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Research Article

Green synthesis and Antimicrobial Activity of Silver Nanoparticles from *Mangifera indica*

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Abstract

Green synthesis of silver nanoparticles and the study of their antimicrobial properties are of fundamental importance in the advancement of recent research. In this paper, we described the synthesis of silver nanoparticles using plant extract of *Mangifera indica*. Synthesized silver nanoparticles were confirmed by sampling the reaction mixture and the absorption maximum was scanned by UV–Visible spectra, at the wavelength of 300 – 700 nm. The nanoparticles size was produced in the average range of 30 - 50 nm some particles were found in the size range of 70 nm. The synthesized silver nanoparticles showed antimicrobial activities as zone of inhibition against *Staphylococcus aureus, Salmonella sp., Pseudomonas aeruginosa and E. coli in the range from 22 mm, 12 mm, 20 mm 22 mm respectively. Aspergillus* sp., *and Candida were* effectively inhibited by the silver nanoparticles but the size of zone was not clearly appear due to second generation of growth of fungus. *Klebsiella sp., and Mucur sp., showed resistance against silver nanoparticles.TEM, XRD, SEM and FTIR analysis were used to characterize the produced nanoparticles.*

Keywords: *Mangifera indica* extract, AgNPs, Antibacterial and Antifungal activity.

Introduction

Nanotechnology is a multidisciplinary research area and the synthesis of nanoparticles is gaining significance worldwide. Nanoparticles have a very large surface area which results in greater chemical reactivity, biological activity and catalytic behaviour as compared to larger particles of the same chemical composition. Nanoparticles are used in application such as catalysts in chemical reactions, electrical batteries and in selective coatings for absorption of solar energy, as optical elements, pharmaceutical components and in chemical sensing and biosensing. The synthesis of nanoparticles has been reported using chemical and physical methods. The present study explains a cost effective and ecofriendly approach for the synthesis of silver nanoparticles (AgNPs) from leaf extract of *Mangifera indica*. In this study aqueous extracts of the leaves of *Mangifera indica* used as reducing and stabilizing agents. Phytochemicals are assumed to be responsible for the formation of AgNPs. The rapid reduction of silver (Ag^+) ions was monitored using UV-visible spectrophotometer. UV-visible spectrum of the aqueous medium containing silver ions demonstrated a peak value at 440 nm .The further characterization of the nanoparticles was done by technique such as X-RAY diffraction (XRD), Scanning Electron Microscopy (SEM), Transmission Electron Microscope (TEM), Atomic Force Microscopy (AFM) and Fourier Transform Infrared Spectroscopy(Vikas Sarsa, 2012).

Materials and Methods

Selection and collection of plant leaves

Healthy and bright greenish (*Mangifera indica*) leaves were collected for the separation of silver nanoparticles.

Optimization of silver nanoparticle production

The synthesis of silver nanoparticles was optimized by using different concentration of $AgNO_3$ solution *viz*., 1

mM, 3 mM and 5 mM. The concentration of culture broth (1 ml, 5 ml) also was optimized.

Synthesis of silver nanoparticles

Ten ml of the extract was added to 100 ml of 1 mM $AgNO_3$ solution and kept in room temperature up to the changing of color into reddish brown. Bioreduction of silver ions in the solution was monitored by measuring UV-VIS spectra of the solution at periodic intervals. The nanoparticle synthesized was confirmed by UV-VIS spectra plasma curve.

Recovery of silver nanoparticles by centrifugation

After bioreduction, the solution consisting of hydrosols of silver nanoparticles was subjected to centrifugation at 5000 rpm for 20 minutes, and the supernatant was discarded. The pellet formed was dissolved in 0.1 ml of deionized water and air dried**.**

UV-VIS spectra analysis

The factor contributes to the measurement uncertainty of the results obtained with UV/VIS spectrophotometry. If UV/VIS spectrophotometry is used in quantitative chemical analysis then the results are additionally affected by uncertainty sources arising from the nature of the compounds and solutions that are measured. These include spectral interferences caused by absorption band overlap, fading of the color of the absorbing species (caused by decomposition or reaction) and possible composition mismatch between the sample and the calibration solution (Soovali *et al*., 2006). The bioreduction of Ag^+ in aqueous solution was monitored by periodic sampling of aliquots (0.2 ml) of the suspension, then diluting the samples with 2 ml deionised water and subsequently measuring UV-Vis spectra, at the wave length of 300 to 700 nm. UV-Vis spectra were recorded at initial, 1 hr, 2 hr, 3 hr, 4 hr and 5 hr.

XRD Measurement

The interaction of the incident rays with the sample produces constructive interference (and a diffracted ray) when conditions satisfy Bragg's Law ($n\lambda=2d \sin\theta$). This law relates the wavelength of electromagnetic radiation to the diffraction angle and the lattice spacing in a crystalline sample. These diffracted X-rays are then detected, processed and counted. By scanning the sample through a range of 2θ angles, all possible diffraction directions of the lattice should be attained due to the random orientation of the powdered material. Conversion of the diffraction peaks to d-spacings allows identification of the mineral because each mineral has a set of unique d-spacings. Typically, this is achieved by comparison of d-spacings with standard reference patterns pMax (Von Laue *et al.*, 1912).

The air dried nanoparticles were coated onto XRD grid and analyzed for the formation of Ag nanoparticle by Philips X-Ray Diffractometer with Philips PW 1830 X-Ray Generator operated at a voltage of 40 kV and a current of 30mA with Cu Kal radiation. The diffracted intensities were recorded from 10' to 80' of 20 angles.

SEM Analysis

After synthesis of nanoparticles, the sample was filtered through millipore filters of 0.2 m pore size, to remove any contaminants interfering with the SEM images. About 25 ml of the sample was pipette out and loaded on a 'stub' provided for SEM analysis. The stub is made of copper, in the shape of a small cylinder about the size of 1 cm dia. One side of the stub was stuck with double sided carbon material. After loading the sample on the carbon material, the stub was fixed to a holder. The holder accommodates about 4 samples at a time. This study was undertaken to know the size and shape of the silver nanoparticles biosynthesized by *Mangifera indica*.

TEM Sample Preparation

Appropriate sample preparation to obtain well-dispersed, isolated particles is a well-recognized, crucial step in the TEM analysis process. The solutions used for the TEM experiments were prepared as described in our earlier papers. Briefly, iridium nanoclusters were obtained from the Standard Conditions 27 hydrogenation of 1.6 M cyclohexene starting with 1.2 mM of the nanocluster precursor complex (a (1,5-COD)Ir+ complex) and 1.2 mM stabilizer in acetone at 22.0 + 0.1°C. After the completion of the nanocluster formation (monitored by GLC determination of cyclooctane evolved from the reduction of the (1,5-COD)Ir+ precursor complex), the Fischer-Porter (F-P) bottle was detached from the hydrogenation line *via* its quick-connects, brought back into an inert (N_2) atmosphere drybox, and its acetone solution was quantitatively transferred with a disposable polyethylene pipette into a clean, 5 mL screw-capped glass vial. The solution was dried under vacuum and the glass vial was then sealed and brought out of the drybox. The dry nanocluster samples in screw-capped glass vials were sent as solids , to obtain the TEM images. There, 1 mL of acetonitrile was added, in air, just before a TEM

was obtained. A drop of this solution was then dispersed on a chloroform cleaned, carbon-coated Cu TEM grid. Purified samples of 2-[2-(2-mercapto-ethoxy)-ethoxy]-ethanol (hereafter mercapto exthoxyethxoy ethanol) stabilized clusters were dissolved in methylene chloride and a few microliters of this solution was aerosoled (from a ne capillary by application of compressed air), onto carbon holey carbon or silicon monoxide films on Cu TEM grids.

Sample TEM Analyses

TEM analyses of samples prepared as described above were performed on a Philips CM-12 TEM with a 70 m lens operating at 100 kV and with a 2.0 Å point-to-point resolution, all as described previously in detail. Typically, TEM pictures of each sample were taken at 3 different magnifications (100, 430, and 580 K) in order to obtain information about the sample in general (100 K), plus a closer visualization of the clusters (580 K). A number of control experiments were performed previously (primarily for the Iron nanoclusters), which provided good evidence that the results are truly representative of the sample (i.e., save any crystallization of the nanoclusters in the electron beam) and that the sample is not otherwise perturbed by application of the TEM beam [e.g., controls showing that varying the sample aerosoling method (in air or under N_2) or depositing the sample as a drop and letting it dry did not change the results; controls showing that changing the beam voltage from 40 to 100 kV, or changing the exposure time (seconds vs. minutes), did not change the images; other controls have been done as well.

FT-IR Analysis

Fourier transform infrared spectroscopy was discovered by Bruker (1967) in Germany is model 3000 Hyperion Microscope with vertex 80 FT-IR system**.** The dried Ag nanoparticles were subjected to FT-IR analysis by KBr pellet (FT-IR grade) method in 1:100 ratios and spectrum was recorded in Nicolet Impa 400 FT-IR Spectrophotometer using diffuse reflectance mode operating at a resolution.

Evaluation of Antibacterial and Antifungal activity

Antibacterial and Antifungal activity of silver nanoparticles against of human pathogenic bacteria and fungi

Silver nanoparticles synthesized using *Mangifera indica* sample were tested for its potential antibacterial and antifungal activity against few human pathogens i.e. Bacteria namely *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Salmonella typhimurium*. Fungus namely *Candida albicans, Mucur* sp, and *Aspergillus* sp, were used as the test organisms.

Disc Diffusion Method

The paper disc (No.1 Whatmann) was cut down into small discs (6 mm diameter) and sterilized at 180^{o}C for 30 minutes in hot air oven. After sterilization, the discs were impregnated with silver nanoparticles and the standard antibiotic solution. The disc was left standing for 1 - 4 hrs, at room temperature for drying. The dried discs were placed on the surface of the pathogenic bacterial and fungal swabbed culture medium. Subsequently incubated for about 18 - 24 hrs at 37^{o}C for bacterial and fungal plates were placed in room temperature. After incubation the diameter of the circular inhibition zones were measured.

Result and discussion

Synthesis of silver nanoparticles by *Mangifera indica* plant leafs

Addition of *Mangifera indica* biomass to a silver nitrate solution led to the appearance reddish brown colour in the solution after a few days, indicating formation of silver nanoparticles (Fig. 1). First, the UV-visible spectroscopy method was used to quantify this process (Fig.2).

The diffraction patterns presented in (Fig. 3) correspond to the amorphic structure of samples. However, a number of Bragg's reflections corresponding to the fcc structure of silver are also seen here. Specifically, the XRD pattern shows four characteristic peaks corresponding to the (109), (200), (205), (211) and (310) sets of lattice planes. The XRD pattern thus clearly shows that the silver nanoparticles formed by the reduction of Ag^{+} ions by *Mangifera indica* are crystalline in nature. It should be noted that the relative crystalline silver content of the *Mangifera indica* biomass was not high, not more than 1%, which was at the sensitivity limit of the XRD analysis. This result confirmed that silver nanoparticles were produced in general extracellularly.

SEM results of *Mangifera indica* leaf

The cells of *Mangifera indica* were imaged by the SEM method after the reaction with the silver nitrate solution

for one week. The SEM images (Fig. 4) illustrate that most of the particles are spherical-like and do not create big agglomerates. There are two types of images got from research institute. One is normal image which contain nanoparticles (Fig.4) and other one is negative film appearance of nanoparticle produced by *Mangifera indica* the nanoparticles were appear in block colour (Fig.5).

The SEM results indicate that the process of formation of silver nanoparticles takes place on the surface of the cells. Reduction and surface accumulation of metals may be a process by which microorganisms protect themselves from the toxic effects of metal ions. In the plants, the metal components were used as induces metabolic reactions in the plants and plant leaves. In this SEM image a result which indicates the *Mangifera indica* will produce silver nanoparticle. Today the exact mechanism for formation of silver nanoparticles by plants is not fully understood also by nanotechnologist.

TEM Analysis

Under TEM, the silver nanoparticles synthesized by *Mangifera indica* plant leaf extract were observed to have an average mean size of 30-50 nm corroborating well the DLS pattern. One or two nanoparticles were appearing in the size of nearby 70nm. The particles appeared to be spherical in shape with weak crystalline structure. TEM analysis results were show in figure 6.

FTIR spectra analysis

The infrared spectra are recorded on Fourier Transform Spectrometer in the mid–infrared region (MIR) within the range (500 - 3500 cm^{-1}). Due to the complex interaction of atoms within the molecule, IR absorption of the functional groups may vary over a wide range. However, it has been found that many functional groups give characteristic IR absorption at specific narrow frequency range. Multiple functional groups may absorb at one particular frequency range but a functional group often gives rise to several characteristic absorptions. Stretching & bending vibrations are varied after formulation can be observed. Thus, the spectral interpretations should not be confined to one or two bands only actually the whole spectrum should be examined. The purified nanoparticles exhibited absorption peaks at 633, 1370, 1563, 1751 and 2589 cm^{-1} due to cyclic C–O–C, C=O and OH functional groups, respectively. Thus, the nanoparticles were stabilized.

Antimicrobial activity of bacterial pathogen.

The biologically synthesized AgNPs inhibited different pathogenic microorganisms. The resulting zones of inhibition formed were mainly due to the destabilization of the outer membrane of microbes by the silver nanoparticles. *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella* sp., *Staphylococcus aureus* were effectively inhibited by the silver nanoparticles but *Klebsiella* sp., was not inhibited by nanoparticles as shown in (Fig - 8). According to the mechanism behind the bactericidal effect of the silver nanoparticles against bacteria is not well known. It has been proposed that AgNPs act similarly to the antimicrobial agents used for the treatment of bacterial infection by different mechanisms.

The sizes of zone inhibition are given below:

A. *Staphylococcus aureus*: Silver nanoparticles (Sn) which produce 22 mm size of zone and Tetracyclin (T) also produce 22 mm of zone of inhibition as sensitive and Methycilline (M) which produce 20 mm of zone. Control did not produce any zone of inhibition because of control do not have any material.

B. S*almonella* sp.: Silver nanoparticles (Sn) which produce 12 mm size of zone but its not sensitive to Salmonella sp., Tetracycline (T) which produce 18 mm of zone, 8 mm of zone only produced by Kanamycin (K) as well as control did not produce any zone of inhibition because of control do not have any material.

***C. Pseudomonas aeruginosa*:** Silver nanoparticles (Sn) which produce 20 mm size of zone as sensitive, Amikasin (A) which produce 13 mm, Norfloxacin(Nx) 10 mm of zone and both are resistant and Ciprofloxacin(Cf) 26 mm size of zone as sensitive but control did not produce any zone of inhibition because of control do not have any material.

D. K*lebsiella* sp.: Silver nanoparticles (Sn) do not produce any zone of inhibition. The testing silver nanoparticles are resistant to Klebsiella so zone was not formed. Norfloxacin (Nx) formed 16 mm of zone of inhibition. Control did not produce any zone of inhibition because of control do not have any material.

E. *Escherichia coli*: Silver nanoparticles (Sn) which produce 22 mm size of zone as sensitive, Amikasin (A) which produce 12 mm size of zone as intermediate and Erythromycin (E) did not produce any zone of inhibition same as control did not produce any zone of inhibition

Figure.1 Siver nitrate solution in normal condition and other one is which contain silver nanoparticle.

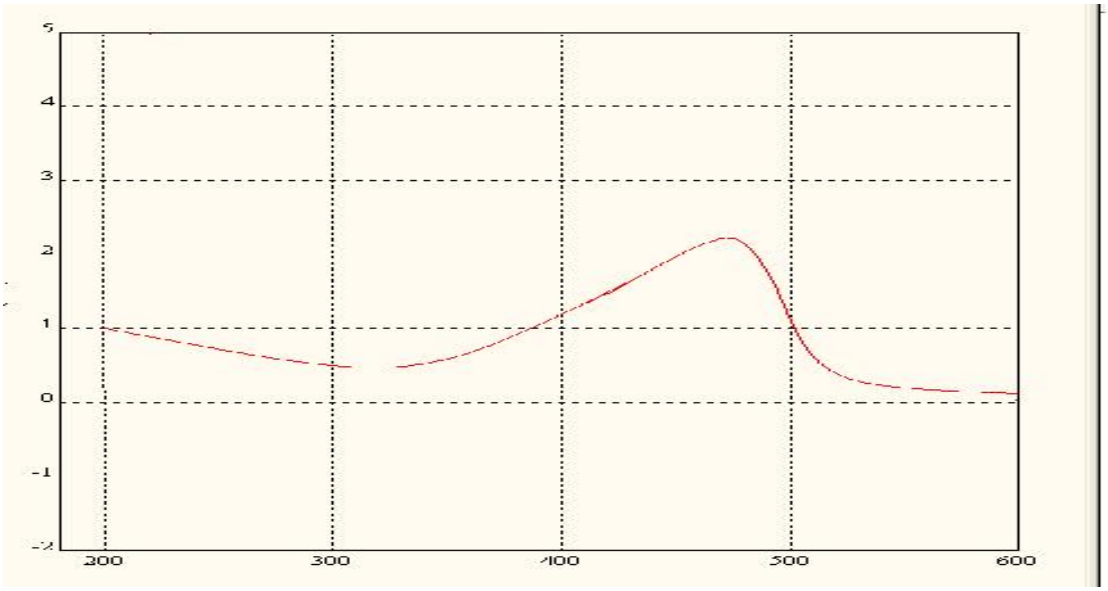

Figure.2–UV-Vis spectra recorded after one week for the reaction mixture prepared using 1mM silver nitrate and 1 g biomass of *Mangifera indica.*

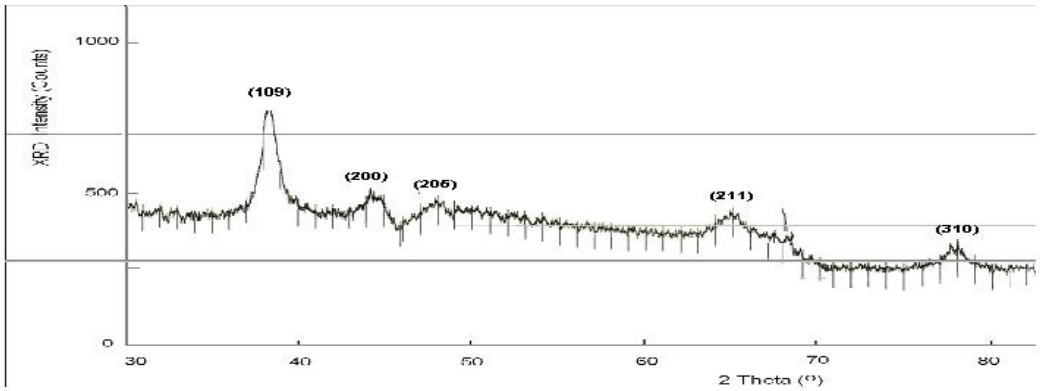

Figure. 3 XRD spectra of silver nanoparticles synthesized using 2 g wet biomass of *Mangifera indica* incubated with 1 mM $AgNO_3$ for 3 days.

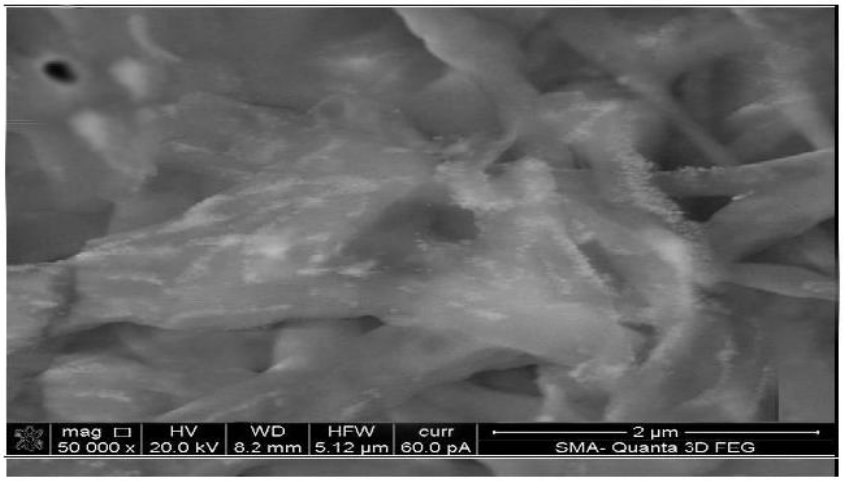

Figure.4 SEM image of nanoparticle produced by *Mangifera indica*, (white color dots).

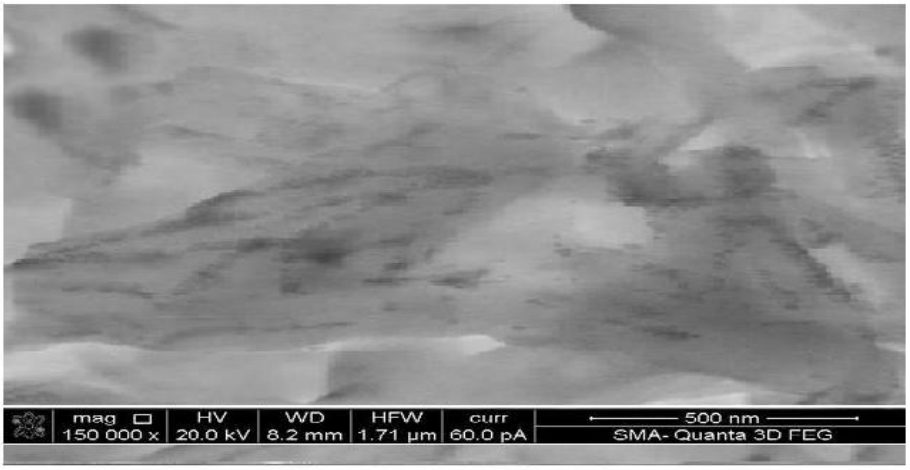

Figure.5 SEM (negative appearance) image of nanoparticle produced by *Mangifera indica* (block dotes)

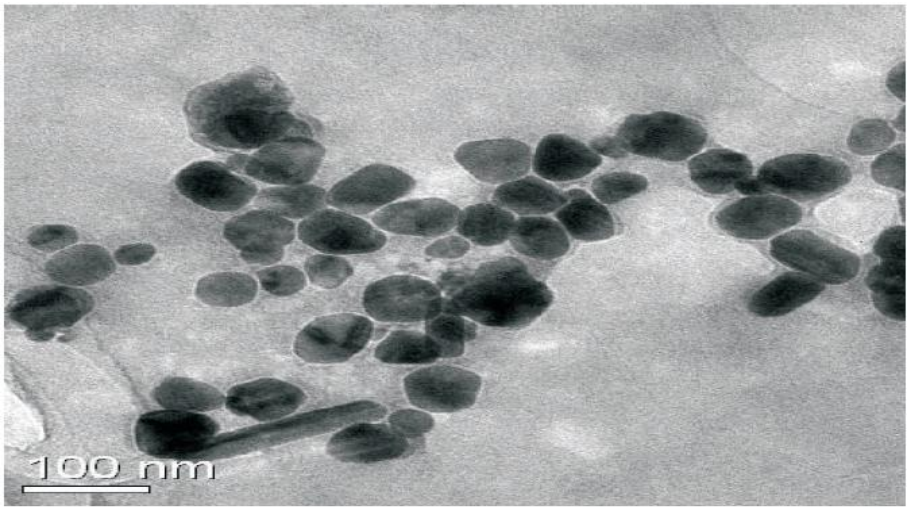

Figure :6 Shows TEM image of silver nanoparticles from *Mangifera indica* leaf

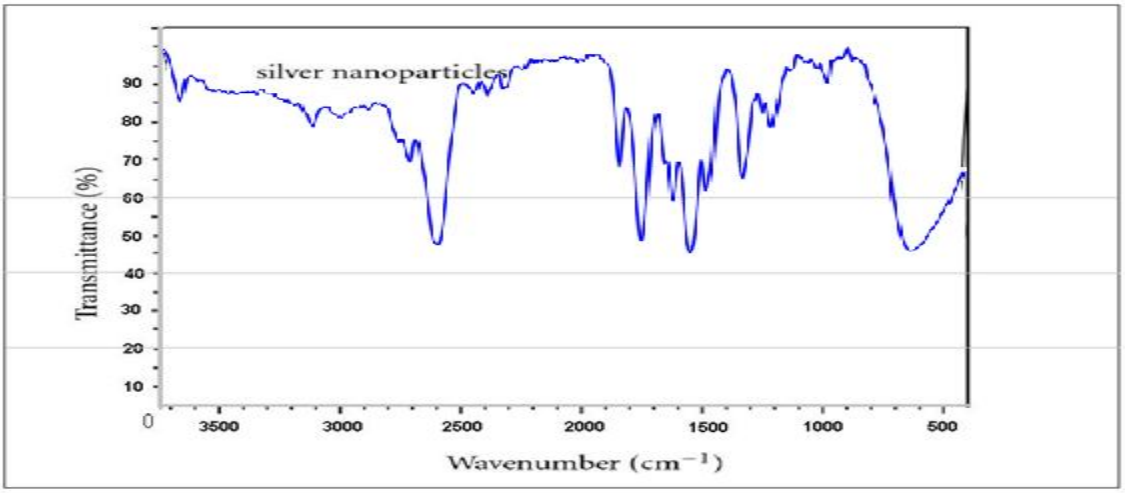

Figure - 7: shows the FTIR spectra of the *Mangifera indica* silver nanoparticles.

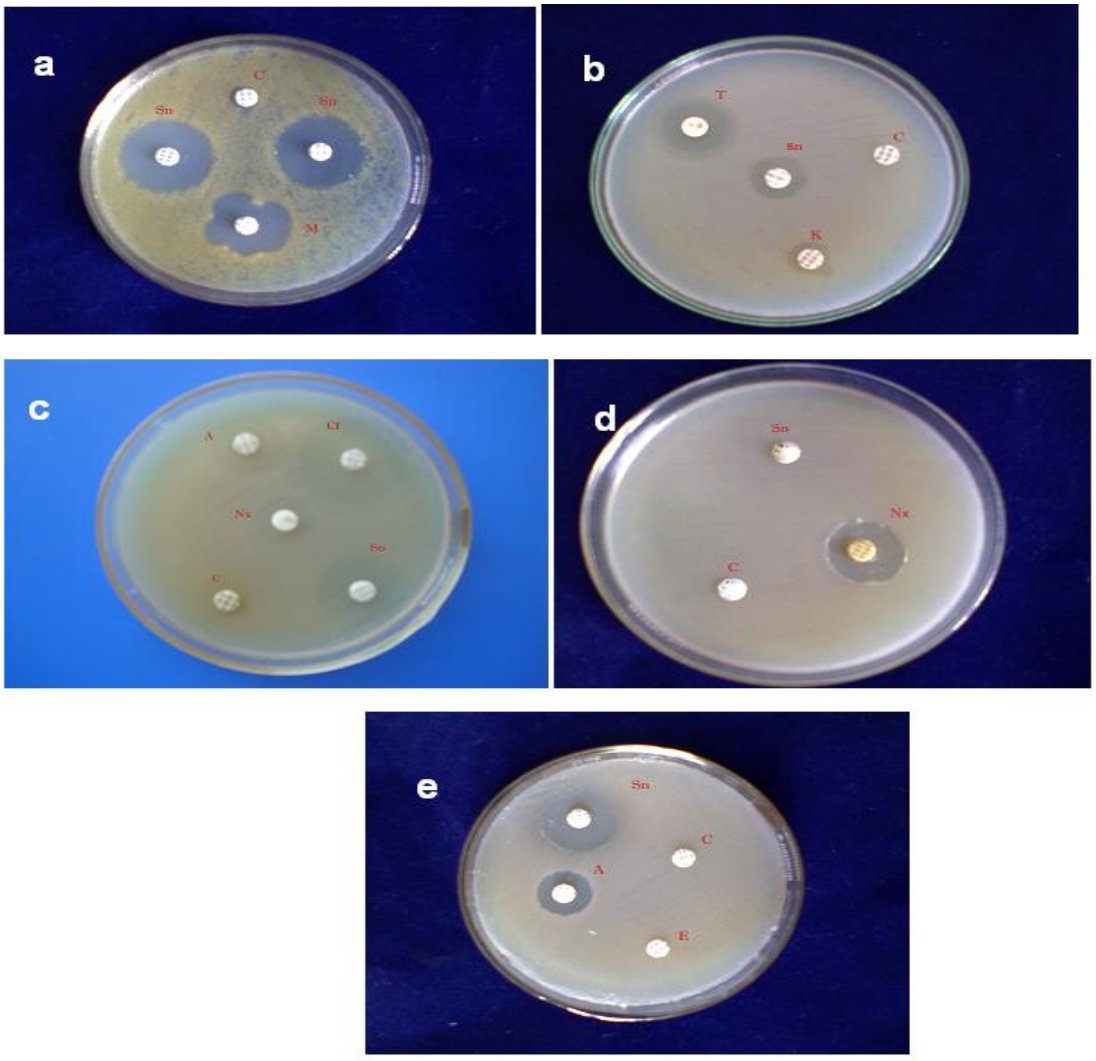

Figure - 8: Shows antibacterial activity of silver nanoparticles
Antimicrobial activity of fungal pathogen.

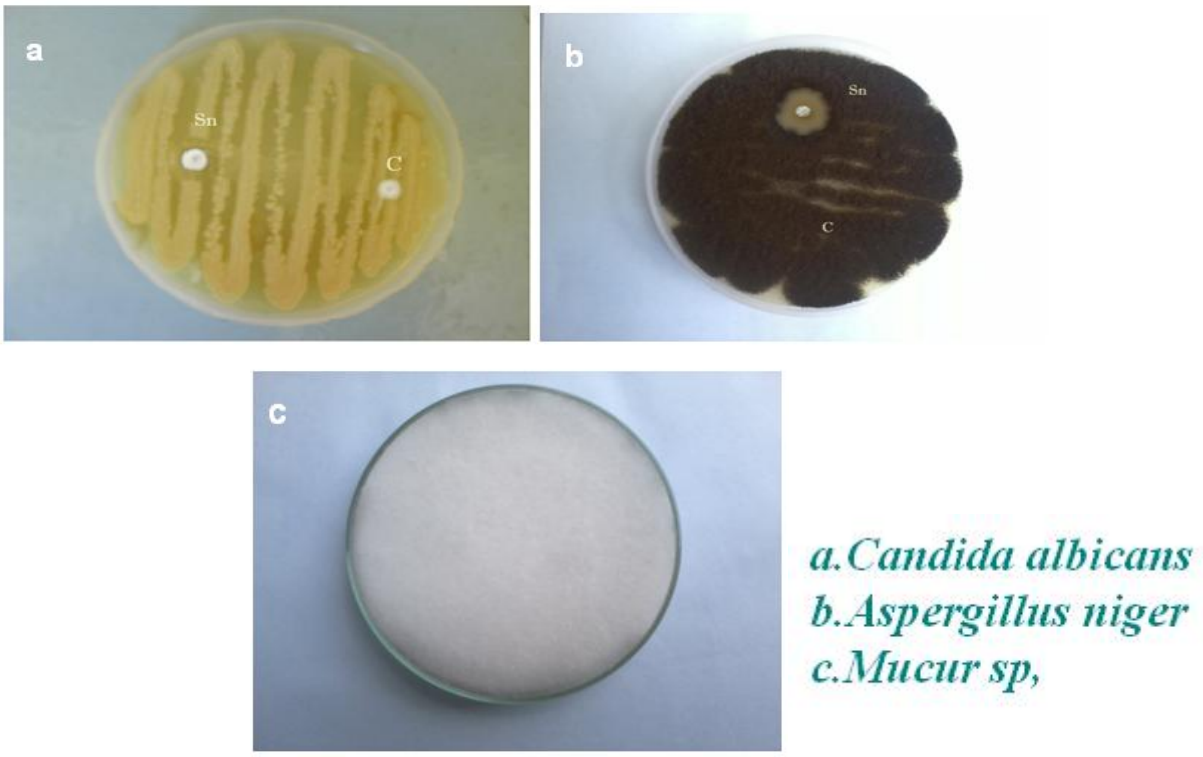

Figure – 9: Antifungal activity of silver nanoparticles

because of control do not have any material.The final find out is silver nanoparticle which produced by Streptomyces glaucus was more or less sensitive to testing pathogens. It was used to treat human pathogens.

Antimicrobial activity of fungal pathogen.

The biologically synthesized AgNPs inhibited different pathogenic microorganisms. The resulting zones of inhibition formed were mainly due to the destabilization of the outer membrane of microbes by the silver nanoparticles. *Aspergillus* sp., *and Candida* were effectively inhibited by the silver nanoparticles as shown in (Fig - 9) but the size of zone was not clearly appear due to second generation of growth of fungus. At the same time *Mucor* was didn't affected by silver nanoparticle. That means *Mangifera indica* plant leaves nanoparticles resistant to *Mucor* sp. Even that plate was appearing with fully grow cotton like formation.

Action of nanoparticles against of microbes

The mechanism behind the bactericidal effect of the silver nanoparticles against bacteria is not well known. There are three different mechanisms explained by. Firstly, Ag NPs attach to the surface of the cell membrane and disturb its power functions, such as permeability and respiration. The binding of the particles to the bacteria depends on the interaction of the surface area available. With a smaller particle size, a large surface area will have a stronger bactericidal effect. Secondly, Ag NPs are able to penetrate the bacteria by possibly interacting with sulphur - and phosphorus - containing compounds such as DNA and cause further damage. Thirdly, the silver nanoparticles release silver ions, which contribute to the bactericidal effect. The mechanism of inhibition by silver ions on microorganisms was partially known. It was believed that DNA loses its replication ability and cellular proteins become inactivated upon silver ion treatment. Furthermore, higher concentrations of Ag^{+} ions have been shown to interact with cytoplasm components and nucleic acids (Tenover *et al.*, 2006).

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