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# INVESTIGATION OF ANTIBACTERIAL PROPERTIES OF SILVER NANOPARTICLES USING AERVA LANATA EXTRACT

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***Abstract:***
*The field of nanotechnology is the most active area of research in modern materials science. Though there are many chemical as well as physical methods, green synthesis of nanomaterials is the most emerging method of synthesis. We report the synthesis of antibacterial Silver nanoparticles (AgNPs) using flower of medicinal herb, Aerva lanata. The synthesized AgNPs have been characterized by UV-Vis spectroscopy, FT-IR, transmission electron microscopy (TEM) and Cyclic Voltammetry(CV). The mean particle of synthesized NPs was found to be 43.5 nm, as confirmed by TEM. FTIR analysis revealed that the AgNPs were stabilized by proteins, polyphenols and other aromatic compounds present in the extract. Such AgNPs stabilized by Aerva lanata flower extract were found to have enhanced antimicrobial activity against well-known pathogenic strains, namely E.Coli, Staphylococus aureus, Bascillus cereus and Pseudomonas aeruginosa.*

***Keywords:*** *Silver Nanoparticles; Aerva lanata; TEM; Antibacterial activity;*

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## INTRODUCTION

Nanoparticles are being viewed as elementary building blocks of nanotechnology. The most important and distinct property of nanoparticles is that they show evidence of larger surface area to volume ratio. The most successfully studied nanoparticles today are those made from noble metals, in particular Ag, Pt, Au and Pd. Metal nanoparticles have marvelous applications in the area of catalysis, optoelectronics, diagnostic biological probes and display devices. [1]. conventionally, nanomaterials are synthesized using either chemical or physical methods which include sol process, micelle, chemical precipitation, hydrothermal method, pyrolysis, and chemical vapour deposition [2]. Some of these methods are easy and provide control over crystallite size by restoring the reaction environment. But problem still exists with the general stability of the product and in achieving monodisperse nanosize using these methods [3]. Moreover, many of the conventional techniques have been found to be capital intensive and inefficient in materials and energy use [4].

Biological methods have emerged as an alternative to the conventional methods for synthesis of NPs. Synthesis of inorganic nanoparticles by biological systems makes nanoparticles more biocompatible and environmentally benign [5].Many plants such as Geranium leaf [6], Alfalfa [7], *Azadirachta indica* [8],*Lemon grass* [9], *Aloe vera* [10], *Cinnamomum Camphora* [11], *Emblica officinalis* [12], *Capsicum annuum* [13], *Diospyros kaki* [14], *Carica papaya* [15], *Coriandrum sp.* [16], *Boswellia ovalifoliolata*[17], *Tridax procumbens, Jatropha curcas, Solanum melongena, Datura metel, Citrus aurantium* [18], *Aegle marmelos*[19], *Cissus quadrangularis*[20], *Morinda tinctoria*[21] and *Couroupita guianensis*[22] have shown the potential of reducing nature for the formation of NPs.

The present study aims at the synthesis of silver nanoparticles from the aqueous extract of *Aerva lanata* flowers. We also attempt to combine the inherent antimicrobial activities of silver nanoparticles for enhanced antimicrobial activity.

## MATERIALS AND METHODS

### Materials:

Fresh plants of *Aerva lanata* were identified and collected from Tamilnadu Agricultural University, Tirunelveli, and Tamilnadu, India and the taxonomic identification was made by Botanical Survey of India, Coimbatore. Silver Nitrate was obtained from the precision scientific co, Coimbatore, India.

### Synthesis of Silver Nanoparticles:

The fresh plant of *Aerva lanata* broth solution was prepared by taking 100 g of thoroughly washed and finely cut plants in a 500 ml Erlenmeyer flask along with 200 mL of sterilized double distilled water and then boiling the mixture for 15 min before finally decanting it. The extract was filtered through Whatman filter paper no 1 and stored at -15$^0$C and could be used within 1 week. The filtrate was treated with aqueous 1 mM $AgNO_3$ solution in an Erlenmeyer flask and incubated at room temperature. As a result, a purple coloured solution was formed; indicating the formation of silver nanoparticles and it was further confirmed by UV-Vis spectrum analysis [23]. It showed that aqueous silver ions could be reduced by aqueous extract of plant parts to generate extremely stable silver nano particles in water (Figure 1).

### Characterization of The Synthesized Silver Nanoparticles Using UV-Spectra:

Synthesis of silver nanoparticles solution with plants extract may be easily observed by ultraviolet-visible (UV-Vis) spectroscopy. The bio-reduction of the $Ag^+$ ions in solutions was monitored by periodic sampling of aliquots (1 mL) of the aqueous component and measuring the UV-Vis spectra of the solution. UV-Vis spectra of these aliquots were monitored as a function of time of reaction on a Vasco 1301 spectrophotometer in 400-600 nm range operated at a resolution of 1 nm.

### FT-IR Spectroscopy:

FT-IR measurements is undertaken in order to confirm the formation of crystalline nanocrystals and identify adsorbed species onto the crystal surface. Generally, FT-IR is recorded using Nicolet FT-IR spectrometer mode impact 400. The spectra were recorded at wave number in the range of 400 and 4000cm$^{-1}$.

### Transmission Electron Microscopy (TEM):

Transmission electron microscopy (TEM) (HITACHI, H-7500) is a microscopy technique whereby a beam of electrons is transmitted through an ultra-thin specimen, interacting with the specimen as it passes through. Ag nanoparticle image was formed from the interaction of the electrons transmitted through the specimen; the image of Ag nanoparticles was magnified and focused onto an imaging device.

### Cyclic Voltammetry Analysis:

Analysis through cyclic voltammetry (CV) confirmed the presence of elemental silver signal of silver nanoparticles .The change in the oxidation state of the metal ion was studied by CV technique, using platinum electrode with fresh surface at the rate of 25mVs$^{-1}$ in the potential range between -1.0 and 1.0V.

### Antimicrobial Activity Study:

Antibacterial activities of the synthesized Ag nanoparticles were determined, using the agar disc diffusion assay method. Approximately 20 mL of molten and cooled Muller Hinton agar media was poured in sterilized petri plates. The plates were left overnight at room temperature to check for any contamination to appear. The test organisms were

grown in selected broth for 24 h.100 mL of broth culture of each test organism was used to prepare lawns. Agar of 5 mm diameter was prepared with the help of a sterilized stainless steel cork borer. Four plates were prepared in the agar plates. Ciprofloxacin was used as standard and positive controls. The plates containing the test organism and Ag nanoparticles were incubated at 37 ºC for 24 - 48 h. The plates were examined for evidence of zones of inhibition, which appear as a clear area around the plates. The diameter of such zones of inhibition was measured using a meter ruler and the mean value for each organism was recorded and expressed in millimeter.

## RESULTS AND DISCUSSION

**UV-VIS Spectra Analysis**

In the present scenario, Ag nanoparticles as antimicrobial agents have come up as a promising candidate in the medical field [24]. Reduction of Ag ion into silver nanoparticles during exposure to the plant extracts could be followed by color change. Ag nanoparticle exhibit dark brown colour in aqueous solution due to the surface plasmon resonance phenomenon. The result obtained in this investigation is very interesting in terms of identification of potential plants for synthesizing the Ag nanoparticles. UV-Vis spectrograph of the colloidal solution of silver nanoparticles has been recorded as a function of time. Absorption spectra of silver nanoparticles formed in the reaction media at 10 min has absorbance peak at 433 nm, broadening of peak indicated that the particles are polydispersed (Figure 2).

**FT-IR Studies:**

FTIR analysis was carried out to identify the possible interaction between the biomolecule and $Ag^+$ during the biogenric reduction reactions. The FTIR data for silver nanoparticles containing *Aerva lanata* plant extract is shown in figure 3. The band at 3598 $cm^{-1}$ is assigned for O-H stretching vibration of phenolic compounds and bands observed at 1761 cm-1 (esters C=O group), 1628 cm-1 (aromatic ring C=C functional groups), 1310 cm-1 (geminal methyls) and 1211 cm-1 (ether linkages). The carbonyl bands at 1761$cm^{-1}$ was shifted to 1628$cm^{-1}$ during the formation of silver nanoparticles. The shifts in bands at 1761, 1310 and 1211$cm^{-1}$ were clearly indicating the coordination of carboxylic acid with silver nanoparticles.

From the analysis of FTIR studies, we revealed that the carbonyl group from the amino acid residues and proteins has the stronger ability to bind metal indicating that the proteins could possibly from the metal nanoparticles (i.e., capping of silver nanoparticles) to prevent agglomeration and thereby stabilize the medium. This suggests that the biological molecules could possibly perform dual functions of formation and stabilization of silver nanoparticles in the aqueous medium. Water soluble heterocyclic compounds such as flavonoids were mainly responsible for the reduction and stabilization of the Nanoparticle. These results imply that proteins, carbohydrates and amino acid present in *Aerva lanata* plant extract are play a major role on reduction of $Ag^+$.

**TEM Analysis of Ag Nanoparticles**:

The resulting silver nanoparticles was analysed with TEM techniques and conclude that the average mean size of Ag nanoparticles was 43.5nm, which seems to be spherical in morphology as shown in (Figure 4).

**Cyclic Voltammetry Analysis:**

In cyclic voltammetric analysis the *Aerva lanata* plant extract free solution makes all the metal ions are reduced to lower oxidation state, since there is no possibility for the formation of NPs. Upon addition of *Aerva lanata* extract in the reaction medium, the cathodic peak shifted towards the negative potential direction, implying that the reduced silver NPs are stabilized by *Aerva lanata* extract (Fig. 5). The extent of decrease in anodic peak current is greater than that of the cathodic peak current due to the fact that the rate of reduction of silver ion may be greater than its oxidation. This might be because of the electron donating hydroxyl and amine groups containing *Aerva lanata* extract can provide a suitable environment for the formation of nanoparticles. The cyclic voltammogram of AgNPs shows the peaks observed at -0.11 and 0.89V.

It is assumed that only the oxidized form $Ag^+$ is present initially. Thus, a negative-going potential scan is chosen for the first halfcycle, starting from a value where no reduction occurred. As the applied potential approaches the redox process, a cathodic current begins to increase, until a peak is reached. The sweep is reversed after traversing the potential region where the reduction process takes place. During the reverse scan, Ag molecules are reoxidized back to $Ag^+$ and it result in an anodic peak.

**Antibacterial Activity Study:**

The antibacterial activity of silver nanoparticle was tested against the following microorganism, viz; *E.Coli, Staphylococus aureus, Bascillus cereus, and Pseudomonas aeruginosa* by disc diffusion method and the results were tabulated in the table 1. The silver nanoparticle has shown antibacterial activity against all tested microorganism and maximum zone of inhibition was found against *Bascillus cereus.*(figure 6)

It is well known that Ag ions and Ag-based compounds have strong antimicrobial effects [25], and many investigators are interested in using other inorganic nanoparticles as antibacterial agents [26-28]. These inorganic nanoparticles have a distinct advantage over conventional chemical antimicrobial agents. The most important problem caused by the chemical antimicrobial agents is

multidrug resistance. Generally, the antimicrobial mechanism of chemical agents depends on the specific binding with surface and metabolism of agents into the microorganism. Various microorganisms have evolved drug resistance over many generations. Thus far, these antimicrobial agents based on chemicals have been effective for therapy; however, they have been limited to use for medical devices and in prophylaxis in antimicrobial facilities. Therefore, an alternative way to overcome the drug resistance of various microorganisms is needed desperately, especially in medical devices, etc. Ag ions and Ag salts have been used for decades as antimicrobial agents in various fields because of their growth-inhibitory capacity against microorganisms. Also, many other researchers have tried to measure the activity of metal ions against microorganism [29,30]. However, Ag ions or salts has only limited usefulness as an antimicrobial agent for several reasons, including the interfering effects of salts and the antimicrobial mechanism of the continuous release of enough concentration of Ag ion from the metalform. In contrast, these kinds of limitations can be overcome by the use of Ag nanoparticles.

## CONCLUSION

In this investigation, the biosynthesized silver nanoparticles were characterized by UV-Vis, HR-TEM, FT-IR and CV measurements. This green synthesis method is alternative to chemical method, since it is cheap, pollutant free and eco-friendly. The potential antimicrobial activity of silver nanoparticles was performed and the maximum antibacterial activity was observed against *Bascillus cereus.* Applications of such eco-friendly nanoparticles in bactericidal, wound healing and other medical and electronic applications, makes this method potentially exciting for the large-scale synthesis of other inorganic nanomaterials. Toxicity studies of silver nanoparticles on human pathogen open a door for a new range of antibacterial agents and anticancer agents.

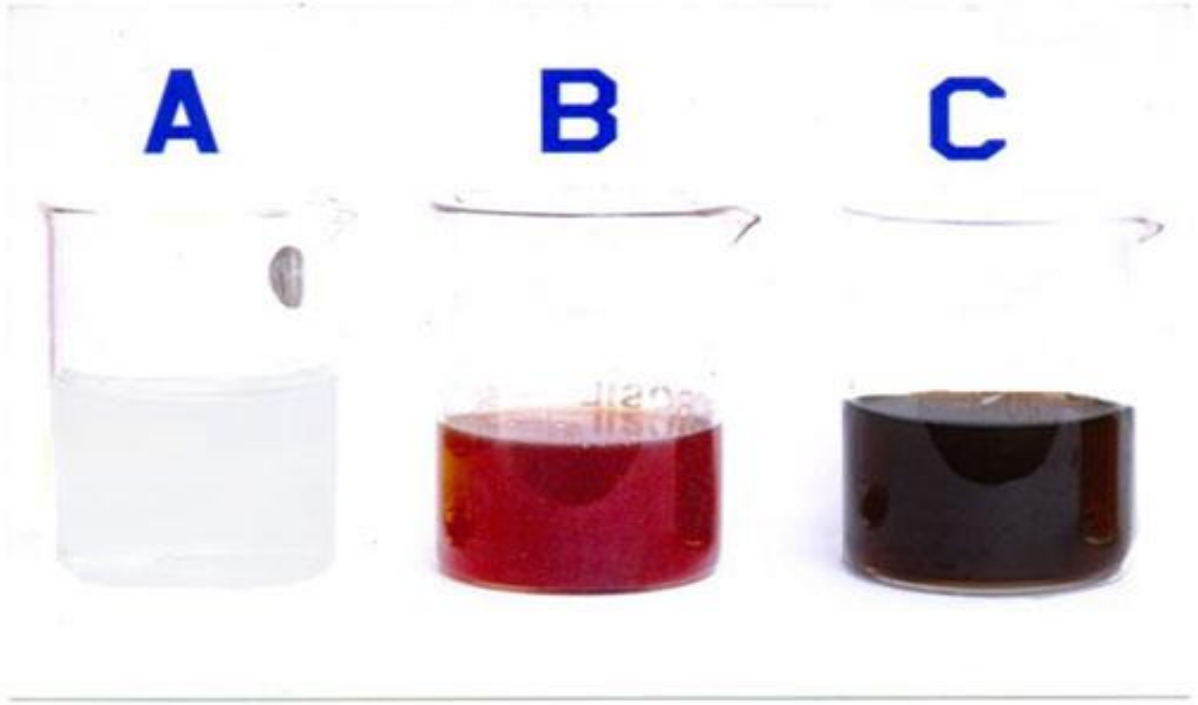


**Fig 1: Photographs Showing A) Pure $AgNO_3$ solution B) Pure *Aerva lanata* Plant Extract C) Colour Changes after Adding Plant Extract with $AgNO_3$ Solution.**

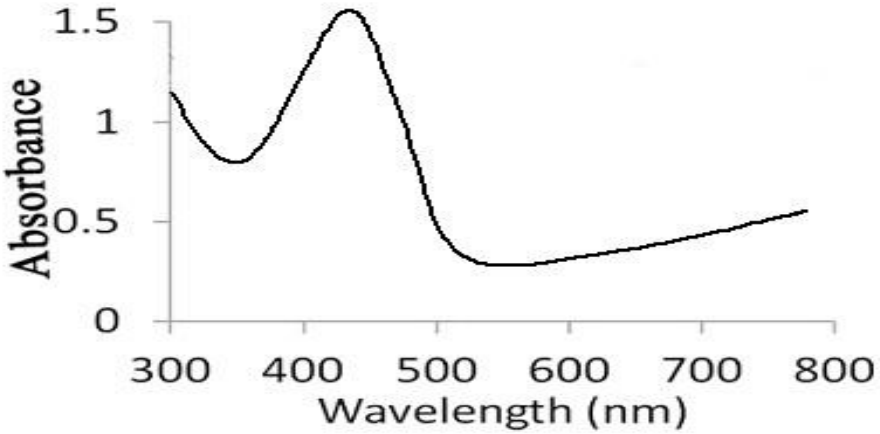


**Fig 2: UV Spectra of Silver Nanoparticles**

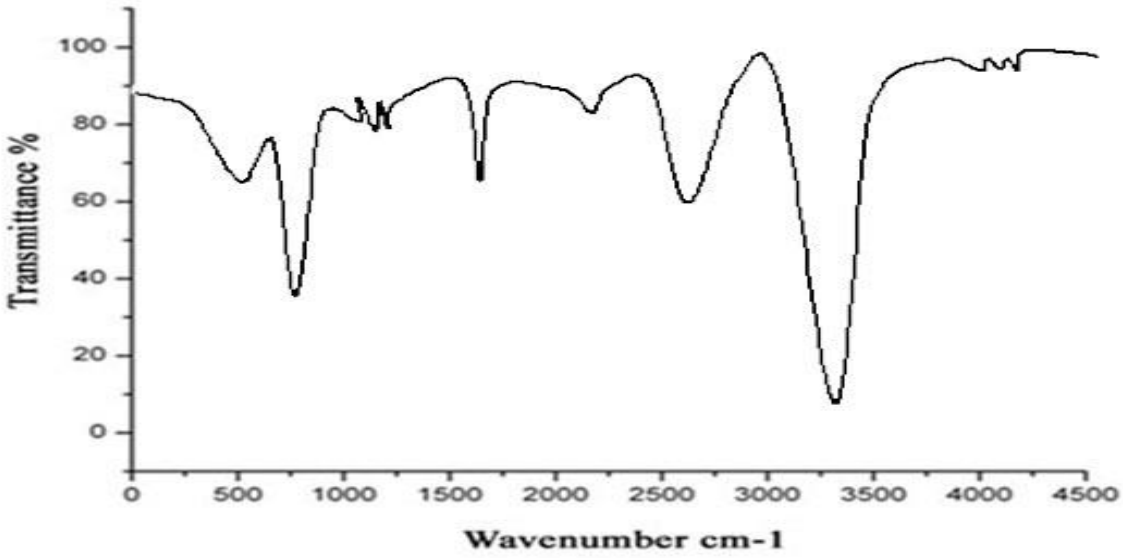


**Fig 3: FT-IR Spectra of Biosynthesized Silver Nanoparticles Using the Plant Extract of *Aerva Lanata***

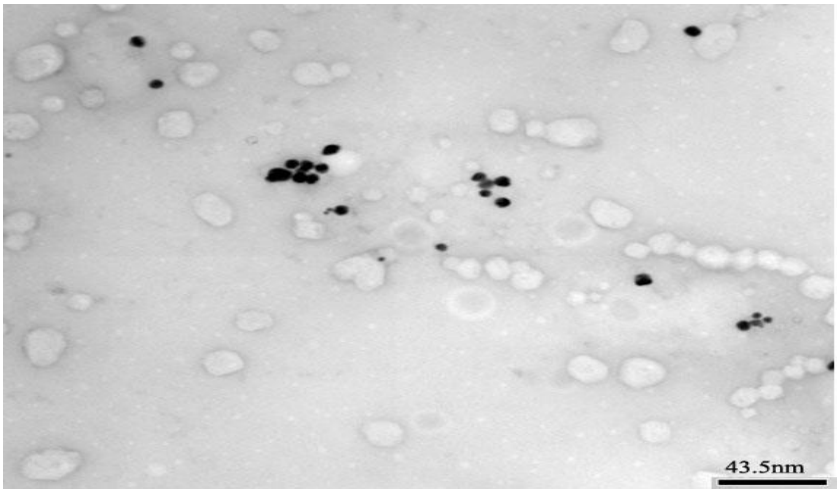


**Fig 4: HR-TEM Image of Silver Nanoparticles Using the Plant Extract of *Aerva lanata***

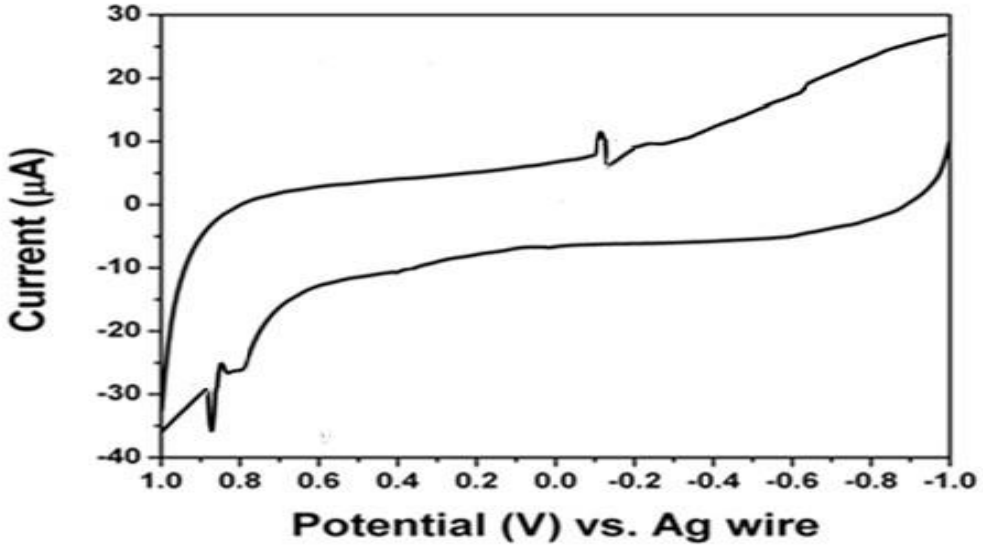


**Fig 5: Cyclic voltammograms of Silver Nanoparticles**

**Table 1: Antibacterial Activity of Silver Nanoparticles**

| Microorganism | Zone of inhibition in mm | | | |
|---|---|---|---|---|
| | 20µl | 40µl | 60µl | Cifrofloxacin |
| *E.Coli* | 8.11±1.32 | 9.08±0.52 | 12.11±0.98 | 15.97±0.25 |
| *Bacillus cereus* | 9.12±0.45 | 12.22±0.74 | 17.29±1.89 | 14.52±1.06 |
| *Staphylococcus aureus* | 8.32±0.79 | 8.98±1.32 | 9.14±0.65 | 14.86±0.01 |
| *Pseudomonas aeruginosa* | 9.08±0.65 | 10.52±0.63 | 12.46 ±0.14 | 13.35±1.85 |


**Fig 6: Antibacterial Activity of Silver Nanoparticles using *Aerva lanata* Plant Extract.**

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