed in terms of electrostatic stabilization and steric stabilization. Coordination of anionic species to metal nanoparticles bring about the electrostatic stabilization. An electrical double layer is formed which result in coulombic repulsions between nanoparticles. Presence of bulky material (typically organic) bring about the steric stabilization of nanoparticles. Due to the bulkiness

of the material, diffusion of nanoparticles together is impeded. The choice of stabilizer affects the solubility of nanoparticles (El-nour, Al-warthan and Ammar, 2010)(Sharma, Yngard and Lin, 2009).

During the synthesis process, silver ion in solution is reduced by the reducing agent to silver atoms. These atoms then undergo the nucleation and growth phases. Colloidal AgNPs are then resulted after coarse agglomeration of oligomeric clusters. Control of shape and the size distribution of AgNPs is difficult when strong reductants are used (Syafiuddin *et al.*, 2017). To obtain AgNPs with uniform size, nuclei of AgNPs should form at the same time, so that the nuclei can undergo the following growth step at the same time (Lee and Jun 2019).

### **Physical synthesis of AgNPs**

Evaporation – condensation approach and laser ablation technique are used to synthesize AgNPs. Without the use of chemicals, large quantities of AgNPs can be produced using these methods (Okafor *et al*, 2013). One big challenge faced when using these methods is the agglomeration of AgNPs as stabilizing agents are not used (Lee and Jun 2019)..

A tube furnace is used in evaporation – condensation approach. A gas phase route at atmospheric pressure is utilized. the chamber in the tube furnace is filled with a carrier gas. The metal source is placed in the center, and which is used to produce the vapor of the metal allowing the final synthesis of AgNPs. By changing the reaction conditions, size, yield and shape of the AgNPs can be changed (Lee and Jun 2019).

Drawbacks of this method are, the apparatus occupies a large space, large energy consumption, needs a longer duration and increase nearby environmental temperature (Chouhan 2012).

In laser ablation process, metal source placed at the center is irradiated

with a pulsed laser. The liquid surrounding collects the formed AgNPs. As only surfactants are used in this method, AgNPs with high purity can be synthesized. Process conditions like type of metal source, duration of irradiation, property of liquid medium, and laser power influence the properties of AgNPs (Lee and Jun 2019).

## **Biological approaches as green synthetic methods;**

### **Need for green synthesis**

The biological methods of synthesis of AgNPs can be used as an alternative method to overcome the drawbacks faced during chemical and physical chemical synthetic methods. These biological methods come under the green chemical approach of synthesis of AgNPs (Sorescu *et al.*, 2016).

Principles of green chemistry require insignificant levels of waste, lower energy demands, use of renewable materials and minimal risk during the synthetic process. Use of biological methods meet above principles than the general chemical synthetic methods.

In green synthesis of AgNPs with algae, *Chlorella vulgaris* has been successfully used to synthesize single crystalline AgNPs at room temperature. The reduction of  $\text{Ag}^+$  was suggested to be conducted by carboxyl groups in aspartic and glutamine amino acids and hydroxyl groups of tyrosine amino acid in proteins of the fungus. The shape of AgNPs were also controlled by the proteins (Sharma, Yngard and Lin, 2009).

A study reports the use of *Verticillium* species to produce AgNPs and the plausible mechanism suggested mentions AgNPs are formed under the surface of the cell wall. The negatively charged carboxylate residues of the enzymes attract the  $\text{Ag}^+$  ions which are then trapped on the surface of the cells. The reduction of  $\text{Ag}^+$  ions occurs in the cell wall and the nuclei

are formed which subsequently grow due to further reduction of Ag<sup>+</sup> ions. The nanoparticles were non-toxic towards the fungal cells and the cells multiplied even in the presence of nanoparticles (Srikar *et al.*, 2016).

Both intracellular and extracellular pathways are used to synthesize AgNPs using bacteria. AgNPs of different shapes like spherical, hexagonal, triangular etc. have been synthesized using bacteria (Sharma, Yngard and Lin, 2009). *Escherichia coli* has been used in the production of AgNPs extracellularly. The nitrate reductase enzyme which is secreted by the bacteria aided in the reduction of Ag<sup>+</sup> ions and other organic compounds present in the medium involved in the stabilization of nanoparticles. The AgNPs were spherical in shape with an average size of 10 nm (Baltazar-Encarnación *et al.*, 2019).

Using microorganisms for synthesis of AgNPs is not economical as aseptic conditions are needed. It shows the importance of using plant extracts for the synthesis of AgNPs because of less cost due to the lack of need of aseptic conditions and ease of improvement. Plant extracts provide natural capping agents for stabilization of AgNPs and contain no toxic chemicals or biohazards (Ahmed *et al.*, 2016). Plant leaves are preferred over other plant parts due to their ability to conduct photosynthesis. (Mousavi *et al.*, 2018).

### **Synthesis of AgNP using plant extracts**

Synthesis of AgNPs using plant extracts provide a single step technique to produce AgNPs in an environmentally friendly manner.

Whole plants, bark, wood, stem, leaves, fruit, peel, seeds, and flowers have been successfully used in the synthesis of AgNPs. However, leaves are predominantly favored for this purpose.. Commonly exercised process of plant extract preparation comprises the following steps. Collection of the plant part of interest, washing and drying of the plant part with tap

water and distilled water and lastly boiling the chopped plant part in sterilized distilled water are the steps of plant extract preparation method (Yasir *et al.*, 2019). This extract is then added with silver nitrate solution and incubated for a certain period of time to generate the AgNPs. With the formation of AgNPs color of the solution changes from yellow to brown color. Formation and the progress of formation of AgNPs confirmed by obtaining UV-Visible spectra. Majority of synthesized AgNPs were spherical in shape. A wide variety of plants have been used to synthesize silver nanoparticles. Table given below summarizes the details of recent articles on synthesis of AgNPs using plant extracts.

Table 01: Preparation of AgNPs using plant extracts as the reducing and the stabilizing agent

Plant Name	Part of the Plant	Extract Preparation Method	Shape	Size	Reference
<i>Adiantum philippense</i>	Fron	Ground frond soaked in double distilled water.	Spherical shape	10 – 18 nm	Sant <i>et al.</i> , 2013
<i>Aloe vera</i>	Leaves	Dried leaves (heated at 80 °C) It was used for both aqueous and ethanolic extracts, using a ratio of 0.1:3, dry material to solvent.	Spherical shape	3-14 nm (aqueous) and 2-7 nm (ethanolic)	Campillo, Morales and Osorio, 2018
<i>Aloe vera</i>	Leaves	Finely cut leaves, boiled in deionized water.	Spherical shape	70-192 nm	Tippayawat <i>et al.</i> , 2016
<i>Alpinia katsumadai</i>	Seed	The dried and chopped seeds were sonicated with deionized water at 30°C.	Quasi-spherical shape	12.6 nm	He <i>et al.</i> , 2017
<i>Amphipterygium adstringens</i>	Bark	Macerated plant part soaked in methanol solution (50%) for 24 hours with stirring.	Spherical shape	25 – 130 nm	Rodríguez-luis <i>et al.</i> , 2016
<i>Annona glabra</i>	Leaves	Fresh leaves, crushed and heated in deionized water	spherical shape	10-100 nm	Amarasinghe <i>et al.</i> 2020

		at 70 °C for 1 hour.			
<i>Annona muricata</i>	Leaves	Powdered dried leaves, soaked in double distilled water.	Quasi-spherical shape	4 – 16 nm	Del Carmen Sánchez-Navarro <i>et al.</i> , 2018
Apple	Fruit	Finely cut fruits, heated at 80 °C in deionized water.	Spherical shape	30 nm	Ali <i>et al.</i> , 2016
<i>Arbutus unedo</i>	Leaves	Finely cut leaves, boiling in distilled water.	Spherical shape	40 – 60 nm	Skandalis <i>et al.</i> , 2017
<i>Avena sativa L.</i>	Whole plant	Aqueous extract - powdered dried plant at 80 °C . Ethanol extract - dissolving the powder in ethanol.	Spherical shape	60-100 nm	Amini, Amin and Azar, 2017
<i>Averrhoa bilimbi</i>	Fruit	Fruits were ground and centrifuged to obtain the extract.	Hexagonal or rhomboidal shape	50 – 175 nm	Isaac, Sakthivel and Murthy, 2013
<i>Azadirachta indica</i>	Leaves	Finely chopped leaves boiled in double-distilled water.	-	65 nm	Roy, 2017
<i>Azadirachta indica</i>	Leaves	Air dried finely cut leaves, boiled in double distilled water.	Spherical shape	34 nm	Ahmed, Ahmad and Swami, 2015
<i>Buddleja globosa</i>	Leaves	-	Spherical shape	16 nm	Carmona <i>et al.</i> , 2017
<i>Caesalpinia ferrea</i>	Seed	Macerating seeds in ethanol in the dark. Extract was lyophilized under 1.8 mBar pressure at -14 °C.	Spheroidal shape	30 - 50 nm	Soares <i>et al.</i> , 2018
<i>Citrullus lanatus</i>	Rind	Crushed rind heated in distilled water at 80 °C.	Spherical shape	17 nm	Ndikau <i>et al.</i> , 2017
<i>Clitoria ternatea</i>	Leaves	Chopped fresh leaves heated in distilled water at 60 °C.	Spherical shape	20 nm	Krithiga, Rajalakshmi and Jayachitra, 2015
<i>Coriandrum sativum</i>	Leaves	Air dried leaves, finely chopped,	Spherical shape	6.45 nm	Khan <i>et al.</i> , 2018

		heated at 60 °C in de-ionized water.			
Cottonseed	Seed oilcake	Sample was soaked in sterile ultrapure water with agitation for 6 hours.	Spherical shape	10 – 90 nm	Govarthanan <i>et al.</i> , 2016
<i>Crocus sativus L.</i>	Flower parts	Finely cut frozen sample, soaked in deionized water.	Spherical shape	15 nm	Bagherzade, Tavakoli and Namaei, 2017
<i>Eriobotrya japonica</i>	Leaves	Powdered dried leaves, heated at 80 °C in distilled water.	Spherical shape	20 nm	Rao and Tang, 2017
<i>Euphorbia Confinalis</i>	Stem	Powdered dried stems, heated for 1 hour at 50 °C in distilled water and shaken for about 3 hours using a shaker.	Spherical shape	12 – 18 nm	Muchanyereyi <i>et al.</i> , 2017
Garlic	Bulb	Chopped fresh garlic soaked in water for 24 hours.	Spherical shape	4 - 6 nm	Ii <i>et al.</i> , 2012
<i>Glycyrrhiza glabra</i>	Root	Macerated plant part soaked in ethanol solution (50%) for 24 hours with stirring.	Spherical shape	25 – 130 nm	Rodríguez-luis <i>et al.</i> , 2016
<i>Hydnocarpus pentandra</i>	Leaves	Finely cut leaves, boiled in sterilized distilled wate.	Spherical shape	141-202 nm	Kumar, Kumar and Punathil, 2018
<i>Ligustrum Ovalifolium</i>	Fruit	Milled fruits, soaked in distilled water	Spherical shape	6 – 13 nm	Moldovan <i>et al.</i> , 2018
<i>Mangifera indica</i>	Leaves	Homogenized leaves. The homogenate was filtered and transferred to a sterile container. The extract was evaporated to obtain dry powder using Soxhlet apparatus. Both the extract and powder were used for the study.	-	100 nm	Bharathi <i>et al.</i> , 2017

Oak	Hull	Air dried powdered hull was extracted by ultrasonic bath for 24 hours in distilled water.	Spherical shape	40 nm	Heydari and Rashidipour, 2015
<i>Ocimum sanctum</i>	Leaves	Finely cut leaves, boiled in distilled water	Spherical shape	3 – 20 nm	Raju, 2011
<i>Padina tetrastromatica</i>	Whole plant	Powdered dried seaweed. Extracted with distilled water and filtered.	Spherical shape	43 nm	Suganya <i>et al.</i> , 2019
<i>Phoenix dactylifera</i>	Seed	Milled seeds were heated at 80 °C in water for 20 mins.	Spherical shape	14 – 30 nm	Ansari, 2018
<i>Pistia stratiotes</i>	Leaves	Finely cut leaves, boiled in deionized water.	Spherical shape	17 nm at neutral pH	Traiwatcharanon, Timsorn and Wongchoosuk, 2015
<i>Polyalthia longifolia</i>	Leaves	Chopped leaves boiled in double distilled water.	Spherical shape	15 – 20 nm	Santhanalakshmi <i>et al.</i> , 2019
<i>Prunus persica</i>	Leaves	Air dried finely cut leaves, heated at 70 °C in distilled water.	Spherical shape	40-98 nm	Ghoshal, 2017
<i>Pterocarpus marsupium</i>	Bark and wood	Powdered dried plant, macerated with distilled water and then allowed to stand for 24 hours.	-	148 nm	Ali <i>et al.</i> , 2016
<i>Punica granatum</i>	Peel	Ground peel, soaked in double-distilled water.	Spherical shape	20 – 40 nm	Devanesan <i>et al.</i> , 2018
<i>Salvia spinosa</i>	Whole plant	Powdered dried plant, boiled in distilled water.	Spherical and Oval shape	19 – 125 nm	Pirtarighat, Ghannadnia and Baghshahi, 2018
<i>Solanum nigrum</i>	Leaves	Chopped fresh leaves heated in distilled water at 60 °C.	Spherical shape	28 nm	Krithiga, Rajalakshmi and Jayachitra, 2015
<i>Syngonium podophyllum</i>	Leaves	Crushed leaves, boiled in de-ionized water	Spherical shape	40 nm	Yasir <i>et al.</i> , 2019
<i>Volkameria inermis</i>	Leaves	Powdered air-dried leaves.	Spherical shape	50 nm	Krishnadhas, R. and S., 2017



		Powder in ethanol was shook using rotary shaker at 190-220 rpm and after 24 hours concentrated using flash evaporator.			
<i>Ziziphus nummularia</i>	Leaves	Crushed leaved soaked and boiled in distilled water.	Spherical shape	4 – 6 nm -	Khan <i>et al.</i> , 2016

Formation and stabilization of AgNPs is done by biomolecules present in the extract such as polysaccharides, proteins, alkaloids etc. Formation of AgNPs are mainly carried out by polyols and other water soluble heterocyclic compounds and stabilization of AgNPs are mainly carried out by flavanone and terpenoid components (Sorescu *et al.*, 2016). The active ingredient responsible for the reduction of Ag<sup>+</sup> ions varies depending on the extract used. Dehydrogenation of acids and alcohols from keto enol conversions give origin to the electrons in mesophytes or both mechanisms in xerophytes (Srikar *et al.*, 2016).

The reduction of Ag<sup>+</sup> is mainly brought about by the water-soluble antioxidant compounds in the plant extracts such as ascorbic acid. Ascorbic acid is a reducing agent therefore it has the capability to neutralize reactive oxygen species. This neutralization result in formation of ascorbate radical and an electron (Isaac, Sakthivel and Murthy, 2013). This free electron can engage in the reduction of Ag<sup>+</sup> ion. Carbohydrates and other compounds with reducing properties also involve in the formation of AgNPs by donating an electron to reduce Ag<sup>+</sup> to Ag.

## **Factors affecting the synthesis of silver nanoparticles**

### **Effect of concentration of precursor**

Silver nitrate is used as the precursor for the synthesis of AgNPs in many studies. The concentration of AgNO<sub>3</sub> affects the nucleation point, size of AgNPs and the quantity of AgNPs formed. AgNP formation starts when the precursor concentration reaches a suitable concentration range for nucleation. This range changes with the approach used to synthesize AgNPs. The increase in amount of AgNPs formed could be attributed to increasing rate of spontaneous nucleation significantly increasing the growth rate of AgNPs (He *et al.*, 2017).

The peak of the SPR band shifts when the silver nitrate concentration is varied. The peak intensity increases with increasing silver nitrate concentration indicating higher formation of AgNPs (He *et al.*, 2017)(Ghoshal, 2017)(Carmona *et al.*, 2017). It has also been reported a blue shift in the peak which is attributed to formation of large amount of small AgNPs (He *et al.*, 2017). One article reports red shift of the peaks from 432 to 467 nm with increasing silver nitrate concentration caused by quantum size effects, indicating a large size distribution given by agglomerated AgNPs (Carmona *et al.*, 2017).

Particle size increases with further increase of precursor concentration, possibly due to agglomeration of formed AgNPs (Ghoshal, 2017).

### **Effect of plant extract concentration**

By changing the concentration of the plant extract, the size and shape of the AgNPs can be changed. The absorbance values and the wavelength change with increasing concentration of the plant extract. This is due to the changes in the quantity of AgNPs formed and the size of AgNPs (Ahmed, Ahmad and Swami, 2015).

A higher and narrower band was observed with higher concentration of plant extract ascribed to greater mean size of AgNPs. Higher quantities of plant extract cause more abrupt nucleation and faster growth while producing small aggregates in some cases. With lower quantities of plant extract, the absorption band broadens and shifts to shorter wavelengths due to smaller size of AgNPs at a wide scatter (Skandalis *et al.*, 2017).

A higher ratio of reducing agent to precursor accelerates the reduction of  $\text{Ag}^+$  to  $\text{Ag}^0$ , followed by capping agents, which prevent aggregation of AgNPs. The size of AgNPs decrease till the optimum concentration of plant extract is reached and then the particle size increases (Ghoshal, 2017).

### **Effect of pH**

The pH of the medium can affect the particle size, shape, growth rate, yield and monodispersity of the AgNPs (Srikar *et al.*, 2016)(Ghoshal, 2017). At low pH, formation of AgNPs is slow and large particles are formed. When the pH of the medium was increased from acidic to basic, absorption peak intensity increases indicating higher concentration of AgNPs in solution due to increased reduction rate of  $\text{Ag}^+$  and highly uniform particles were obtained (Ghoshal, 2017).

One article reports synthesis of AgNPs using *Alpinia katsumadai* seed extract, a rich source of flavonoids and chalcones. The hydroxyl groups in these, as powerful reduction and stabilizing functional groups, could participate in the synthesis of AgNPs. In acidic pH values, the competing biosorption process and the hydroxyl protonation might slow down the formation of AgNPs. At higher pH values, a greater degree of ionization could generate strong complexing ligands for silver ions, so that more complexed silver ions are formed, which would have an effect on the capping ability of biomolecules in plant extract and promote the reduction of  $\text{Ag}^+$  (He *et al.*, 2017).

AgNPs synthesized using *Pistia stratiotes* leaf extract, at acidic pH values, a higher driving force for AgNPs dissolution exists, which balances the repulsive force to maintain the dispersion of nanoparticles which result in smaller sizes. In basic medium, hydroxyl ions enhance the complete reduction of  $\text{Ag}^+$  to  $\text{Ag}^0$ . At a higher ion density, Ag atoms tend to diffuse between adjacent adsorption sites on a surface and form bonds with nearest neighbor atoms via Brownian motion due to high surface energy and thermodynamic instability of the nanoparticle surfaces, leading to the increase of particle size (Traiwatcharanon, Timsorn and Wongchoosuk, 2015).

Very high pH ( $\text{pH} > 11$ ) was associated with the drawback of the formation of agglomerated and unstable AgNPs (Srikar *et al.*, 2016).

### **Effect of temperature**

The size and shape of nanoparticles are determined by the temperature of the reaction mixture which is a precarious factor. Temperatures up to  $100^\circ\text{C}$  were used by many researchers for AgNP synthesis using plant extracts. The temperature increase ( $30^\circ\text{C} - 90^\circ\text{C}$ ) resulted in an increased rate of AgNP synthesis and also promoted the synthesis of smaller AgNPs (Srikar *et al.*, 2016).

Kumar *et al* assessed the biosynthesis of AgNPs at different temperatures ( $20^\circ\text{C} - 100^\circ\text{C}$ ). When the temperature of the reaction mixture was increased, an increase in the AgNP concentration was observed due to increase in reduction rate of  $\text{Ag}^+$  ions and color of the solution turned light brown to dark brown within 5 minutes and also decreased the average particle size. The optimum temperature was obtained at  $40^\circ\text{C}$ , whereas with an increase in temperature above  $40^\circ\text{C}$  showed very broad absorbance peak which showed an increasing particle size (Ghoshal, 2017).

It has been reported that the gradual increase in temperature of the reaction mixture results in an increase in the rate of biosynthesis in addition to the transformation of silver ions to AgNPs. The final transformation of silver ions was reported to be 60% at 25°C, which increased to almost 100% at 55°C (Anjum, Abbasi and Shinwari, 2016).

### **Effect of reaction time**

When the time is increased, the intensity of the SPR peak increases, indicating an increasing number of AgNPs formed. After a certain time period, the intensity shows no obvious increase, indicating the AgNPs synthesis reaction tended towards equilibrium.

In some reports, the abrupt increase in intensity is considered to be the nucleation step of the AgNP formation and the gradual increase in intensity is considered to be the growth step of AgNP synthesis. Nucleation implies an increase in the number of scattering centers (number of particles) for a given system; therefore, it increases the scattered intensity. On the contrary, the growth of particles is associated with a decrease of the scattered intensity since the observation window corresponds to the diffraction of smaller particles that are disappearing during the growth process dissolution of the unstable nucleus. It is consistent with the mechanism of the reduction of Ag<sup>+</sup> ions and the association of Ag<sup>0</sup> atoms to produce metallic Ag particles (Mehta, Chaudhary and Gradzielski, 2010)(Ramirez and Jaramillo, 2016).

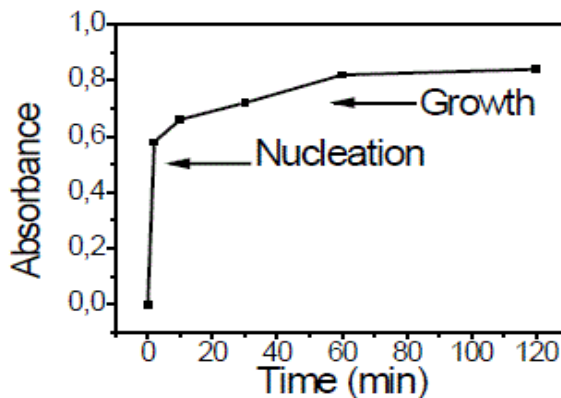


Fig. 02: Absorbance of the SPR peak with time  
(Ramirez and Jaramillo, 2016)

## APPLICATIONS OF SILVER NANOPARTICLES

Nanotechnology aides the growth and sustainable competitiveness of numerous fields of industrial applications. Useful functions are provided by the physical and chemical properties of AgNPs, which are used in fields like medicine, energy storage, agriculture and material science (Beyene *et al.* 2017). Due to its interesting antibacterial properties, AgNPs are used vastly in the medical field to produce products like antibacterial medicine, antibacterial water filters, antibacterial air filters, etc. (Sharma, Yngard and Lin, 2009). A few of the applications of AgNPs are given below.

Due to the antimicrobial effects of AgNPs, it can be used to treat infections. It has the ability to disinfect antibiotic-resistant bacteria and a broad range of bacterial and fungal species as a nonspecific antimicrobial agent. At a certain dosage, these AgNPs are nontoxic to mammalian cells. Due to these properties, the drive to use AgNPs in orthopedic implants

was raised into the field of orthopedics to reduce infection of implanted devices (Gosheger *et al.*, 2004).

A significant source of morbidity in orthopedic surgeries is implant based infections. AgNPs have been used to reduce the biofilm formation on these implants to reduce the risk of infection. AgNPs can be incorporated into tumor prostheses, external fixator pins, dressings, bone cement, hydroxyapatite coatings and coating of trauma implants (Gosheger *et al.*, 2004).

In orthopedic oncology, peri-prosthetic infection is a major issue. These infections can lead to amputations in these patients. With infection rates of 9% to 29%, this patient group is at elevated risk of infection compared with standard arthroplasty patients, due to chemotherapy and radiotherapy which cause immunosuppression (Gosheger *et al.*, 2004).

A study has been conducted to investigate the antimicrobial activity and side effects of silver coated mega prosthesis in a rabbit model. These silver coated endoprostheses were inoculated with *Staphylococcus aureus*. Rabbits showed significantly lower signs of inflammation and lower rates of infection compared to titanium endoprostheses (Suganya *et al.*, 2019).

Due to the tedious process of current cancer treatments and possible enormous side effects, interest towards the development of therapeutic agents that can target cancer cells has grown widely. Nanomaterials have gained fame for this application and many researchers are studying the viability of using nanomaterials and tuning its surface properties to synthesize cancer cell targeted therapeutic agents (Zhang *et al.*, 2016).

AgNPs biosynthesized using *Padina tetrastromatica* seaweed extract has been assessed for their cytotoxicity against breast cancer MCF-7 cells. The synthesized AgNPs were predominantly round shaped and the size range was between 40–50 nm. These AgNPs have shown dose dependent

cytotoxicity effects on breast cancer MCF-7 cells with 24h incubation period. The inhibitory concentration (IC<sub>50</sub>) was recorded as 86.7 µg mL<sup>-1</sup>. The cytotoxic effects of the AgNPs were brought about by DNA fragmentation and have finally induced apoptosis by activating Caspase 3 enzyme (Moldovan *et al.*, 2018).

AgNPs synthesized using aqueous extract of *Ligustrum ovalifolium* L. fruits as reducing and capping agents have shown dose dependent cytotoxic effects against two human ovarian cancer cell lines, A2780 and A2780Cis. The IC<sub>50</sub> values were recorded as by 50% (IC<sub>50</sub>) were: 7 µg/mL for A2780 and 14.04 µg/mL for A2780-Cis respectively (Lee *et al.*, 2016).

Humidity sensing is an important measurement to maintain optimum conditions for different processes. Ceramics, polymers, metal oxides and hybrid nanostructures have been used to produce humidity sensors (Chen and Lu 2005). Higher sensitivity, shorter response time, wider range of humidity detection, and better repeatability are the properties that have gained interest to increase the performance of humidity sensors (Traiwatcharanon, Timsorn, and Wongchoosuk 2015).

Humidity sensors detect the presence of water molecules in the air by changing properties like resistance, capacitance, impedance, piezoelectric voltage and surface acoustic wave signal. Resistive humidity sensors are the widely used type of humidity sensors due to their many advantages over the other types like ease of measurement, cost effectiveness and simple processing (Chen and Lu 2005).

AgNPs synthesized using *Pistia stratiotes* aqueous leaf extract at pH 10 with blue light irradiation has been used as the sensing material for a humidity sensor. Adsorption and desorption of water molecules on to AgNPs were analyzed real time at humidity levels like 20% and 85%. A drastic resistivity has been observed for 20% and 85% humidity levels,



showing full recovery of AgNPs when humidity drops back to 20%. Response time and the recovery time of this humidity sensor were relatively short compared to other sensors (Rao and Tang, 2017).

The resistance of AgNPs was found to be decreased with the adsorption of water. This humidity sensing property is attributed to the charge transfer process. At room temperature and in dry air, oxygen anions are adsorbed on to AgNP surface. This induces electron depletion layers on the surface due to the transfer of electrons from AgNPs to oxygen anions. This forms a silver oxide layer which is semi-insulative causing the high electrical resistance in dry air. When water molecules are physisorbed by AgNPs, the oxygen molecules are displaced from the AgNP surface. This causes the electrons to fill up in the conduction bands of the silver oxide surface layers. Electrical conductivity is thus increased when the fermi level moves toward the conduction band. This causes a drop in the electrical resistance. The amount of water molecules physisorbed increases with increasing humidity. Therefore, the resistance of AgNPs also varies linearly with the level of humidity (Rao and Tang, 2017).

TiO<sub>2</sub>, the conventional semiconductor photocatalyst, is only active under UV light due to the presence of the large bandgap. As mentioned earlier, AgNPs can be used as photocatalysts, which could make use of visible and UV light (Chen *et al.*, 2010).

The degradation of Reactive Red 120 and Reactive Black 5 has been assessed using AgNPs biosynthesized using *Eriobotrya japonica* leaf extract. These dyes are commonly used to dye cellulosic yarns and fabrics. It is biodegradable by the activated sludge process. AgNPs with lower sizes have shown to be more efficient in the degradation of the dyes than the larger particles. This is due to the higher surface area available, which causes faster electron transport to break azo bonds in the dyes. A degradation of 92% for Reactive Red 120 has been observed within 30

minutes. A degradation of 94% has been observed for Reactive Black 5 (He *et al.*, 2017).

AgNPs synthesized using ethanolic Aloe vera leaf extracts have been assessed for the ability to remove mercury (Hg<sup>2+</sup>). AgNPs were incorporated into an agar plate count matrix which was then added with Hg<sup>2+</sup>. A removal percentage of 96% has been reported. Concentration of Hg<sup>2+</sup> remaining in the system was 0.75 mg/mL which is lower than the limit set by the WHO and EPA organizations (Kumar and Punathil 2018). The combination of the insecticidal activity of the botanicals with nano technology has resulted in plant-mediated nano formulations as larvicides. Combinatorial effect of these characteristics has enabled to achieve their insecticide efficacy at very low concentrations (<30 mg/L) (Benelli *et al.*, 2017). Benelli (2016) gives a comprehensive review on plant-mediated biosynthesis of nanoparticles as an emerging tool against mosquitoes. Silver nanoparticles synthesized using *A. glabra* leaf extract has been successfully tested to control the larvae of *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) (Amarasinghe *et al.*, 2020).

## CONCLUSION

Nature has elegant and ingenious means to create the most effective miniaturized functional materials. A growing awareness of green chemistry and the use of the greenway for the synthesis of metallic nanoparticles is generating a desire to develop environmentally friendly techniques. The advantages of synthesizing silver nanoparticles from plant extracts is that it is economical, energy efficient; provides healthier workplaces and communities, protects human health and the environment, and generates less waste and safer products. The green synthesized silver nanoparticles present important aspects of nanotechnology through

incomparable applications. For nanoparticle syntheses, the use of plants may be advantageous over other biological entities, which can overcome the tedious process of using microbes and maintaining their culture, which can cause them to lose their synthesis potential of nanoparticles. Hence, in this respect, the use of plant extracts for synthesis can have a considerable impact in the decades to come.

This review addressed recent publications on synthesis of AgNPs using plant extracts and their applications. Many plant extracts made using different plant parts have been successfully used to synthesize AgNPs. Factors such as plant extract concentration, silver nitrate concentration, pH of the medium, incubation time and temperature can be changed to tune the properties of the formed AgNPs. These parameters have an optimum value that could be used to obtain AgNPs with smaller size and narrow size distribution.

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