



## Plant based synthesis of silver nanoparticles using *Musa paradisiaca* and evaluation of its antimicrobial potential

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### Abstract

There are reports which described the formation of silver nanoparticles using the reduction mechanism with the help of extracts of different plants. This study emphasizes the synthesis of silver nanoparticles using *Musa paradisiaca* extracts. The Silver nanoparticles synthesized were characterized using UV visible spectroscopy, SEM, particle size analyser and Zeta potential. Due to the presence of aqueous plant extracts, silver nanoparticles were synthesized with size 100 to 120 nm. These particles were stabilized using PEGylation. Food pathogens are known to reduce productivity in agricultural fields. These nanoparticles were tested for antimicrobial potential against food pathogens and excellent activity was observed.

**Keywords:** green synthesis, plant based extracts, silver nanoparticles, zeta potential, zone of inhibition, food microorganisms, antimicrobial activity

### Introduction

Plants have been used as small factories for the synthesis of metal nanoparticles. Among different types of plants banana shows a distinct advantage due to the presence of different reductants which causes synthesis of metal nanoparticles. Rapid synthesis of silver nanoparticles is observed due to high metal uptake capacity in banana and this is enhanced due to the presence of different other components too. Banana is a tropical crop which is known for its biological activity [19]. As food is the measure source of energy as high as the population is increasing day by day the requirement of variety of different types of food also increasing. But previous study showed that food is also the measure of energy or nutrient for many food pathogens which are dangerous or disease causing agents for humans. Food poisoning diseases results from ingestion of contaminated water and wide variety of food contaminated with pathogenic organisms (bacteria, viruses, parasites and fungus), their toxins and chemicals [1]. Some chemicals that causes foodborne illness are natural components of food while other may be accidentally added during production and processing, either through carelessness or pollution. The main causes of foodborne illness are bacteria (66%), chemicals (26%), viruses (4%) and parasites (4%). The most common types of foodborne illness are intoxication and infection. Intoxication occurs when toxin produced by the pathogens cause food poisoning, while infection is caused by the ingestion of food containing pathogens [2, 3]. For this reason nowadays scientists are doing research on how to detect contaminated food due to presence of pathogens before it will be packed for sell at industry level. Therefore this research paper was designed to review information about antimicrobial activity of green-synthesized nanoparticles for the detection of food pathogens like *Staphylococcus aureus*, *Escherichia coli* and *Bacillus. S.*

*aureus* is gram negative cocci that occur in singles, short chain tetrads and irregular grape like cluster. Only the strains that produce enterotoxin can cause food poisoning. The food handler with an active lesion or carriage later initiates infection [4, 5]. *E. coli* are bacterium which belongs to the family enterobacteriaceae and are gram negative up to 3µm in length ferment glucose and wide range of sugars. These lactase fermenters produce pink colonies on Macconkey agar [6]. *E. coli* O157:H7 is one of the more than 60 serotypes of veretoxin producing *E. coli* that cause a variety of human illness such as mild diarrhea, hemorrhagic colitis and hemolytic-uremic syndrome [7]. Two species of bacillus are considered medically significant, *B. anthracis* which causes anthrax and *B. cereus* which causes food poisoning similar to that caused by *S. aureus* [8]. Different drugs are used to kill food pathogens like Streptomycin for bacteria and Ketoconazole for fungus. The recent study showed that antimicrobial activity of synthesized nanoparticles was higher than that of the standard drug i.e. Streptomycin and Keticonazole [9]. Nanoparticles are synthesized from clusters of atoms which are nanoscopic in nature [10]. These particles are not visible to naked eyes hence require large magnification instruments for observation. Nanoparticles are fine entities having size ranging from 100 to 2500 nanometers [11]. Silver nanoparticles in hydrosols are one of the most attractive inorganic materials due to tremendous application in photography [12], catalysis [13], biosensor [14], biomolecular detection [15], diagnostics [16] and particularly antimicrobial activity [17, 18, 19]. Different methods have been used in past for the synthesis of silver nanoparticles which include reduction in solution [20], radiation assisted [21], chemical and photoreduction in reverse micelles [22], thermal decomposition of silver compound [23] and recently by biogenic or green synthesis route [24, 25, 26]. Biological

synthesis of silver nanoparticles using different leaf extracts is investigated and dispersed particles size has been reported [27, 28, 29]. The use of plant extract is more beneficial for the synthesis of silver nanoparticles after various benefits of ecofriendliness and compatibility for pharmaceutical and other biomedical applications [30].

## Material and Methods

### Reagents

Nutrient broth, Agar-Agar type I (source should be mention), Distilled water, Analytical grade silver nitrate (AgNO<sub>3</sub>), Polyethylene glycol (PEG) Phosphate buffered saline (PBS), 100% ethanol.

### Sample collection

Banana plants belonging to the species *Musa paradisiaca* were collected from the fields near Pune, Maharashtra located in South India. Collected samples were immediately brought to the laboratory to prevent desiccation. They were identified by the local botanist and then were exposed to drying. The leaves of *Musa paradisiaca* were identified by consulting botanical experts in Sinhgad College of Science, Pune

### Biosynthesis & PEGylation of silver nanoparticles

Silver nanoparticles were synthesized using reduction method induced by polyphenols from the leaves of *Musa paradisiaca*. The leaf materials were washed to remove excess dirt and contaminants, chopped into pieces and oven

dried at 150 °C until the dry weight was uniform. Then dried leaves were crushed into powder form. Then 5 g of powder leaves were boiled into 250 ml glass beaker containing 100 ml deionized water for 5 min. The aqueous extract was filtered using Whatman no. 1 filter paper to remove larger plant material clumps. The extract was stored at 4 °C to be used for biosynthesis of silver nanoparticles from silver nitrate solution. An aqueous solution of AgNO<sub>3</sub> (1 mM) was prepared in 250 ml Erlenmeyer flask and 0.3% PEG was added to induce PEGylation of nanoparticles and increase stability. The aqueous extract of *Musa paradisiaca* was added to above solution to initiate reduction of Silver ions. The reaction (1 ml aqueous extract + 99 ml 1mM AgNO<sub>3</sub> with 0.3% PEG) was carried out in light at room temperature. The reaction mixture was observed for colour changes from slight yellowish to red and finally into brown colour, which indicated the synthesis of silver nanoparticles. Further the AgNPs were filtered, centrifuged, washed with 100% ethanol and freeze dried.

### Characterization of AgNP's

This is an important aspect in nanoparticles research to achieve better interpretation of results with primitive understanding. The colour changes were recorded along with periodic sampling and scanning using UV-Vis spectrophotometry (Systronics type 108-double beam) ranging from 200 to 680 nm for a maximum time period upto 120 min.

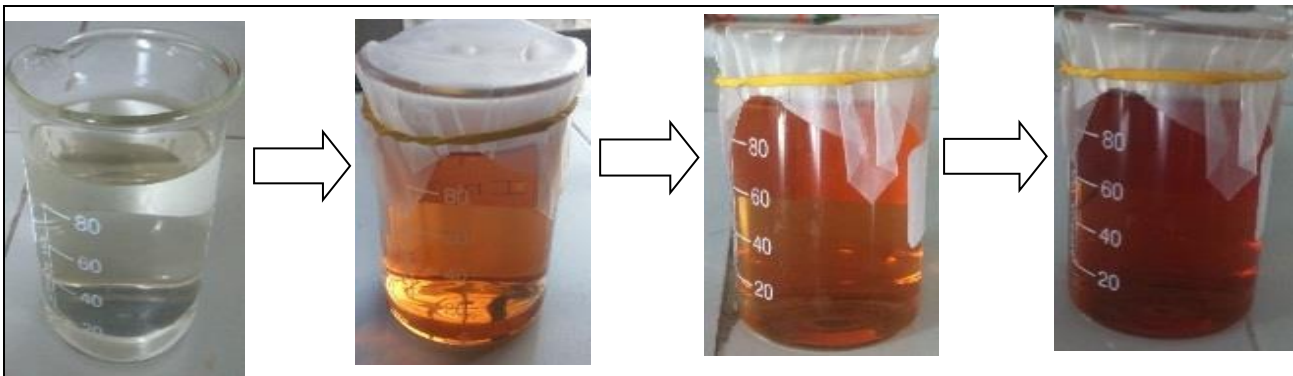


Fig 1

Further reaction mixture was centrifuged at 10000 rpm for 10 mins at 4°C, dissolved in distilled water and washed by centrifugation to remove plant impurities. Particle size analysis (Nanophox NX0088, Sympatec GmbH) and zeta potential measurement (Delsa Tm Nano, Beckman Coulter) were carried to analyse the size and stability of AgNPs. An FTIR spectrum (Bruker) of the AgNPs was measured in the transmittable mode at the range 4000 to 500 cm<sup>-1</sup> in KBr pellets. Further the FESEM analysis (Nova Nano SEM 450) i.e. field emission scanning electron microscopy was performed to confirm the size and morphology of the AgNPs.

### Antimicrobial assay

The antimicrobial activity of these synthesized nanoparticles were performed by using agar diffusion method at different parameter.

## Results and Discussion

### Biogenic synthesis and characterization of AgNPs

This study includes a biogenic method for synthesis of AgNPs using plant based polyphenols reduction. Stabilization of AgNPs synthesized by plant extract requires coating with polymer such as PEG (Poly ethylene glycol), PVP (Poly vinyl pyrrolidone). The presence of such polymers inhibits nucleation and retards the rate of particle growth. PEG (Polyethylene glycol) mediated surface coating was performed. In addition, of aqueous plant extract to silver nitrate, the reaction mixture turned transparent to light yellow, further into reddish brown. This was a clear indication for AgNPs synthesis using polyphenol-reduction method. In this study, it is suggested that PEG coating creates thiol group on the surface of AgNPs, increasing the stability.

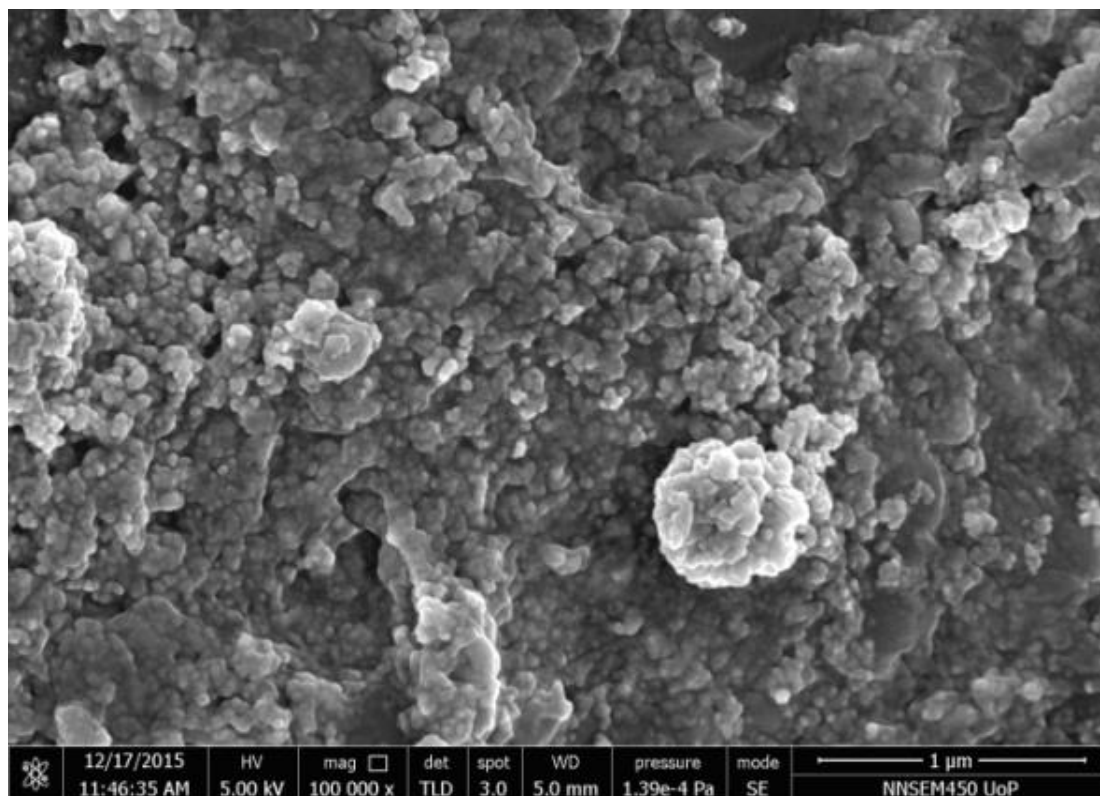


Fig 1: SEM images of Ag Nps

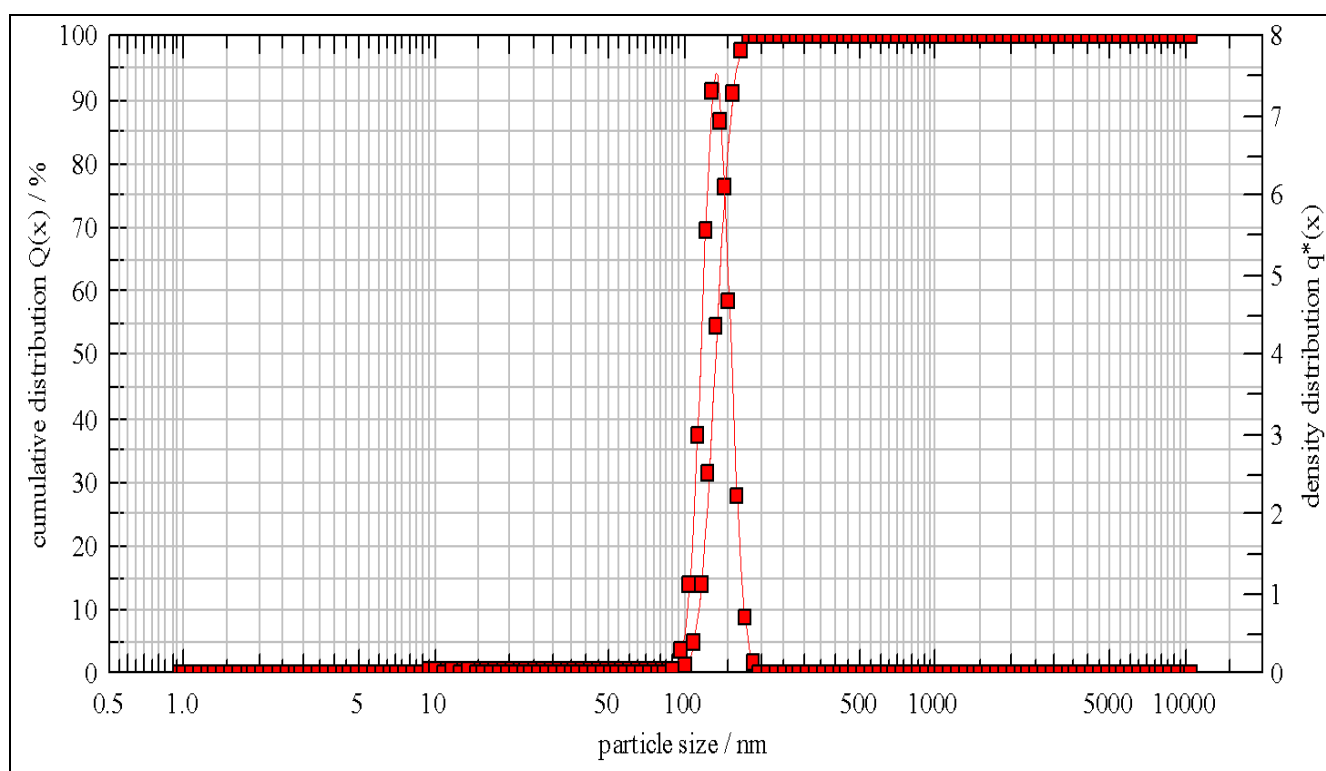


Fig 3: PSA for Ag Nps

UV-Vis spectroscopy plays an important role in the characterization of silver nanoparticles synthesis. A characteristic surface plasmon peak was observed at 418nm with a sharp peak for treating PEG-AgNPs. It is well understood that the color of Ag NPs influences the size of particles due to which a characteristic surface plasmon peak is observed in the solution. An FTIR spectrum confirms the presence of glycolated (PEG lyated) thiol groups due to

peak observed at 2865 and 3157  $\text{cm}^{-1}$ . This spectra explains the presence of free AgNP's coated with PEG i.e functional hydroxyl groups. 2865  $\text{cm}^{-1}$  peak represents CH stretching alkanes while 3157  $\text{cm}^{-1}$  represents Alcohol/Phenol -OH stretch which indicates presence of AgNPs coated with glycol group.

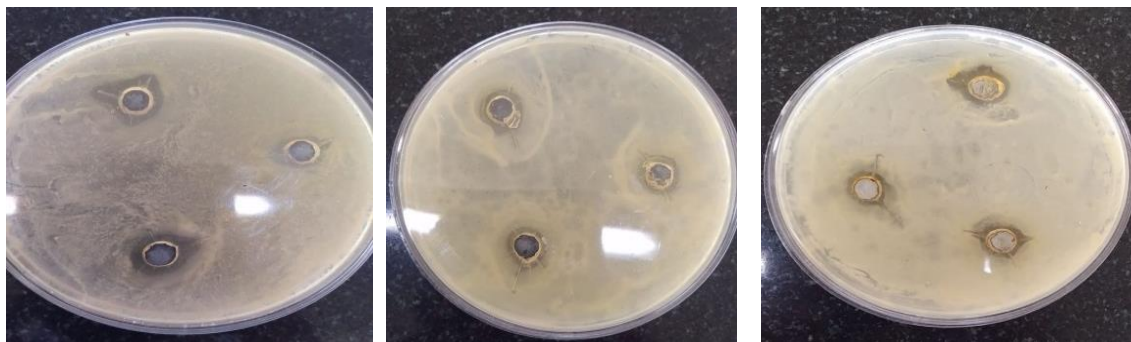
The diameter and zeta potential of PEG-AgNPs were detected using Nanophox and Beckman coulter Delsa Tm. It

was observed that uniform AgNPs were formed with average size of  $131\pm 18$  nm (Fig). The negative Zeta potential of AgNPs was observed to be at  $-15.8$  mv which confirms the higher stability of particles. Greater zeta potential indicates higher negative charge which creates stronger repulsive forces. This avoids agglomeration and increase the stability. The characterization of nanoparticles by FESEM revealed that PEG-AgNPs had a spherical shape observed with uniformity and monodispersity. This confirms that PEG-AgNPs had narrow particle size

distribution due to PEG coating as compared to other plant mediated synthesis.

#### Antimicrobial activity of Nanoparticles

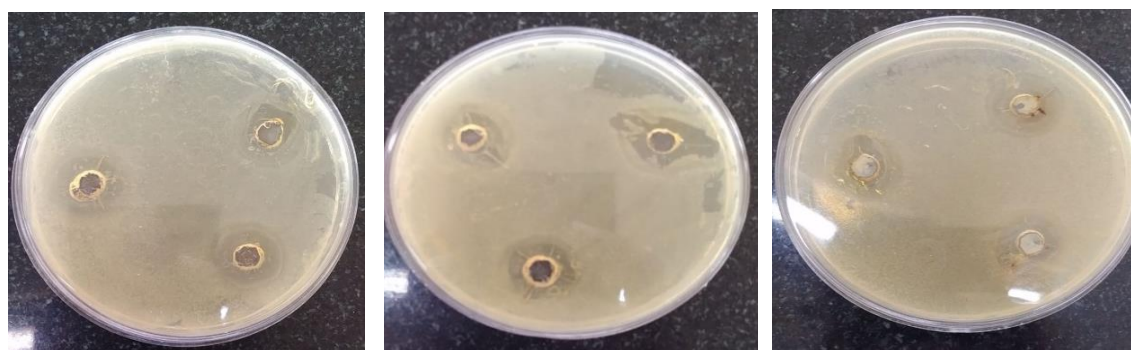
The green-synthesized nanoparticles shows the great antimicrobial activity by showing zone of inhibitions at different concentrations of nanoparticles. We can use the nanoparticles for the detection of food pathogens from the contaminated food at industry level especially in case of canned food.



*E. coli* Zone of Inhibition



*S. aureus* Zone of inhibition



Bacillus Zone of Inhibition

**Table 1**

Microorganism Name	Nanoparticle concentration	Zone of inhibition
E. Coli	400 (6ml Distilled water+4ml Np's solution)	10mm
E. Coli	500 (5ml Distilled water+5 ml Np's solution)	13mm
E. Coli	1000 (Pure solution of 1 $\mu$ l of nanoparticles+1ml of distilled water)	14mm
S. Aureus	400 (6ml Distilled water+4ml Np's solution)	10mm
S. Aureus	500 (5ml Distilled water+5 ml Np's solution)	11mm
S. Aureus	1000 (Pure solution of 1 $\mu$ l of nanoparticles+1ml of distilled water)	10mm
Bacillus	400 (6ml Distilled water+4ml Np's solution)	18mm
Bacillus	500 (5ml Distilled water+5 ml Np's solution)	15mm
Bacillus	1000 (Pure solution of 1 $\mu$ l of nanoparticles+1ml of distilled water)	19mm

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