

# Synthesis and characterization of Silver nanoparticles (AgNPs) and their antibacterial effect on gram positive bacteria (*Staphylococcus aureus*) and gram negative bacteria (*Escherichia coli*) by using synthesized silver nanoparticles from *Lantana camara*

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## ABSTRACT

**AIM:** The aim of the present study is to investigate the synthesis and characterization of Silver nanoparticles (AgNPs) and their antibacterial effect on gram positive bacteria (*Staphylococcus aureus*) and gram negative bacteria (*Escherichia coli*) by using synthesized silver nanoparticles from *Lantana camara*.

**MATERIAL AND METHODS:** AgNPs were prepared by green synthesis process from 1mM AgNO<sub>3</sub> solution the aqueous silver ions when exposed to the *L.camara* were reduced and stabilized over long periods of time resulting in the green synthesis of surface functionalized silver nanoparticles. The bio-reduced silver nanoparticles were appropriately characterized by using UV-Vis spectroscopy, Dynamic Light Scattering and Scanning Electron Microscope. Antibacterial activity of synthesized silver nanoparticles (AgNPs) was carried out on both Gram negative and Gram positive bacteria.

**RESULT:** From UV-Visible spectroscopy the peak of the spectra was found at 472 nm. In Dynamic Light Scattering the size of the particle diameter ranges at 179.3nm. From the Scanning Electron Microscope analysis the average size of synthesized silver nanoparticles at 80.2nm was analysed.

**Keywords:** Nano particles, Silver Nano particles, Bio particles, Nano particles production, *Lantana camara*.

## INTRODUCTION

The development of new resistant strains of bacteria to current antibiotics has become a serious problem in public healthcare. Therefore, there is a strong incentive to develop new bactericides. Elemental silver and silver salts are being used for decades as antimicrobial agents in curative and preventive healthcare. Microbes are unlikely to develop resistance against silver as they do against conventional and narrow-target antibiotics, because the metal attacks a broad range of targets in the organisms, which means that they need to develop a range of mutations simultaneously to protect themselves. Delivering therapeutic compound to the target site is a major problem in treatment of many diseases. A conventional application of drugs is characterized by limited effectiveness, poor biodistribution, and lack of selectivity. These limitations and drawbacks can be overcome by controlling drug delivery. In controlled drug delivery systems (DDS) the drug is transported to the place of action thus, its influence on vital tissues and undesirable side effects can be minimized. In addition, DDS protects the drug from rapid degradation or clearance and enhances drug concentration in target tissues; therefore, lower doses of drug are required. This modern form of therapy is especially important when there is a discrepancy between a dose or concentration of a drug and its therapeutic results or toxic effects. Cell-specific targeting can be achieved by attaching drugs to individually designed carriers. Recent developments in nanotechnology have shown that nanoparticles (structures smaller than 100 nm in at least one dimension) have a great potential as drug carriers. Due to their small sizes, the nanostructures exhibit unique physicochemical and biological properties (e.g., an enhanced reactive area as well as an ability to cross cell and tissue barriers) that make them a favorable material for biomedical applications.

Silver based compounds have been used in recent years to prevent bacterial growth in application such as burn care. Silver doped polymer fabrics, catheters and polyurethane are well known for their antibacterial functionality. Silver has long been acknowledged as having inhibitory effect on microbes present in medical and industrial process. Specific surface area is an important for catalytic reactivity and other related properties such as antimicrobial activity in silver nanoparticles.

Silver nanoparticles find wide application in various fields like catalysis, photonics, optoelectronics, information storage, antibacterial applications, etc. Silver powders, having ultra-fine and uniformly distributed particle size, are of considerable use in the electronics industry as thick film conductors in integrated circuits due to their unique properties such as high electrical and thermal conductivity, high resistance to oxidation. Apart from electronic applications, it has been known for centuries that silver has bactericidal properties. Silver is a safe and effective bactericidal metal because it is non-toxic to animal cells and highly toxic to bacteria such as *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S.aureus*).

*Lantana camara* is a flowering ornamental herb found in tropical and subtropical countries and otherwise called as Lantana, Wild Sage, Surinam Tea Plant, Spanish flag and West Indian lantana. It's leaves are rich in essential oil. It has wide traditional claims for treatment of various illness and recent scientific studies have emphasized the possible use of *L. camara* in modern medicine. It have medicinal

properties like anticancer activity, anti-inflammatory activity, antidiabetic activity, anthelmintic, antibacterial activity, antifungal activity, hepatoprotective activity, antioxidant activity, larvicidal activity.

Some common approaches such as physical processes of atomization or milling, chemical methods of chemical reduction, biological irradiation, water-in-oil microemulsions, and green synthesis methods have been utilized. Among the different living organisms used for nanoparticles synthesis, plants are of particular interest in metal nanoparticles synthesis because of its advantage over other environmentally benign biological process as it eliminates the elaborate process of maintaining cell cultures. Plant mediated synthesis of nanoparticles is gaining importance due to its simplicity and eco-friendliness. The use of environmentally affable materials like plant extract, bacteria, fungi and enzymes for the synthesis of silver nanoparticles offers various advantages of eco-friendliness and cohesiveness for pharmaceutical and different biomedical applications as they do not use toxic chemicals for the synthesis algorithm. Chemical synthesis method results in presence of some toxic chemical absorbed on the surface that may have unfavorable impact in the medical applications. Compared with chemical and physical method of synthesis, green synthesis method provides a low cost, environment friendly, easily scale up for large scale synthesis. Green synthesis method there is no need to use high pressure, energy, temperature and toxic chemicals.

## MATERIALS AND METHODS

### MATERIALS REQUIRED

#### GLASSWARES

Beaker, Conical flask, Micropipettes with tips, Measuring cylinder, L-rod, Forceps, polypropylene tubes, Mortar and pestle, Petri plates, Inoculation loop, Microsyringe filter, Whatman filter paper, Funnel.

#### CHEMICALS

Silver nitrate ( $\text{AgNO}_3$ ), Mueller – Hinton agar (MHA), Mueller – Hinton Broth (MHB) was purchased from HI media, Mumbai, India.

#### BACTERIAL STRAINS

*Escherichia coli* and *Staphylococcus aureus* were obtained from the Microbiology Research Laboratory at K.A.P. Vishwanathan government medical college, Trichy district, Tamil Nadu, India.

#### INSTRUMENTATION

UV-Vis spectrophotometer, Scanning Electron Microscope (SEM), Dynamic Light Scattering (DLS) were done by Archbishop Casimir Instrumentation Centre (ACIC), St. Joseph's College (Autonomous), Trichy district, Tamil Nadu, India.

#### COLLECTION OF PLANT MATERIAL

The fresh leaves of *Lantana camara* were collected from surrounded villages in Marandhalli, Dharmapuri district, Tamil Nadu, India.

## METHODS

### STERILIZATION TECHNIQUES

All glasswares were washed with detergent, rinsed thoroughly with distilled water. All polypropylene tubes and tips used were sterilized as well as media and solutions prepared were sterilized by autoclaving at 121°C for 15 - 25 min. Inoculations were done with flame sterilized loops and all experiments were performed wearing sterile disposable hand gloves and Laminar Air Flow chamber were sterilized with ethanol.

### PREPARATION OF THE LEAF EXTRACT

*Lantana camara* leaf extract was used for nanoparticles synthesis. 25g leaves of *Lantana camara* were weighed and thoroughly washed in distilled water to remove dirt and other adherent material. Then the leaves were crushed and boiled in 100 ml distilled water for 10 minutes at 60°C. After it was cooled to room temperature, filtered using Whatman filter paper 1 (pore size 25  $\mu\text{m}$ ) followed by 0.45  $\mu\text{m}$  syringe filter and centrifuged at 10000 rpm for 10 minutes. Filtrate solution was referred as stock solution and used directly of silver nanoparticles synthesis.

### PREPARATION OF 1mM SILVER NITRATE SOLUTION

Accurate concentration of 1mM silver nitrate can be prepared by dissolving 0.0421gms of silver nitrate in 250ml of double distilled water and stored in amber colored bottle to prevent auto oxidation of silver.

### SYNTHESIS OF SILVER NANOPARTICLES

#### OPTIMIZATION AND SYNTHESIS OF AgNPs

1ml, 3ml, 5 ml and 10 ml of *Lantana camara* leaf extracts were taken in a separate conical flask and to this 10 ml of 1mM silver nitrate ( $\text{AgNO}_3$ , MW 169.87) solution was added with constant stirring under room temperature. The color change of the leaf extract was checked periodically and the color changes from green to dark brown indicate the synthesis of AgNPs from the leaves.

### PRODUCTION AND RECOVERY OF AgNPs

For the bulk production of silver nanoparticles from leaf extract of *L.camara*. 50mL extract was added into the aqueous solution of 1mM silver nitrate in a 1:1 ratio at room temperature. Initially the plant extract was light green. Upon providing the silver salt, it turned dark brown. Then the mixture was stored in the refrigerator for the antibacterial activity test and further analyzed by using UV-Vis spectrophotometer and DLS. After reduction, the extract consisting of NPs was subjected to centrifugation at 10,000rpm for 20 minutes and the supernatant was discarded. The pellet was air dried and stored for SEM analysis.

## CHARACTERIZATION OF SILVER NANOPARTICLES

### VISUAL OBSERVATION

The synthesis of silver nanoparticles (the reduction process  $\text{Ag}^+$  to  $\text{Ag}^0$  nanoparticles) was confirmed by visual observation of color change.

### UV-VISIBLE SPECTROSCOPY

The UV-Vis absorption was analyzed after centrifugation followed by redispersing the particles in deionized water. The reduction of silver ions in the aqueous solution of nanoparticles in the solution could be correlated with the respective UV-Vis spectra of the colloidal solution was analyzed for the absorption ranges from 200 to 600 nm by using UV-Visible Spectrophotometer.

### DYNAMIC LIGHT SCATTERING (DLS)

DLS gives information about the size of the synthesized nanoparticles. Particle size is measured by the SZ-100 using dynamic light scattering (DLS). The sample particles in the cell experience Brownian motion. A light source is introduced into the cell and the scattered light is collected. The system automatically selects the optimum angle and cell position depending on the sample concentration and intensity.

### SCANNING ELECTRON MICROSCOPE (SEM)

The synthesized silver nanoparticles were monitored morphologically using SEM (Hiyachi S-4500). A thin film of each synthesized silver nanoparticles samples were prepared on a carbon copper grid by dropping a small amount of sample on the grid were allowed to dry by putting it under a mercury lamp for 5 minutes. Then the sample was scanned by high energy electron beam, the beam which passes through the sample produce signal in a raster that contain information of sample surface topography composition and other properties.

### ANTIBACTERIAL ACTIVITY OF AgNPs USING DISC DIFFUSION METHOD

Synthesized Nanoparticles (AgNPs) was screened in vitro by disc diffusion assay method against Gram- negative bacteria *Escherichia coli* and Gram-positive bacteria *Staphylococcus aureus*. The target microorganisms were cultured in Mueller– Hinton broth (MHB). After 24 h the suspensions were adjusted to standard sub culture dilution. The Petri dishes containing Muller Hinton Agar (MHA) medium were cultured with diluted bacterial strain (*Escherichia coli* and *Staphylococcus aureus*). Then sterile discs (Whatman No.1, diameter 4mm) were kept and the samples like Leaf extract, Silver nitrate solution, Synthesized AgNPs in different concentrations of 10 $\mu\text{g}$  and 20 $\mu\text{g}$  were added to the disc and plates were incubated at 37°C for 24 hours. After 24h of incubation, the inhibition zones were observed.

## RESULTS AND DISCUSSION

### VISUAL OBSERVATION OF SYNTHESIZED AgNPs

Silver nanoparticles have shown dark brown colour in aqueous solution was visually observed as shown in (Figure 2D). The addition of leaf extract to the aqueous solution of silver nitrate results gradual change in the colour of the solution from slightly green to pale yellow, deep yellow, brown, dark brown. This colour change was due to excitation of surface plasma vibrations in silver nanoparticles. After added the extract with silver nitrate the colour of the solution changed from watery to dark brown due to the reduction of  $\text{Ag}^+$  into  $\text{Ag}^0$  which had shown the formation of silver nanoparticles. Silver nanoparticles exhibit interesting optical properties directly associated with localized surface Plasmon resonance which is highly depends on the morphology of the nanoparticles. Control experiments without the addition of extracts showed no formation of brown color, indicating that the color change is due to the presence of extracts. This indicates the synthesis of silver nanoparticles, (Figure 2D) was used for further confirmed by UV- Visible Spectroscopy.

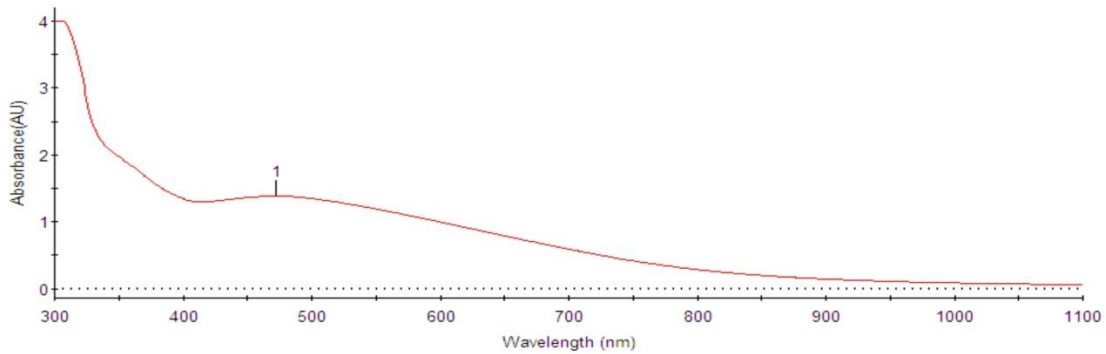


**FIGURE 1: (A) Prepared *Lantana camara* leaf extract; (B) Prepared 1mM silver nitrate Solution; (C) Initial (pale yellow); (D) 10 minutes (deep yellow); (E) 30 minutes (brown); (F) 1 hour (dark brown)**

### UV-VIS SPECTROSCOPY

The optical and structural properties were determined by UV-VIS. The UV-VIS Spectroscopy was the preliminary technique for the characterization of the silver nanoparticles. The UV-VIS absorption was analyzed after centrifuging and redispersing the particles in deionized water. Reduction of  $\text{Ag}^+$  ions during exposure to the extract of *L. camara* leaf was easily followed by UV-spectroscopy. In (Graph 1) shows the peak of the above spectra was found at 472 nm and this peak is due to Surface Plasmon Resonance (SPR) property of silver nanoparticles.

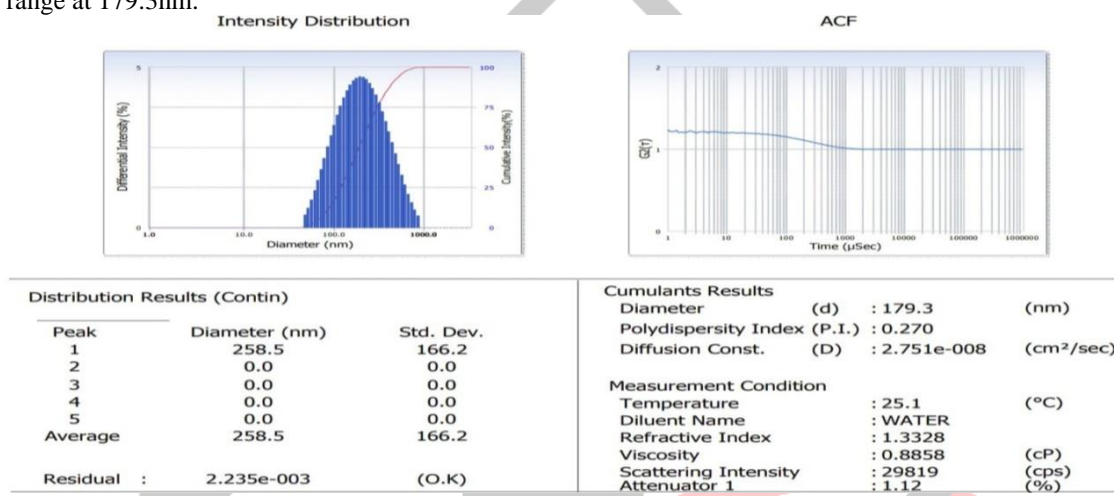




**GRAPH 1: Graphical representation of UV visible Spectroscopy analysis for the formation of silver nanoparticles at 472 nm**

**DYNAMIC LIGHT SCATTERING**

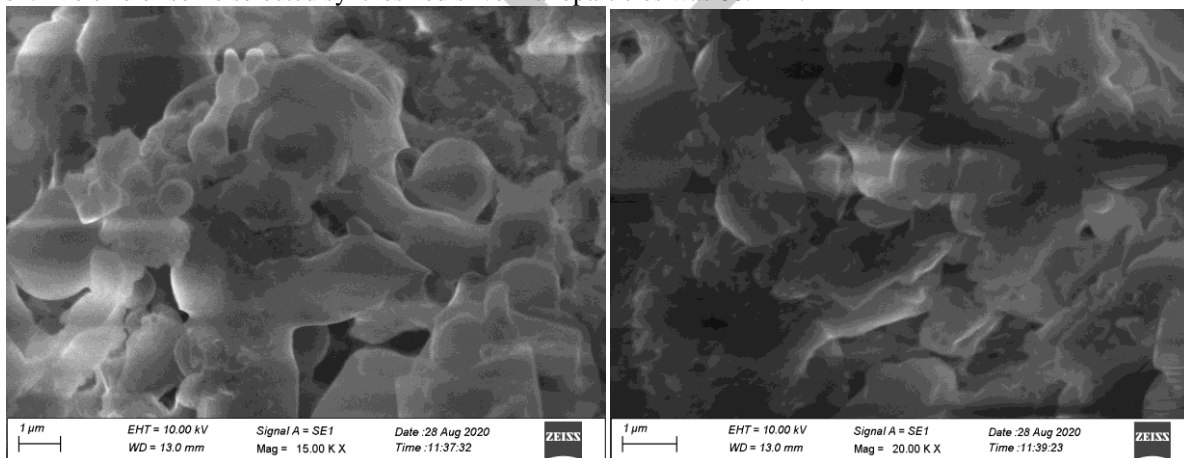
The Dynamic light scattering reflects the reaction of silver nanoparticles and also the activity towards agglomeration or settlement. A light source is introduced into the cell and the scattered light is collected and measured for the particle size. Synthesized AgNPs using *L.camara* is measured diffuse light scattering method from to analyse the particle size and their settlement. In (Graph 2) shows diameter of the particle range at 179.3nm.



**GRAPH 2: Graphical representation of Intensity distribution and ACF of DLS analysis of non-filtered colloidal synthesized silver nanoparticles showing distribution result part in the form of peak, diameter(nm), average with poly dispersity index and diffusion constant**

**SCANNING ELECTRON MICROSCOPE (SEM)**

The morphological features (shape and size) of synthesized silver nanoparticles were studied by SEM analysis. SEM analysis suggested that most of the particles are oval shape AgNPs as shown in (Figure 2). The synthesized silver nanoparticles had been spread thoroughly in the solution. The size of some selected synthesized silver nanoparticles was 80.2nm.



**FIGURE 2: SEM analysis of synthesized silver Nanoparticles using *Lantana camara* with different magnification**

**ANTIBACTERIAL ACTIVITY**

The antibacterial activity of synthesized AgNPs was studied against tested microorganism by using disc diffusion method. Antibacterial activity of that synthesized nanoparticles were more effective for *Staphylococcus aureus* (gram positive organisms) when compared

with *Escherichia coli* (gram negative organisms). In (Table 1) shows the maximum zone of inhibition of  $20.2 \pm 0.3$ ,  $23.3 \pm 0.7$  were observed against *Escherichia coli*, *Staphylococcus aureus* at the concentration of  $20 \mu\text{g/mL}$  of silver nanoparticles (AgNPs). Likewise the minimum zone of inhibition of  $18.3 \pm 0.3$ ,  $18.1 \pm 0.7$  were observed against *Escherichia coli*, *Staphylococcus aureus* at the concentration of  $10 \mu\text{g/mL}$  of silver nanoparticles (AgNPs). Zone of inhibition was not observed in control plates loaded with deionized water while leaf extract has showed inhibition effect against *Escherichia coli*, *Staphylococcus aureus* with inhibition zone of  $5.8 \pm 0.3$ ,  $7.8 \pm 0.9$ . The silver nitrate ( $\text{AgNO}_3$ ) showed inhibition effect of *Escherichia coli*, *Staphylococcus aureus* with inhibition zone of  $8.0 \pm 1.4$ ,  $9.5 \pm 0.5$  respectively.

The effects of Leaf extract, Silver nitrate ( $\text{AgNO}_3$ ) and Synthesized AgNPs on the bacterial growth inhibition were monitored and zone of inhibition was measured after 24 hours of incubation and depicted in (Table 1).

ZONE OF INHIBITION (mm)				
BACTERIA	LEAF EXTRACT	$\text{AgNO}_3$	AgNPs ( $10 \mu\text{g/mL}$ )	AgNPs ( $20 \mu\text{g/mL}$ )
<i>Escherichia coli</i>	$5.8 \pm 0.3$	$8.0 \pm 1.4$	$18.3 \pm 0.3$	$20.2 \pm 0.3$
<i>Staphylococcus aureus</i>	$7.8 \pm 0.9$	$9.5 \pm 0.5$	$18.1 \pm 0.7$	$23.3 \pm 0.7$

**TABLE 1: Measurement of Zone of inhibition of AgNPs against *E.coli* and *S.aureus***

## CONCLUSION

In conclusion, the leaf extract of *L. camara* are capable of synthesize silver nanoparticles has been demonstrated. Green synthesis of silver nanoparticles is an eco-friendly and low cost method. Synthesized silver nanoparticles were characterised by visible observation, UV-Visible spectroscopy, DLS and SEM analysis. The synthesized silver nanoparticles are appeared dark brown at visual observation. In UV-Visible spectroscopy the the peak of the spectra was found at  $472 \text{ nm}$  and this peak is due to Surface Plasmon Resonance (SPR) property of silver nanoparticles. From the Dynamic Light Scattering the size of the particle diameter ranges at  $179.3 \text{ nm}$ . From the Scanning Electron Microscope analysis the average size of synthesized silver nanoparticles at  $80.2 \text{ nm}$  was analysed. Study also reveals antibacterial activity for *Staphylococcus aureus* as gram positive and *Escherichia coli* as gram negative bacteria. It has been generally believed that the mechanism of the antibacterial effects of silver ions  $\text{Ag}^+$  involves their absorption and accumulation by the bacterial cells that would lead to shrinkage of the cytoplasm membrane or its detachment from the cell wall. At lower concentration, silver nanoparticles directly damage the cell envelope by penetrating the cell and then silver binds to the DNA, this complex prevents the DNA replication by displacement of hydrogen bonds between adjacent nitrogen of purines and pyrimidines. As a result, DNA molecules become condensed and lose their ability to replicate upon the infiltration of  $\text{Ag}^+$  ions. Antibacterial activity concluded that the synthesized nanoparticles were more effective for *Staphylococcus aureus* (gram positive organisms) when compared with *Escherichia coli* (gram negative organisms). The antibacterial result states Silver nanoparticles as a strong antibacterial agent which can be useful for antimicrobial applications.

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