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GREEN BIOSYNTHESIS OF SILVER NANOPARTICLES USING FLOWER OF *Musa paradisiaca* L. WITH REFERENCE TO ANTIMICROBAL AND ANTIOXIDANT POTENTIAL

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ABSTRACT

Biosynthesis of nanoparticles is under exploration is due to wide biomedical applications and research interest in nanotechnology. Bioreduction of silver nitrate (AgNO_3) for the synthesis of silver nanoparticles using *Musa paradisiaca* extract. The plant extract is mixed with AgNO_3 incubated and studied the synthesis of nanoparticles using UV-Vis spectroscopy. The nanoparticles are characterized by FTIR, XRD and SEM. The silver nanoparticles synthesized are found to be cubical in shape with size 35 nm. The results showed that the flower extract is very good bioreductant for the synthesis of silver nanoparticles and synthesized nanoparticles are active against clinically isolated human pathogens *Candida albicans*, *Staphylococcus aureus* and *Escherichia coli*. Antioxidant activity is also confirmed by DPPH radical scavenging activity.

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1. INTRODUCTION

Nanoparticles, generally considered as particles with a size of up to 100 nm, exhibit completely new or improved properties as compared to the larger particles of the bulk material that they are composed of based on specific characteristics such as size, distribution, and morphology [1]. Nanoparticles of noble metals, such as gold, silver, and platinum, are widely applied in products that directly come in contact with the human body, such as shampoos, soaps, detergent, shoes, cosmetic products, and toothpaste, besides medical and pharmaceutical applications. Therefore, there is a growing need to develop environmentally friendly processes for nanoparticle synthesis without using toxic chemicals. Biological methods for nanoparticle

synthesis using microorganisms, enzymes, and plants or plant extracts have been suggested as possible ecofriendly alternatives to chemical and physical methods [2]. There have been recent reports on phytosynthesis of silver nanoparticles by employing coriander leaves [3], sundried *Cinnamomum camphora* leaves [4], *phyllanthin* extract [5], and purified apiin compound extracted from henna leaves [6]. Keeping in view, in the present study to explore the novel approaches for the biosynthesis of silver nanoparticles using *Musa paradisiaca*.

Musa paradisiaca Linn (Musaceae) is prominently used in the form of Hemanta Rasa in traditional system of medicine. The term banana is Spanish-Portuguese from Guinea. Plantain refers in

India to a coarse banana. Though the two terms are regarded as almost synonymous banana refers botanically to *Musa paradisiaca*, the most familiar of tropical fruits. From its origin in India/Malaysia it spread to the tropical world. It has been cultivated for *Musa Paradisiaca* more than 4000 years, the original varieties have increased to 300 [7, 8].

2. MATERIAL AND METHODS

Chemicals

Materials used for the synthesis of silver nanoparticles are AR grade silver nitrate (AgNO_3), purchased from Merck, India.

Collection of plant materials

The fully mature flower of *Musa paradisiaca* are collected in April 2016 from Lalgudi, Trichy District, Tamil Nadu, India. The flowers are identified and authenticated by Botanist, Dr. S. John Britto, Department of Botany, St. Joseph's College, Tiruchirappalli, Tamil nadu, India. A Voucher specimen has been deposited at the Rapinat Herbarium, St. Joseph,s College, Tiruchirappalli, Tamil nadu, India.

Preparation of extract

The flowers of *Musa paradisiaca* is rinsed with water thrice to remove the fine dust materials. The flowers are air dried for 15 days and then they are kept in air hot oven at 60°C for 36 hrs. The flowers are ground to a fine powder. 50g of powdered material of sample is packed in soxhlet thimble and it is extracted using water as a solvent. The extract is filtered with the help of filter paper and solvent is evaporated from extract in rotary evaporator to get the syrupy consistency. Then, the extract is kept in refrigerator at 4°C for future experiments.

Synthesis of Silver Nanoparticle

The 5ml of aqueous filtrate extract of *Musa paradisiaca* is taken into 250ml Erlenmeyer flask. Extract is mixed with silver nitrate (AgNO_3) to make final volume concentration of 1mM solution for *Musa paradisiaca*. The reaction mixture is kept in dark room condition until the onset of colour change is observed. The colour changes in the reaction solution is watched carefully for the characterization of silver nanoparticles [9]. Then the solution is centrifuged at 18,000 rpm for 30 min at room temperature to precipitate the nanoparticles. The resulting pellet is dissolved in deionized water and filtered through whatman filter paper No: 42.

Characterisation of Silver Nanoparticles

Silver nanoparticles are characterized by UV-Vis schimadzu 1600 spectrophotometer. The bioreduction is monitored in the UV absorption spectrometer from 300 to 700 nm range. An aliquot of the filtrate containing silver nanoparticles are used

for Fourier transmission Infrared spectroscopy (FTIR).

SEM analysis of silver nanoparticles

Scanning electron microscopic (SEM) analysis is done using VEGA3 LMU machine. Thin films of the sample is prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid. Extra solution is removed using a blotting paper and then the films on the SEM grid is allowed to dry by putting it under a mercury lamp for 5 min.

Microorganisms

Escherichia coli, *Staphylococcus aureus* and *Candida albicans* are the microorganisms used and they are obtained from the Rontgen Diagnostic Laboratory, Thanjavur. These microorganisms are identified and confirmed by Microbiologists, Rontgen Diagnostic Laboratory, Thanjavur.

Preparation of dried filter paper discs

Whatman filter paper (No:1) is used to prepare four discs approximately 6 mm in diameter, which are placed in hot air for sterilization. After sterilization, discs are loaded with $30\mu\text{l}$ of plant extract, AgNO_3 solutions. AgNPs and Standard solution as Chloromphenical respectively and again kept under refrigeration for 24 hrs.

Application of discs to inoculated agar plates

Previously prepared paper discs are dispensed onto the surface of the inoculated agar plate. Each disc is pressed down firmly to ensure complete contact with the agar surface. The discs are placed on the medium suitably apart and the plates are incubated at 5°C for 1 hr to permit good diffusion and then transferred to incubator at 37°C for 24 hrs. After completion of 24hrs, the plates are inverted and placed in an incubator set to respective temperature for 24 hrs.

Antimicrobial assay

Antibiogram is done by disc diffusion method [10, 11] using plant extracts. Petri plates are prepared by pouring 30 ml of NA medium for bacteria/fungi. The test organism is inoculated on solidified agar plate with the help of micropipette and spread and allowed to dry for 10 min. The surfaces of the media are inoculated with bacteria/fungi from a broth culture. A sterile cotton swab is dipped into a standardized bacterial/ fungi test suspension and used to evenly inoculate the entire surface of the Nutrient agar/PDA plates. Briefly, inoculums containing *Escherichia coli*, *S. aureus* and *Candida albicans* on Nutrient agar plates for bacteria and potato agar for fungi. Using sterile forceps, the sterile filter papers (6 mm diameter) containing each $30\mu\text{l}$ of plant extract, AgNO_3 solutions. AgNPs and Standard solution as Chloromphenical are laid down on the surface of

inoculated agar plates. The plates are incubated at 37°C for 24 h for the bacteria and at room temperature (30±1) for 24-48 hr. for fungi strains. Each sample is tested in triplicate.

Measurement of zone of inhibition

The antimicrobial potential of test compound is determined on the basis of mean diameter of zone of inhibition around the disc in millimeters. The zones of inhibition of the tested microorganisms by the extracts are measured using a millimeter scale. The diameter sizes in mm of the zone of inhibition are shown in the table 1.

DPPH radical-scavenging activity

DPPH radical-scavenging activity is determined by the method of Shimada, *et al.*, [12]. Briefly, a 2ml aliquot of DPPH methanol solution (25µg/ml) is added to 0.5 ml sample solution at different concentrations. The mixture is shaken vigorously and allowed to stand at room temperature in the dark for 30min. Then the absorbance is measured at 517nm in a spectrophotometer. Lower absorbance of the reaction mixture indicated higher free-radical scavenging activity.

$$\text{Radical scavenging activity (\%)} \\ = 100 - \left[\frac{A_C - A_S}{A_C} \right] \times 100$$

Where A_C = control is the absorbance and A_S = sample is the absorbance of reaction mixture (in the presence of sample).

3. RESULTS AND DISCUSSION

Synthesis of silver nanoparticles

The green synthesis of silver nanoparticles through plant extract is carried out. Silver nitrate is reduced as silver has distinctive properties such as good conductivity, catalytic and chemical stability. 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 materials (nanomaterials). The aqueous silver ions when exposed to herbal extracts are reduced in solution, there by leading to the formation of silver hydrosol. The time duration of change in colour varies from plant to plant. The phytochemicals present in the flower extract are considered responsible for the reduction of silver ions. It is well known that silver nanoparticles exhibit yellowish - brown colour in aqueous solution due to excitation of surface plasmon vibrations in silver nanoparticles. The appearances of yellowish-brown colour in the reaction vessels suggest the formation of silver nanoparticles (SNPs) [13].

Silver nanoparticles are being extensively synthesized using many different biological sources including fungi, bacteria and plants [14, 15]. Among them the plant mediated nanoparticle synthesis is getting more popular because of the high reactivity of plant extract and easy availability of plant materials. This method of nanoparticles synthesis involves no toxic chemicals and termed as green chemistry procedure. In this present study, *Musa paradisiaca* extract is used for the synthesis of silver nanoparticles. The aqueous AgNO_3 solution turned to brown colour in 30 min with the addition of flower extract (Figure 1 shows - AgNO_3 and AgNPs), indicating the formation of AgNPs in the reaction solution probably as a result of the excitation of surface plasmon resonance (SPR) bands [16]. The control tubes (AgNO_3) showed no change in colour when incubated in a similar condition.

SEM analysis is carried out to understand the topology and the size of the Ag-NPs, which showed the synthesis of higher density polydispersed cubical Ag-NPs of various sizes. The SEM image showing the high density silver nanoparticles synthesized by the flower extract further confirmed the development of silver nanostructures. Most of the nanoparticles aggregated and only a few of them are scattered, as observed under SEM. The SEM analysis showed the particle size around 35nm as well the cubic, face-centred cubic structure of the nanoparticles (Figure 2 and 3).

EDX analysis gives qualitative as well as quantitative status of elements that may be involved in formation of nanoparticles. The elemental profile of synthesized nanoparticles using *Musa paradisiaca* flower extract shows higher counts at 3 keV due to silver, confirms the formation of silver nanoparticles. Generally metallic silver nanocrystals show typical optical absorption peak approximately at 3 keV due to their surface Plasmon resonance (Figure 4).

The crystalline nature of silver nanoparticles is confirmed by the analysis of XRD pattern as shown. The four distinct diffraction peaks at 2θ values of 38.15°, 44.30°, 64.53° and 76.96° can be indexed to the (1 1 1), (2 0 0), (2 2 0) and (3 1 1) reflection planes of face centred cubic structure of silver. In addition to the Bragg peaks representative of silver nano crystals, additional peaks are also observed at 27.89°, 32.24°, 46.26°, and 54.79°. (Figure 5) These peaks are due to the organic compounds which are present the extract and responsible for silver ions reduction and stabilization of resultant nanoparticles.

It is generally recognized that UV-Vis spectroscopy could be used to examine size- and shape-controlled nanoparticles in aqueous

suspensions. Figure 6 shows the UV-Vis spectra recorded from the reaction medium after 5 hours. Absorption spectra of silver nanoparticles formed in the reaction media has absorbance peak at 413 nm, broadening of peak indicated that the particles are polydispersed.

FTIR is an important tool which enables us to understand the involvement of functional groups in the interactions between metal particles and biomolecules. In the present work, FTIR spectra are used in the identification of biomolecules responsible for capping and stabilizing the silver nanoparticles. FTIR spectrum of *Musa paradisiaca* extract shows bands at 540,746, 919, 1040, 1428, 1633 and 3540 cm^{-1} . The FTIR spectra of the *Musa paradisiaca* is given in the Figure 7. which show the presence of silver nanoparticles, peak at 3540 cm^{-1} which are assigned as -OH stretching in alcohols and phenolic compounds^[17]. The band appeared at about 1040 cm^{-1} can be assigned for aromatic rings. The strong broad band appearing at 3540 cm^{-1} can be associated to the stretching vibrations of alcoholic and phenolic O-H. At 1633 cm^{-1} a peak is observed that could be for plant ascribed to multiplet C=O group.

Antimicrobial activity

Silver has been known to have a disinfecting effect and has been found in applications ranging from traditional medicines to culinary items. Moreover, several salts of silver and their derivatives are commercially manufactured as antimicrobial agents^[18]. In small concentrations, silver is safe for human cells, but lethal for bacteria and viruses^[19]. Reduction of the particle size of the materials is an efficient and reliable tool for improving their biocompatibility that can be achieved using nanotechnology.

Toxicity studies on pathogen opens a door for nanotechnology applications in medicine. Biological synthesis of metal NPs is a traditional method and the use of plant extract has a new awareness for the control of disease, besides being safe and no phytotoxic effects^[20]. The biologically synthesized silver nanoparticles using medicinal plants are found to be highly toxic against different pathogenic bacteria of selected species. The SNPs of *Musa paradisiaca* shows highest antimicrobial activity is observed against *E. coli*, *S. aureus* and *C. albicans*. The inhibitory activities in culture media of the Ag nanoparticles reported in Table 1 are comparable with standard antimicrobial viz. chloramphenicol.

In this study, to evaluate the antimicrobial effects Ag nanoparticles against three microorganisms, *S. aureus*, *E. coli* and *C. albicans*.

There are distinct differences among them. When Ag nanoparticles are tested they effectively inhibited bacterial growth. In this results, Ag nanoparticles showed antimicrobial activity against *E. coli* (Figure 8) that is similar to that found by^[21]. In contrast, the inhibitory effect of Ag nanoparticles is mild in and *S. aureus* (Figures 9 and 10) as compared with other microorganisms; these results suggest that the antimicrobial effects of Ag nanoparticles may be associated with characteristics of certain bacterial species. The growth of microorganisms is inhibited by the green synthesized SNPs showed variation in the inhibition of growth of microorganisms may be due to the presence of peptidoglycan, which is a complex structure and after contains teichoic acids or lipoteichoic acids which have a strong negative charge. This charge may contribute to the sequestration of free silver ions. Thus gram positive bacteria may allow less silver to reach the cytoplasmic membrane than the gram negative bacteria^[22]. The result shows that the lower efficacy of the Ag nanoparticles against *S. aureus* may derive from the difference as a point of membrane structure. To confirm this hypothesis, further comparative study between various gram-negative and gram-positive bacterial species are needed. The peptidoglycan layer is a specific membrane feature of bacterial species and not mammalian cells. Therefore, if the antibacterial effect of Ag nanoparticles is associated with the peptidoglycan layer, it will be easier and more specific to use Ag nanoparticles as an antibacterial agent. The SNPs synthesized from plant species are toxic to multi-drug resistant microorganisms. It shows that they have great potential in biomedical applications.

DPPH Radical scavenging activity

The identification of antioxidant is beneficial to biological system against ROS ravage. Recently importance has been given for in vitro antioxidant study to understand the pharmacological role of medicinal plant and its isolate. *In vitro* techniques have been used for detection of antioxidants, which are based on the ability of compounds to scavenge peroxy radicals^[23, 24].

The DPPH antioxidant assay is based on the ability of DPPH a stable free radical, to decolorize in the presence of antioxidants. The DPPH radical contains an odd electron, which is responsible for the absorbance at 517 nm and also for visible deep purple colour. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized which can be quantitatively measured from the changes in absorbance.

Fig 1 The colour change due to formation of silver nanoparticles by the addition of *Musa paradisiaca* flower extract to silver nitrate

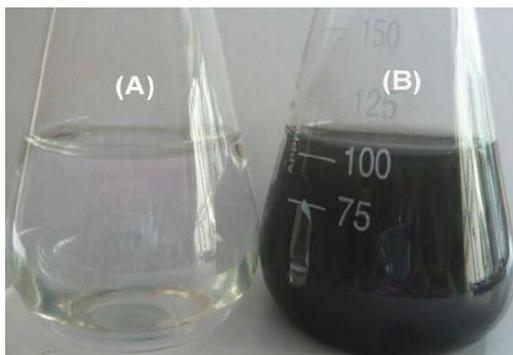


Figure 2 High resolution scanning electron microscopic (SEM) image of silver nanoparticles (AgNPs). Polydispersed (Cluster) AgNPs around 35nm.

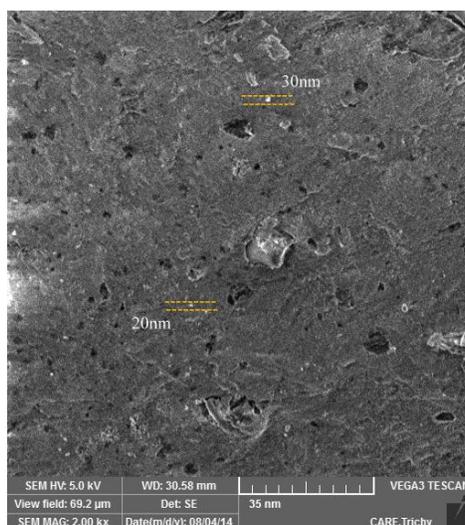


Figure 3 Capturing a high resolution scanning electron microscopic image of Ag nanoparticles

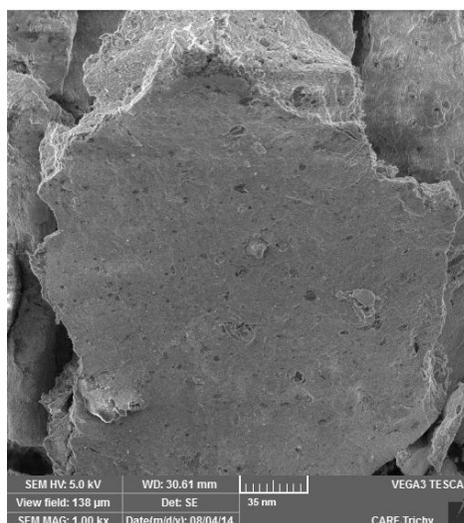


Figure 4 EDX analysis of silver nanoparticles synthesized with *Musa paradisiaca* flower extract

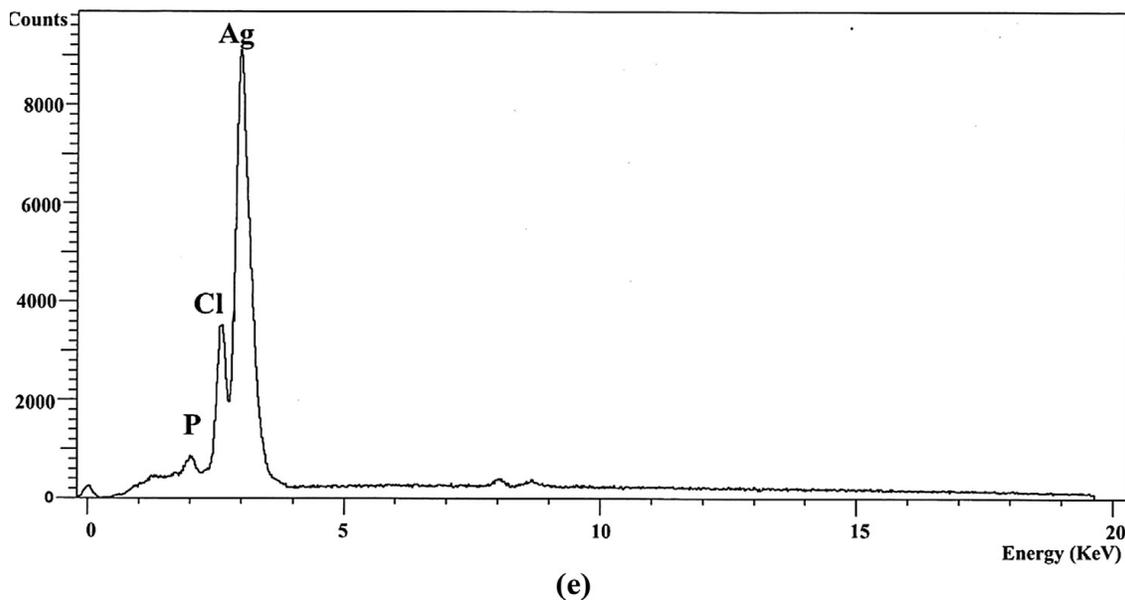


Figure 5 XRD pattern of silver nanoparticles of *Musa paradisiaca* flower extract

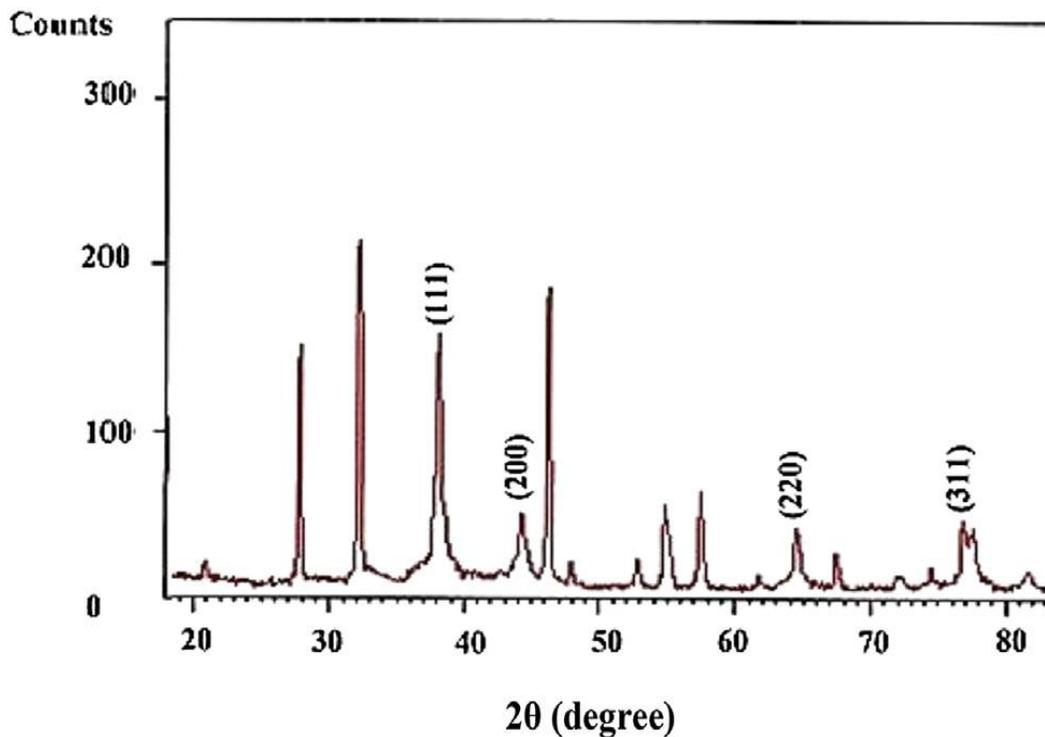


Figure 6 UV-Vis absorption spectrum of silver nanoparticles synthesized by treating 1mM aqueous AgNO_3 solution with *Musa paradisiaca* flower extract.

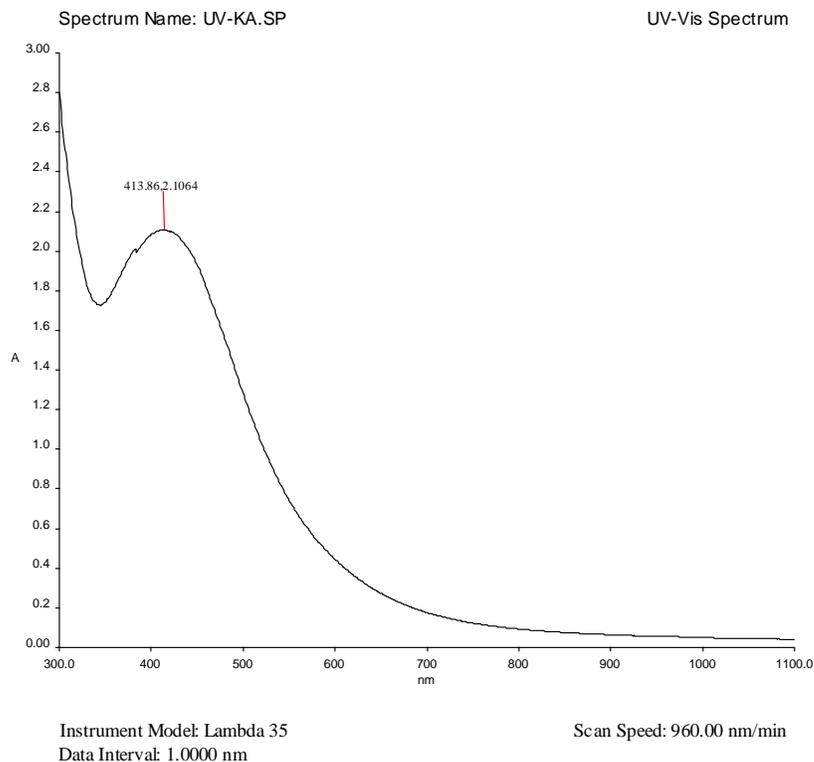


Figure 7 FTIR analysis of silver nanoparticles synthesized by treating 1mM aqueous AgNO₃ solution with *Musa paradisiaca* extract.

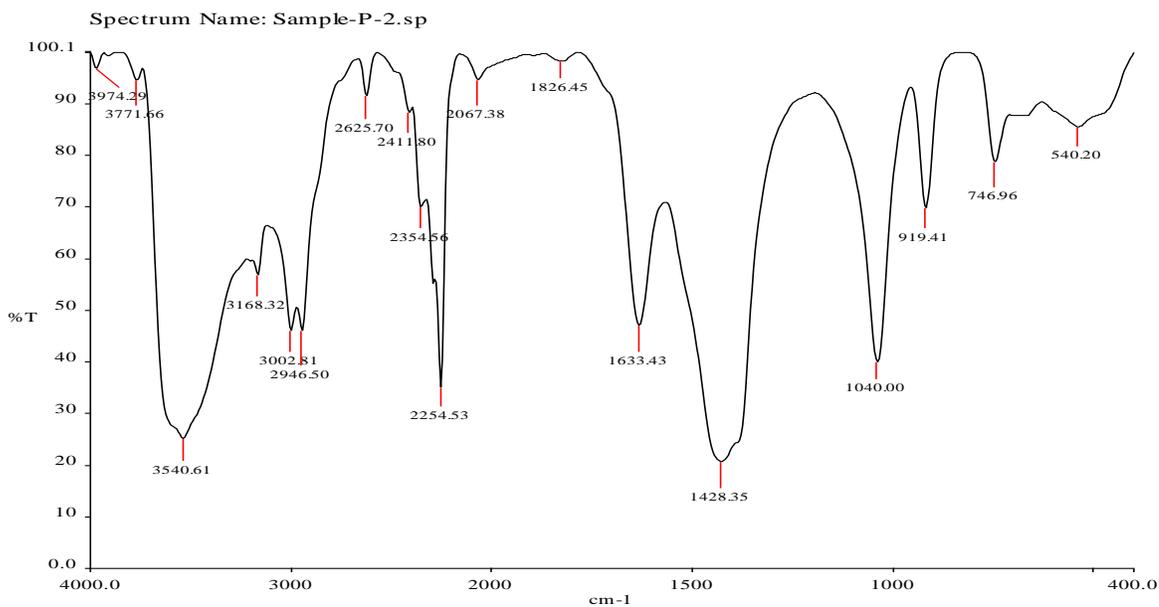


Table 1 Antimicrobial activity of AgNPs, AgNO₃ and *Musa paradisiaca* flower extract

Sample	Concentrations	<i>Escherichia Coli</i> (mm) (30µl)	<i>Staphylococcus aureus</i> (mm) (30µl)	<i>Candida albicans</i> (mm (30µl))
AgNO ₃	30µl	2±0.14	1±.07	4±0.28
Plant extract	30µl	4±0.25	1±.07	1±0.07
AgNPs	30µl	6±0.42	4±0.28	6±0.42
Standard (Chloromphenical)	30µl	8±0.56	12±0.84	---
Standard (Flucanazole)	30µl	---	---	5±0.35

Values are expressed as Mean ± SD.

AgNO₃ = Silver Nitrate; AgNPs = Silver Nanoparticles

Figure 8 shows the antimicrobial (*E. Coli*) activity of AgNPs and *Musa paradisiaca*

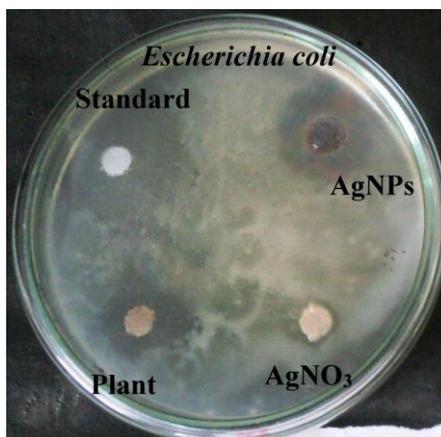


Figure 9 shows the antimicrobial (*S. aureus*) activity of AgNPs and *Musa paradisiaca*

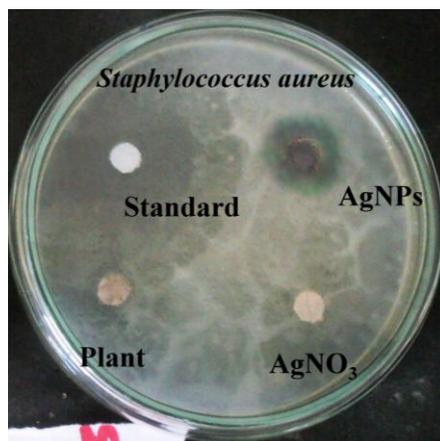


Figure 10 shows the antimicrobial (*Candida albicans*) activity of AgNPs and *Musa paradisiaca*

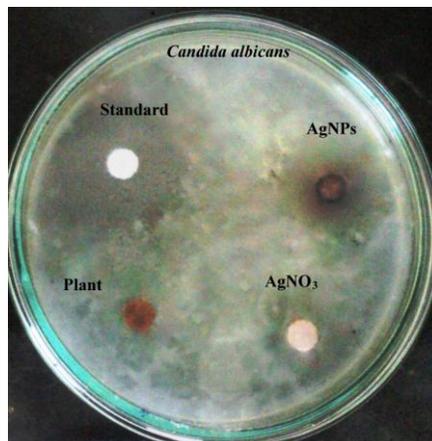
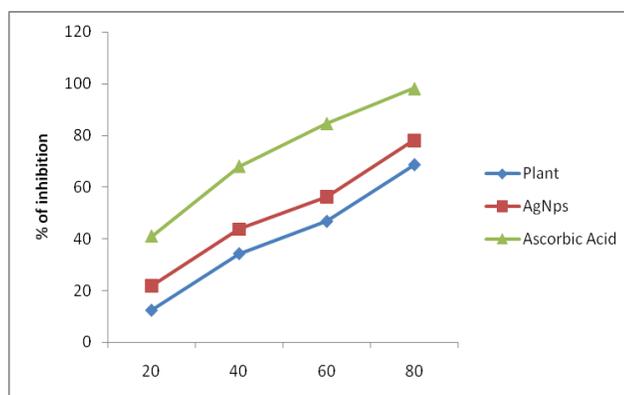


Figure 11 DPPH radical scavenging activity of *Musa paradisiaca* and standard (Ascorbic acid)



The antioxidant activity of *Musa paradisiaca* and AgNPs are shown in Figure 11. The *Musa paradisiaca* and AgNPs exhibited a significant dose dependent inhibition of DPPH activity. AgNPs possess probable antioxidant activity as compared with plant extract.

DPPH free-radical scavenging activity Free radicals are harmful by-products generated during normal cellular metabolism, which could initiate oxidative damage to body [25]. Antioxidants are believed to play a significant role in the body's defense system against free radicals. Recently, numerous reports have described antioxidants and compounds with radical-scavenging activity present in fruits, vegetables, herbs and cereals extracts [26]. The DPPH radical is widely used to evaluate the free-radical scavenging capacity of antioxidants [27].

The present study concluded that the bio-reduction of silver ions through *Musa paradisiaca*

extract and testing for their antimicrobial activity. The aqueous silver ions exposed to the extract, the synthesis of silver nanoparticles are confirmed by the change of colour of plant extract. These environmentally benign silver nanoparticles are further confirmed by using SEM and XRD. The SEM and XRD analysis showed the particle size around 35nm as well the cubic structure of the nanoparticles. The results indicated that silver nanoparticles have good antimicrobial and activity against different microorganisms such as *S. aureus*, *E. coli* and *C. albicans*. It is confirmed that silver nanoparticles are capable of rendering high antibacterial efficacy and hence has a great potential in the preparation of drugs used against bacterial diseases. The Antioxidant activity is also confirmed by DPPH radical scavenging activity. Applications of Ag nanoparticles based on these findings may lead to valuable discoveries in various fields such as medical devices and antimicrobial systems.

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