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Study of antimicrobial activity in silver nanoparticles from *Musa paradisiaca*

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Abstract
The biosynthesis of nanoparticle is widely spreading around researchers. The leaf and flower extract of *Musa paradisiaca* when subjected to silver nitrate solution shows the colour change from pale yellow to dark brown at room temperature. The siver nanoparticles were identified by UV-Visible spectrophotometer, Fourier transform infrared spectrometer (FTIR), Atomic force microscopy (AFM), and X-Ray diffraction (XRD) was performed to calculate the leaf and flower extract of *Musa paradisiaca*. The silver nanoparticles received showed respectively higher antimicrobial activity against *bacillus subtilis, Escherichia coli and bacillus cereus* in leaf extract of *Musa paradisiaca*. Hence, the silver nanoparticles is made widely antibiotic and medical science.

Keywords: *Musa paradisiaca*, silver nanoparticle synthesis, UV, FTIR, AFM, Antimicrobial activity

Introduction
Musa paradisiaca is a perennial plant widely spread in the tropical region. The herbal extract and it's effective ingredient have been used for the treatment of huge number of human disorder [1]. According to World Health Organisation (WHO) [2] about 80% of the globe natives rely on customery therapeutics for the health maintenance. Most of the remedy require the use of herb extracts and it's active constituents [3].
The nanomaterial synthesis showing highlight of the intersection of nanotechnology and biotechnology has experienced increasing attention due to growing environment benign technologies in biosynthesis of nanoparticles. The appearance and dimension of the nanoparticle synthesized using plants can be managed and modulated by salt solutions of metal ion [4]. Silver nanoparticles widely employed in shampoos, cosmetics and medical products [5, 6].
The antimicrobial constituents isolated from higher plants seem to be one of the important alternative approaches to antibiotic resistance and in the treatment of ailment. The interest firstly that green medicine is safe and trustworthy, compared with costly synthetic drug that have adverse effects. The plant *Musa paradisiaca* comes under the family *Musaceae*, it has antiulcerative, antimicrobial and antioxidant trait [7].
This is one of the most valued traditional Indian medicines. Therefore the present study was carried out with the aim of effects on frequently encountered microorganism such as *Bacillus subtilis, E. coli and Bacillus cereus* [8].

Materials and Methods
Materials
Silver nitrate, Double distilled water, *Musa paradisiaca* plant leaves and flower, Muller Hinton Agar, Nutrient agar, sterile cotton swabs, Sterile Disc dispenser, sterile discs, Sterile loop.

Methods
Silver nanoparticle generation
Silver nitrate solution
Dissolve 0.017g of silver nitrate in 100ml of sterile distilled water.

Boiling/ Collection of the Extract
The herbal plants were collected naturally from various places in south India. The plant was authentified by the herbarium of botany directorate in National Institution of the Herbal science, by plant anatomy, Chennai. A voucher specimen no, PARC-2019/4150. The product of plant stored in the centre. Firstly the parts leaves, flowers of *Musa paradisiaca* were washed and cleaned, were dried with water absorbent paper.

Then it was cut into small pieces (do not grind), dispensed in 100 ml of sterile distilled water and boiled for 1 hour at 80 ºC, the extracts were collected in separate conical flask by standard filtration method. The prepared extract was stored in airtight container for further uses.

Synthesis of silver nanoparticles

10^{-3}M silver nitrate solution was prepared and stored in brown bottles. 5 ml of leaves extract was added to the 95 ml of silver nitrate solution in BOD bottles. The color change from pale yellow to dark brown was checked periodically. Then the BOD bottles were incubated at normal room temperature for further incubation till 28 hours. The color change to brown indicated that the silver nanoparticles were synthesized from the leaves and flowers of *Musa paradisiaca* and then it was centrifuged at 10,000 rpm for 25 minutes where pellets used for antibacterial activities.

UV-Visible Spectra Analysis

UV-Visible spectra analysis was carried out on a Systronic UV-Visible absorption spectrophotometer 117 with a resolution of +1 nm between 200-1000 nm processing a scanning speed of 200nm/min. The progress of the reaction between metal ions and the leaves extracts were monitored by UV-Visible spectra of silver nanoparticles in aqueous solution after diluting a small aliquot of 100µl of the sample with 1 ml deionized water in different wavelengths i.e. 340, 380, 420, 460, 500, 540, 580 and 620nm. The leaves and flower extracts of *musa paradisiaca* synthesize silver nanoparticles.

Ftir analysis of silver nanoparticles

For FTIR measurements, the synthesized silver nanoparticles solution was centrifuged at 10000 rpm for 30 minutes. The pellet was cleaned thrice with 5 ml of deionized water to get rid of the free proteins or enzymes that are not capping the silver nanoparticles. The pellet was dried by using vacuum drier. This was then inspected by FTIR for silver nanoparticle synthesis from leaves and flower of *Musa paradisiaca*.

Xrd analysis of silver nanoparticles

A thin film of the silver nanoparticles was made by dipping a glass plate in the solution and carried out the X-ray studies. The diffraction pattern was recorded by Co–K_1 radiation with a wavelength of 1.78 Å. The scanning was done in the region of 20_ to 90_ for 2_ at 0.02_/min and the time constant was 2 s. The extract obtained from leaves and flowers of *musa paradisiaca* were then analysed through X-Ray Diffraction.

Afm analysis of silver nanoparticles: The synthesized silver nanoparticles using leaves and flowers of *Musa paradisiaca* extracts were detected by AFM i.e Advanced Surface Microscopy.

Antibacterial activity

Muller-hinton agar

Dissolve 19g of MHA in 500ml of sterile distilled water.

Culture media used

Muller-Hinton Agar and Nutrient broth/agar were prepared as described in the media constituents used were purchased from Hi- media chemicals.

Test Microbes

The synthesized silver nanoparticle solutions were examined against pathogenic bacteria by utilizing the agar well diffusion method. The microorganism includes *Bacillus subtilis, Escherichia coli, Bacillus cereus, Proteus mirabilis*.

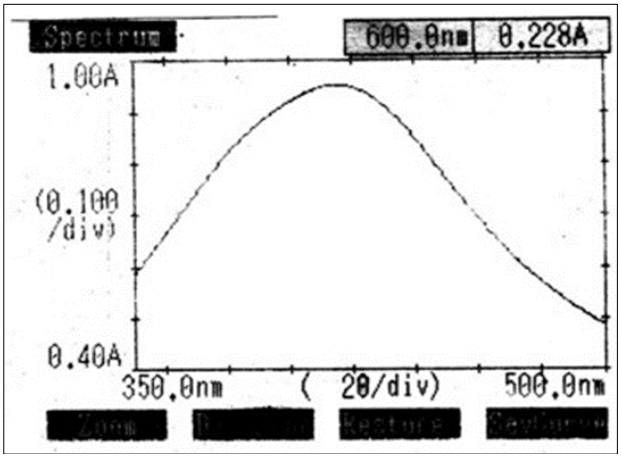
Disc diffusion method

The antibacterial activities were done on above discussed bacterial organisms by using disc diffusion method. Nutrient broth medium was used to cultivate bacteria. Fresh over night culture of inoculums (100 µl) of each culture was spread on to Muller Hinton Agar (MHA) plates. Sterile paper disc of 5 mm diameter containing 10 mg/liter of silver nanoparticles containing discs were placed in each plate at different concentrations (0.2, 0.4, 0.6, 0.8, 1.0 ml of extract which is make up to 1 ml with distilled water). The plates were incubated at 37 ºC for overnight. Next day, the inhibition zones around the discs were observed.

Result and Discussion

UV-visible spectra analysis of silver nanoparticles

The synthesized silver nanoparticles using leaf and flower of *Musa paradisiaca* extracts were detected by UV-Visible spectrophotometer at various nm i.e. 340, 380, 420, 460, 500, 540, 580 and 620 nm. The particle has increasingly sharp absorption maximum peak at 415- 420 nm (Figure 1 showing UV analysis of *musa paradisiaca* leaf extract & Figure 2 showing UV analysis of *musa paradisiaca* flower extract). The peak detected at 420nm of silver nanoparticles in *musa paradisiaca* leaf extract and its absorbance is about 0.583A. Then at about 416nm peak is observed by flower extract of silver nanoparticles from *musa paradisiaca* with absorbance of 0.955A. Similarly, earlier researchers have also observed the spectral absorption between 420-460 nm (9, 10). The maximum peak is obtained by leaf extract containing silver nanoparticles from *musa paradisiaca*.



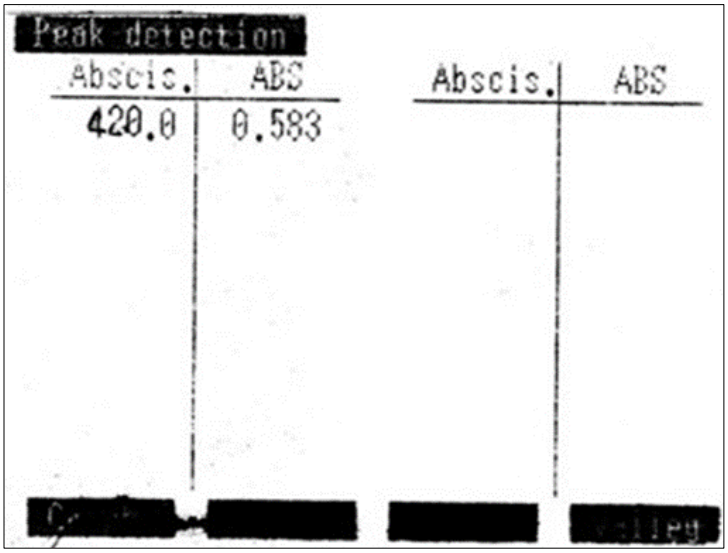


Fig 1: UV Spectra analysis of Silver Nanoparticles in *Musa paradisiaca* Leaf extract

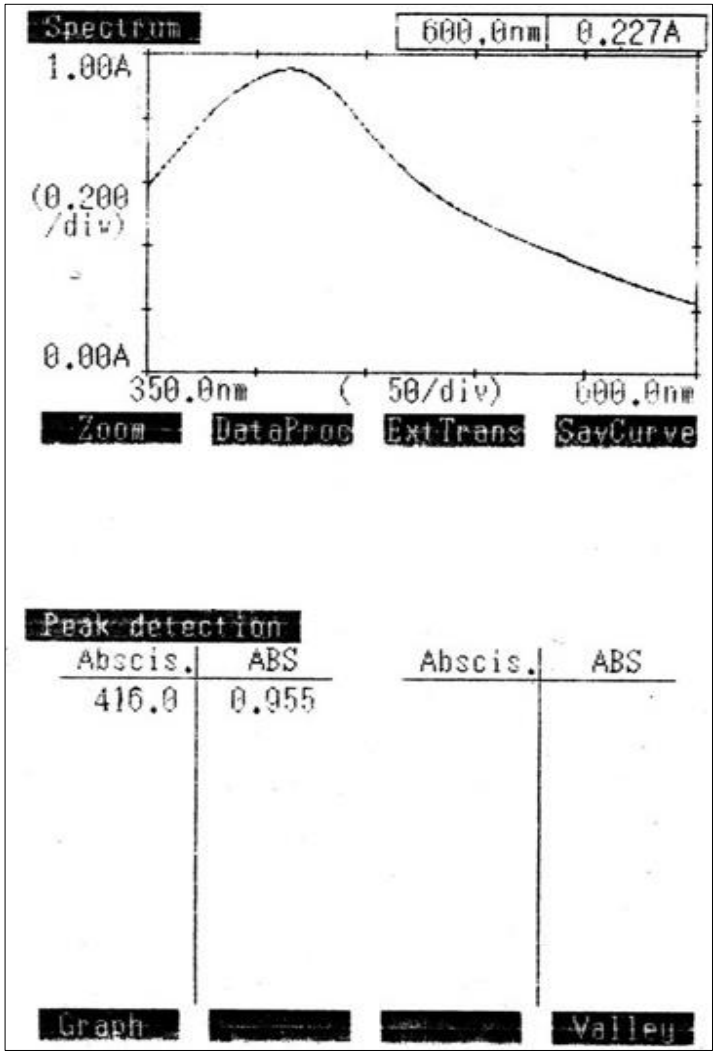


Fig 2: UV Spectra analysis of Silver Nanoparticles in *Musa paradisiaca* Flower extract

Ftir analysis of silver nanoparticles

The synthesized silver nanoparticles using leaf and flower of *Musa paradisiacal* extracts were detected by FTIR spectrophotometer. The FTIR analysis of silver nanoparticles. (Figure 3 showing FTIR analysis of silver nanoparticles in *musa paradisiaca* leaf extract & Figure 4 showing FTIR analysis of silver nanoparticles in *musa paradisiaca* flower extract). The prominent bands of absorbance were absorbed at around 994.52, 1038.83, 1089.96, 1406.28, 1642.53, 3459.49 cm $^{-1}$ in the leaf extract containing silver nanoparticles from *musa paradisiaca.* The absorbance band of silver extract in silver nanoparticles from *musa paradisiaca* is around 992.50, 1037.81, 1087.94, 1404.27, 1641.51, 3458.46 cm^{-1}.

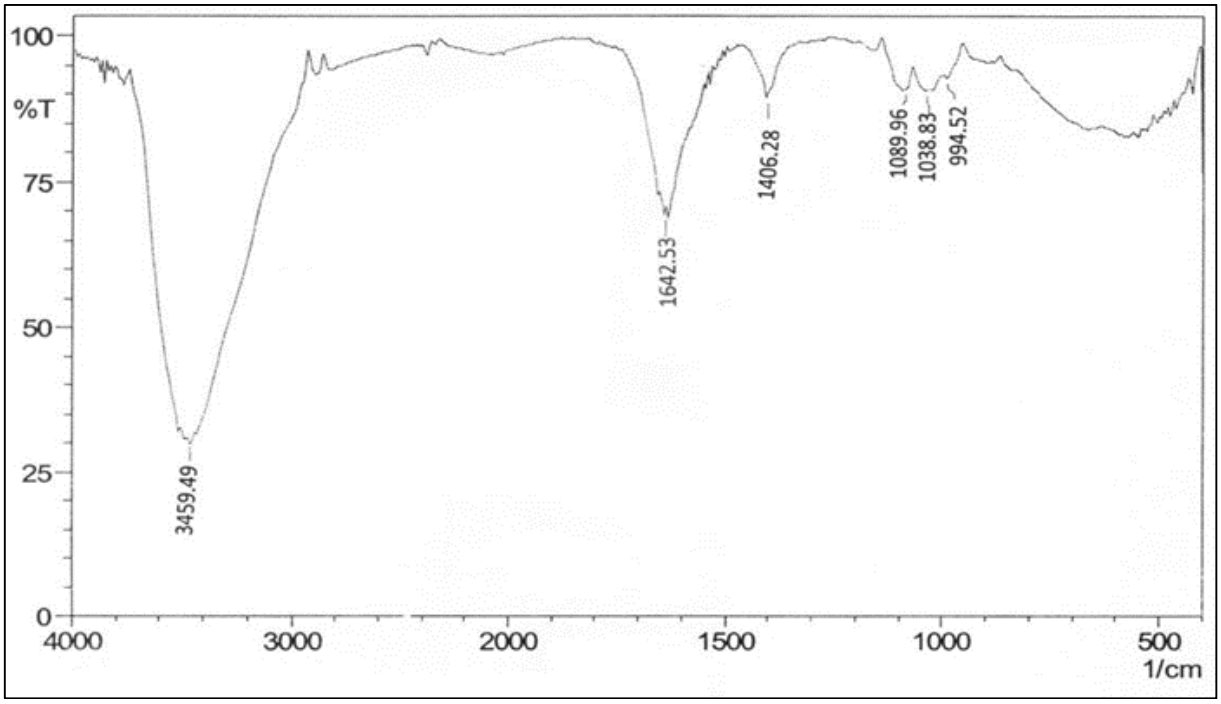


Fig 3: FTIR analysis of Silver nanoparticles in *Musa paradisiaca* Leaf extract

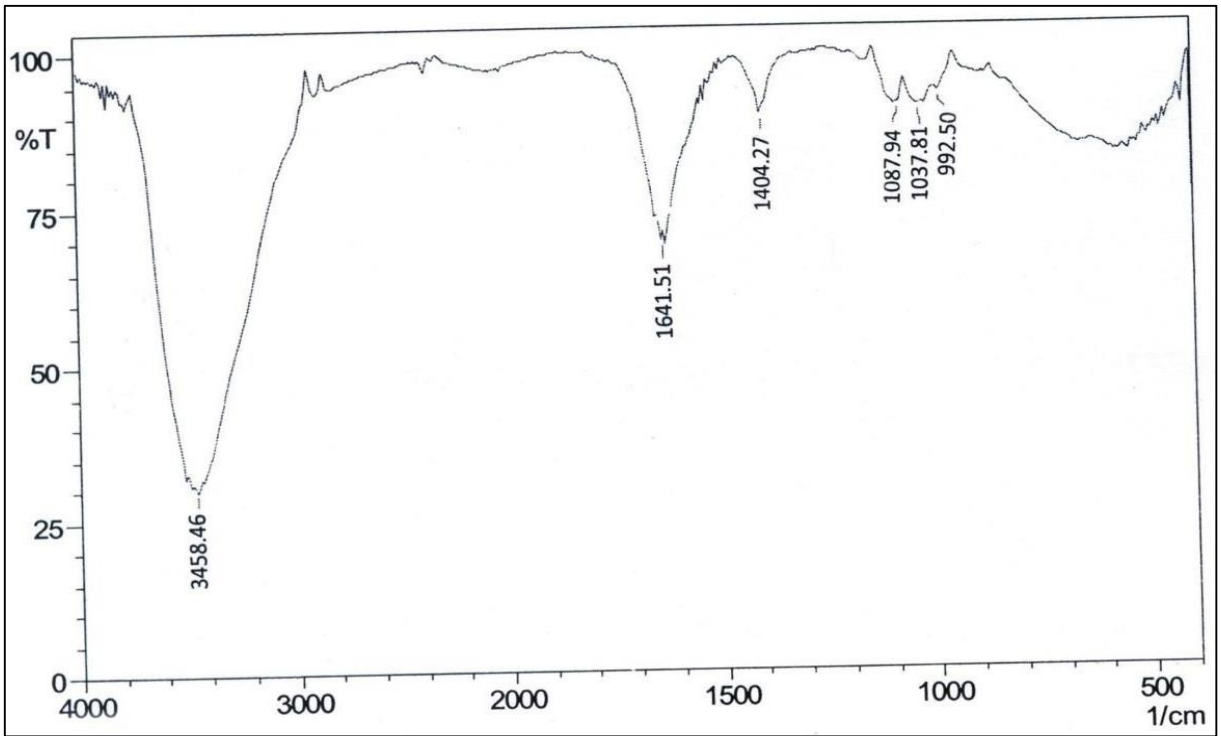


Fig 4: FTIR analysis of Silver nanoparticles in *Musa paradisiaca* Flower extract

XRD analysis of silver nanoparticles

The crystalline nature of Ag nanoparticles was confirmed from the X-ray diffraction analysis. XRD pattern with the diffraction peaks at 44.50, 52.20 and 76.7 corresponding to the (111), (200) and (220) facets of the face centred cubic crystal structure. The broadening of the Bragg peaks indicates the formation of nanoparticles. In addition to the Bragg peaks representative of fcc silver nanocrystals, additional, and yet unassigned, peaks were also observed suggesting that the crystallization of bio-organic phase occurs on the surface of the silver nanoparticles (Figure – 5 showing XRD analysis of silver nanoparticles from *musa paradisiaca*). The XRD shows maximum absorbance of about 32.218 A of diameter 2.7701 at 91 ℃ in silver nanoparticle from *musa paradisiaca*.

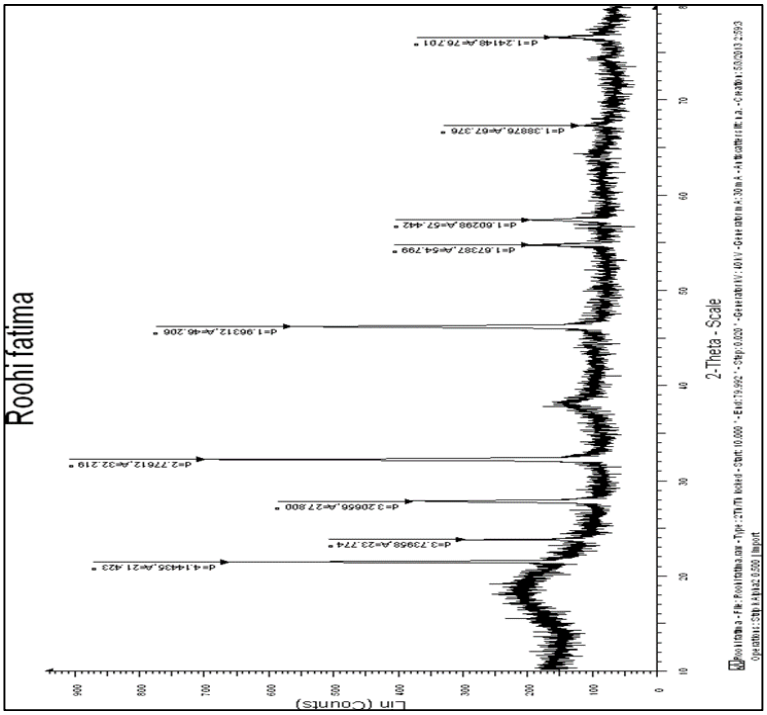


Fig 5: XRD Analysis of silver nanoparticles from *Musa paradisiaca*

Afm analysis of silver nanoparticles

The synthesized silver nanoparticles using leaves and flowers of *Musa paradisiaca* extracts were detected by AFM. (Figure - 6 showing analysis of silver nanoparticles through Atomic force microscopy). It shows silver nanoparticle with an average particle of 65 nm with image size of about 0.6µm. At about 1.2 µm image size, the particle size is observed as 34.4 nm.

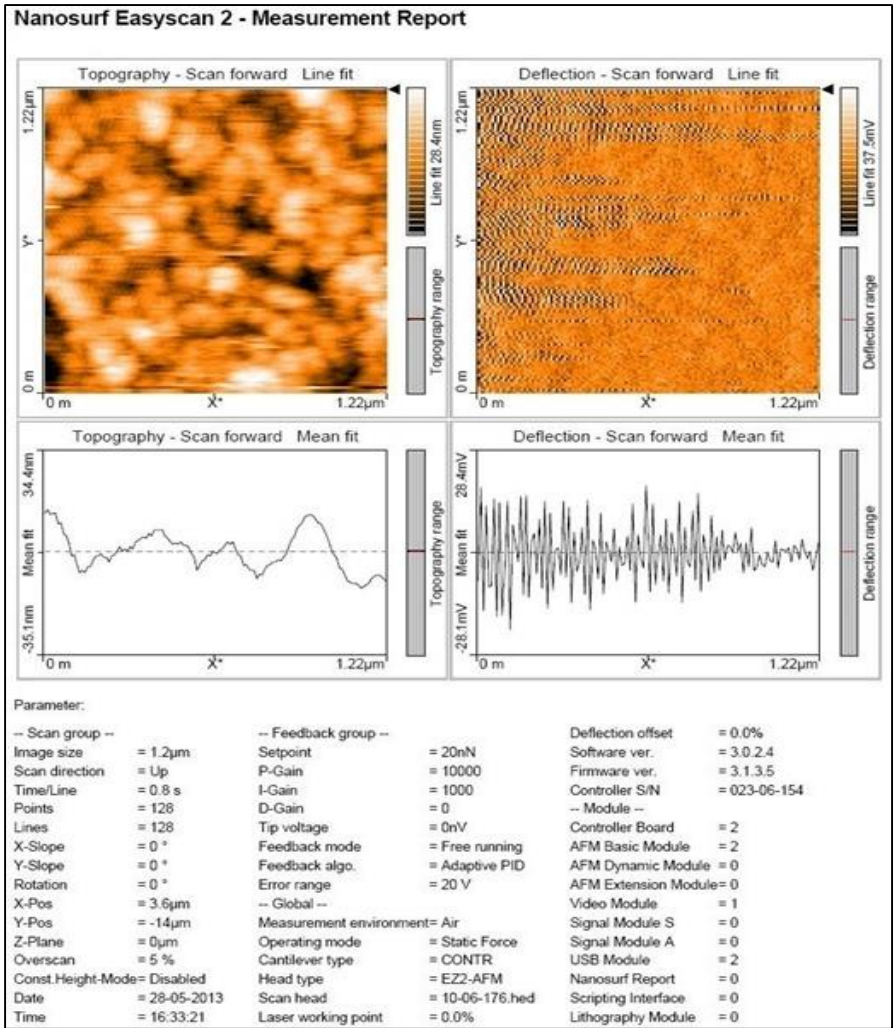


Fig 6 (1a): AFM result image size 1.2 µm

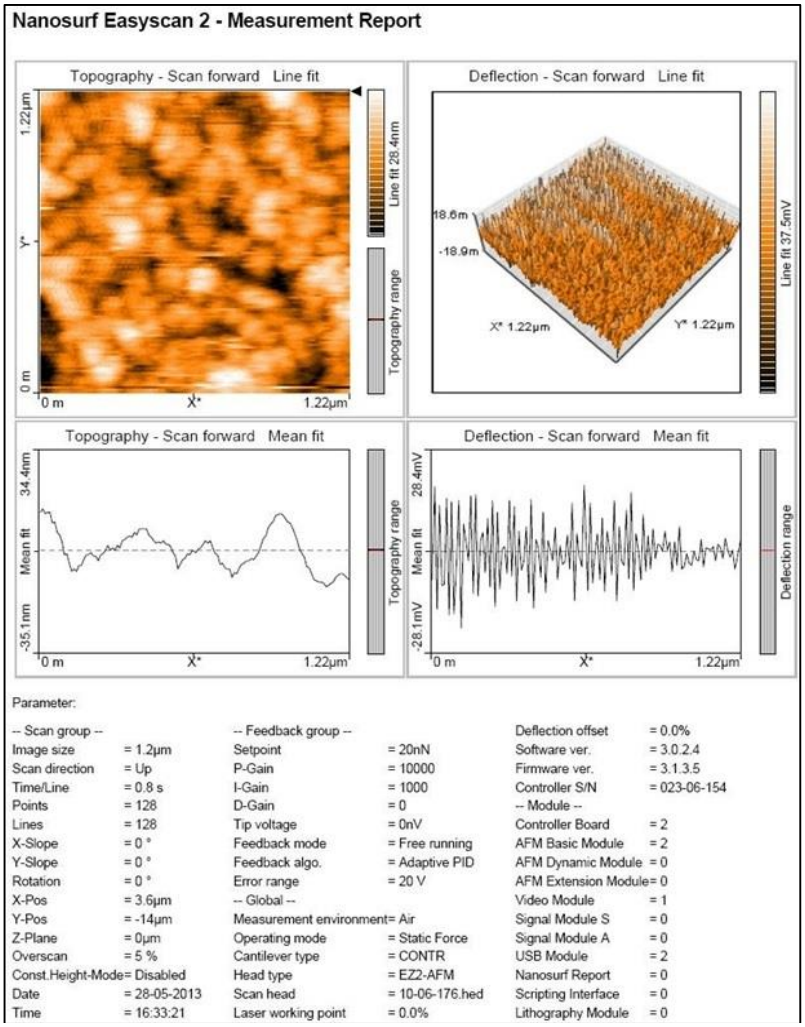


Fig 6 (1b): AFM result image size 1.2 µm

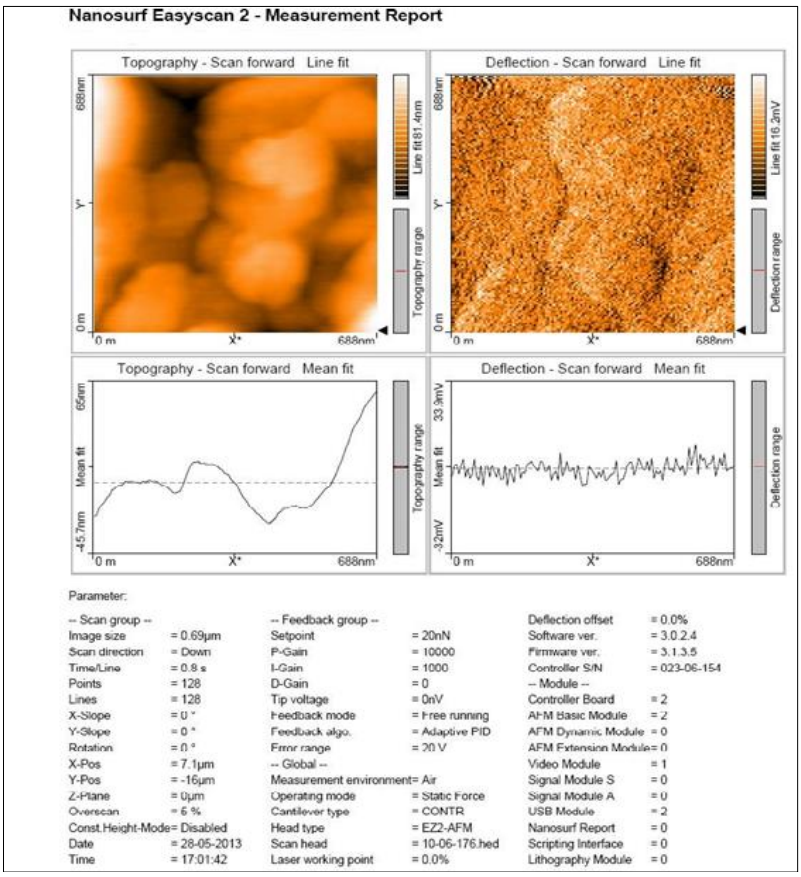


Fig 6 (2a): AFM result image size 0.6 µm

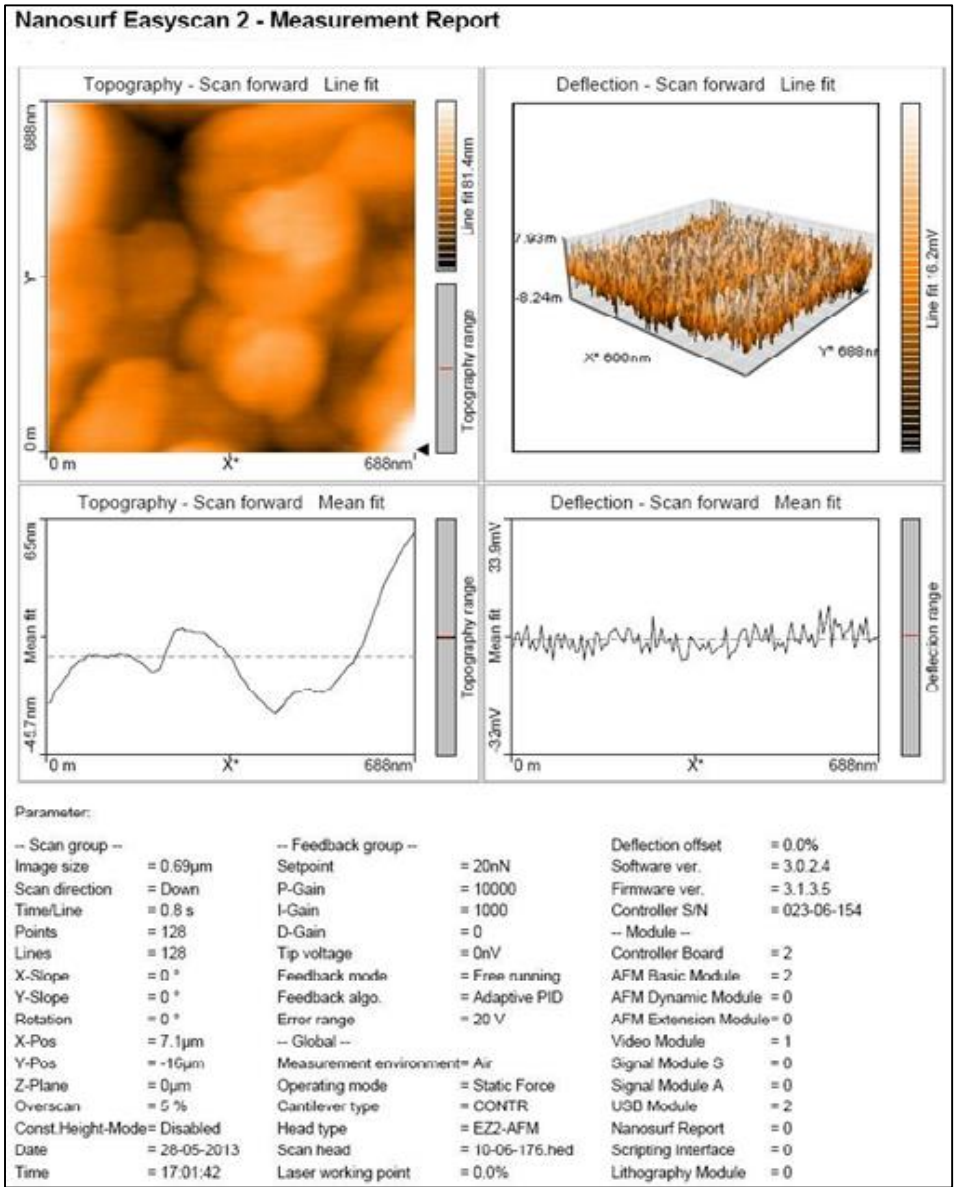


Fig 6(2b): AFM result image size 0.6 µm

Antibacterial activity of silver nanoparticles

In the antibacterial screening, the extract showed average zone of inhibition. At low concentration (25µl/disc) the extract was ineffective against *Proteus mirabilis.*

Bacillus subtilis, E. coli and Bacillus cereus showed higher inhibitory effect in different concentrations (Table 1 showing silver nanoparicles *musa paradisiaca* leaf extract (SMPLE) & Table 2 showing silver nanoparticles *musa paradisiaca* (SMPFE) flower extract).

The highest zone of inhibition (22.0mm) at a concentration (10µg/ml) was noted with *bacillus cereus* and minimum zone observed at (6.0 mm) at concentration (2µg/ml) with *escherichia coli* in the leaf extract of *musa paradisiaca*. Then highest zone of inhibition in flower extract was found to be (12.0 mm) with *bacillus cereus* at a concentration of (10µg/ml) with *escherichia coli* least zone as (4.0mm) at a concentration of (2µg/ml) with *musa paradisiaca*. However, the higher antimicrobial activity observed in leaf extract comparing to flower extract of *musa paradisiaca*.

The synthesized silver nanoparticles had antimicrobial activity against bacterial strains. The tested bacterial strains of *bacillus subtilis, E. coli and Bacillus cereus* were most susceptible towards silver nanoparticles extract of both leaf and flower extract. Disc diffusion method was used for the plant leaf extract containing silver nanoparticles [11].

Table 1: Antibacterial activity against Silver nanoparticles *Musa paradisiaca* Leaf Extract (SMPLE) at various concentrations, zone of inhibition (mm ± SE) against pathogenic bacteria is shown:

Pathogens	2µg/ml	4µg/ml	6µg/ml	8µg/ml	10µg/ml
B. subtilis	7 ± 0.02	10 ± 0.04	11 ± 0.06	12 ± 0.07	15 ± 0.10
E. coli	6 ± 0.03	9 ± 0.11	10 ± 0.14	12 ± 0.16	14 ± 0.17
B. cereus	10 ± 0.08	12 ± 0.12	16 ± 0.15	20 ± 0.16	22 ± 0.17
P. mirabilis	_	_	_	_	_

- indicates the negative result, mm – mean, SE – Standard error.

Table 2: Antibacterial activity against Siver nanoparticles *Musa paradisiaca* Flower Extract (SMPFE) at various concentrations, zone of inhibition (mm ± SE) against pathogenic bacteria is shown:

Pathogens	2µg/ml	4µg/ml	6µg/ml	8µg/ml	10µg/ml
B. subtilis	4 ± 0.01	5 ± 0.02	6 ± 0.04	7 ± 0.06	8 ± 0.07
E. coli	5 ± 0.03	6 ± 0.04	8 ± 0.06	10 ± 0.07	9 ± 0.08
B. cereus	6 ± 0.03	7 ± 0.05	8 ± 0.08	11 ± 0.07	12 ± 0.08
P. mirabilis	_	_	_	_	_

-- indicates negative result, mm – mean, SE – Standard error.

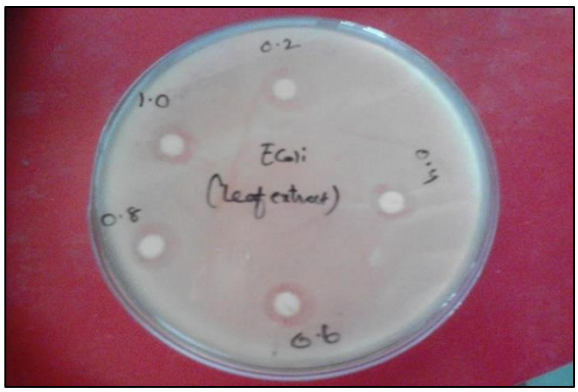


Fig 7: Antibacterial effect of *E. coli* on *Musa paradisiaca* leaf extract

Fig 8: Antibacterial effect of *E. coli* on *Musa paradisiaca* flower extract

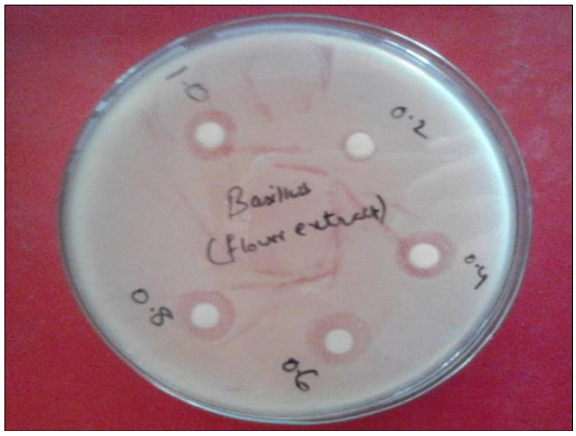


Fig 9: Antibacterial effect of *B. subtilis* on *Musa paradisiaca* flower extract

Fig 10: Antibacterial effect of *B. cereus* on *Musa paradisiaca* leaf extract

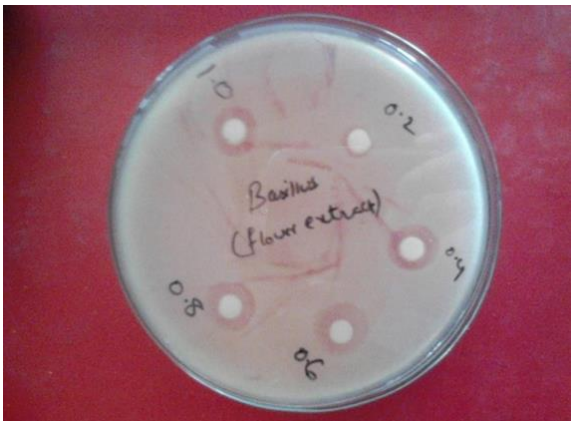


Fig 11: Antibacterial effect of *B. cereus* on *Musa paradisiaca* flower extract

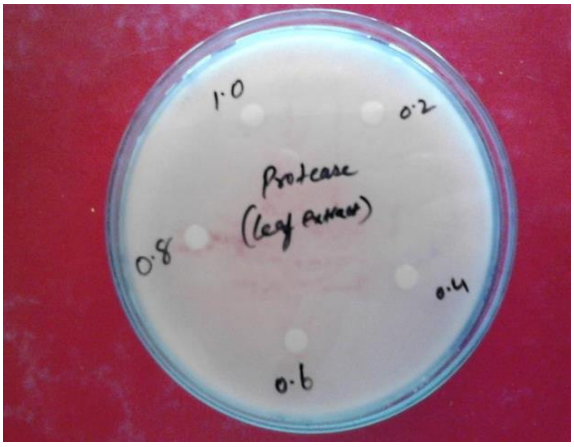


Fig 12: Antibacterial effect of P. *mirabilis* on *Musa paradisiaca* leaf extract

Fig 13: Antibacterial effect of P. *mirabilis* on *Musa paradisiaca* flower extract

Fig 14: Antibacterial effect of B. *subtilis* on *Musa paradisiaca* leaf extract

Summary and Conclusion

From this study it was concluded that**,** the aqueous silver ions exposed to the herbs were reduced and the nanoparticles were synthesized. The presence of nanoparticles was confirmed by the brown colour formation. The brown colour was observed after 30 minutes in *Musa Paradisiaca*. Leaf shows faster colour change than flower.

Synthesized nanoparticles from *Musa paradisiaca* can be used as antibacterial agents for *E. coli, B. subtilis* and *B. cereus.* Leaf and flower shows higher activity. These synthesized nanoparticles can be used as bactericidal and in wound healing [12], water purification and also in the field of medicine.

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