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STUDIES ON MORPHOLOGICAL CHARACTERIZATION AND ANTIMICROBIAL ACTIVITY OF SILVER NANOPARTICLES SYNTHESIZED BY BIO AND CHEMOREDUCTIVE METHODS

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ABSTRACT

In this study, we report the synthesis of silver nanoparticles (SNP) by plant mediated (*Azadiracta indica*) and chemical methods. The synthesized SNP were characterized by UV-Visible spectroscopy, Scanning Electron microscopy(SEM) and Fourier transform infrared(FT-IR) spectroscopy. The antibacterial effect of SNP produced by both the methods was evaluated by growth inhibition study which showed strong antibacterial activity against gram positive and gram negative bacterial strains.

Key words: Silver nanoparticles ; characterization; Scanning Electron microscopy(SEM); Antibacterial activity.

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INTRODUCTION

Currently, there is a growing need to develop environmentally benign nanoparticle synthesis processes that do not use toxic chemicals in the synthesis protocol [1]. Among the various known biosynthesis methods, plant-mediated nanoparticles synthesis is preferred as it is cost-effective, environmentally friendly , safe for human therapeutic use [2] and among the various plants, neem leaves extract was chosen for the present study, since (i) Neem is a quite commonly available plant, (ii) it excludes addition of external stabilizing agent during synthesis and (iii) antimicrobial properties of the synthesized silver nanoparticles (one of the major end uses) might be enhanced due to synergistic effects of neem leaves [3].

Biosynthesized silver nanoparticles are used in label-free colorimetric assay to detect enzymatic reactions [4], water-filtering apparatus [5], catalysis [6], surface enhanced Raman scattering, surface plasmon resonance studies [7], optical receptors for biolabelling [8], antimicrobial material [9], plasmonics [10], anti-viral and anti-HIV studies [11].

Recently, the development of resistant or even multiresistant pathogens has become a major problem, for instance *Staphylococcus aureus* resistance to methicillin and *Candida albicans* resistance to fluconazole have to be mentioned [12]. Therefore, antibacterial agent effective against multiresistant pathogens need to be developed. It is well known that silver ions and silver-based compounds are highly toxic to microorganisms. Silver compounds have also been used in the medical field to treat burns and a variety of infections. Several salts of silver and their derivatives are commercially employed as antimicrobial agents. Commendable efforts have been made to explore this property using electron microscopy, which has revealed size dependent interaction of silver nanoparticles with bacteria[13].

In the present study we synthesized SNP by two methods namely bio and chemoreductive methods and compared the morphological characteristics and antimicrobial activity of plant mediated and chemically synthesized silver nanoparticles.

MATERIALS AND METHODS

Chemicals and Plant material

Analytical grade silver Nitrate was purchased from RANBAXY pharmaceuticals, Pvt ltd. Mumbai, India. The plant material was collected from our college campus and was identified as *Azadiracta indica (Family:Meliaceae)* by Siddha central research institute ,Arumbakkam, Chennai .Sodium citrate was procured from Sisco Research Laboratories, Pvt Ltd., Mumbai, India. Nutrient agar was purchased from Himedia Laboratories,Pvt ltd., Mumbai, India.

Plant mediated synthesis of silver nanoparticles

The *Azadiracta indica* leaves were shade dried for 12 days and ground to fine powder using mixer grinder and sieved. 1% of leaf extract was prepared using triple deionised water. The solution was boiled and cooled to room temperature.5ml of this yellow colour filtrate was mixed with preheated 125 ml of dilute silver nitrate solution prepared from 25ml of 5mM silver nitrate. The change of colour from yellow to reddish brown indicates the synthesis of silver nanoparticles [3].

Chemical method for synthesis of Ag nanoparticles

25 ml of 5Mm AgNo3 solution was prepared and it was diluted to 125ml. The solution was boiled and 5 ml of the 1% sodium citrate solution was added to this solution and heating was continued till colour change was observed

UV – VIS Spectral analysis

The preliminary characterization of SNP produced by both the methods was carried out by UV-Visible spectral analysis (300 to 1000nm) using Shimadzu UV-1800 double beam spectrophotometer.

SEM analysis of silver nanoparticles

Purified SNP in suspension were characterized for their morphology using a HR-FEG SEM (High Resolution Scanning Electron Microscope) at Sophisticated Analytical Instrument Facility, IIT-Madras . The clear water suspended SNP solution was placed onto carbon tape mounted on an aluminium stub for SEM imaging. The samples were dried at room temperature in a controlled environment. The images were captured in SEM mode at 400nm magnification.

FTIR analysis

To identify the biomolecules associated with the synthesis of nanoparticles by plant mediated method, Fourier Transform Infra Red spectroscopy (Sophisticated Analytical Instrument Facility, IIT-Madras) was performed. For FTIR measurements the synthesised nanoparticle solutions were centrifuged at 8500 rpm for 30 minutes. The pellets were washed and redispersed in 10ml of distilled water. The process was repeated for three times. The final solutions obtained after centrifugation were analysed by FTIR by coating the films on a carbon coated copper grid by placing a small amount of the sample and allowing it to dry. The scanning was performed at 400-4000 cm^{-1}range with a resolution of $4cm^{-1}$.

Antimicrobial activity

The silver nanoparticles synthesised by both methods were tested for antimicrobial activity by agar well diffusion method [15] against various bacterial strains such as Escherichia coli, Pseudomonas aeruginosa and Klebsiella (gram negative), Staphylococcus aureus and Bacillus subtilis (gram positive). Wells of 5mm diameter were made on nutrient agar plates using gel puncture. Using a micropipette, 20µL of nanoparticle solution was added to each well Standard antibiotic Streptomycin solution at a concentration 1µg/ml was used as positive control and neem leaf extract as negative control. After incubation at 37ºc for 24 hours, the diameter of zone of inhibition was measured.

RESULTS AND DISCUSSION

Synthesis of silver nanoparticles

In *plant mediated method* the change of colour from yellow to reddish brown colour indicates the synthesis of silver nanoparticles(fig:1).

Figure:1

Synthesis of silver nanoparticles by plant mediated method

In *chemical method* the the change of colour from white to a pale reddish brown colour indicates the synthesis of silver nanoparticles (fig:2).

Figure:2
Synthesis of silver nanoparticles by chemical method

UV-Visible spectral analysis

The reduction of AgNO3 into nanoparticles was visibly evident from the colour change as well as Reaction mixture showed an absorbance peak at around 425 nm, which is characteristic of silver nanoparticles, due to its surface plasmon resonance absorption [14]. The peak absorbance for SNP sythesized by plant mediated and chemical method was observed at 445 nm and 411 nm respectively. (fig:3 & fig:4)

Figure:**3**
UV spectra of silver nanoparticles synthesised by plant mediated method

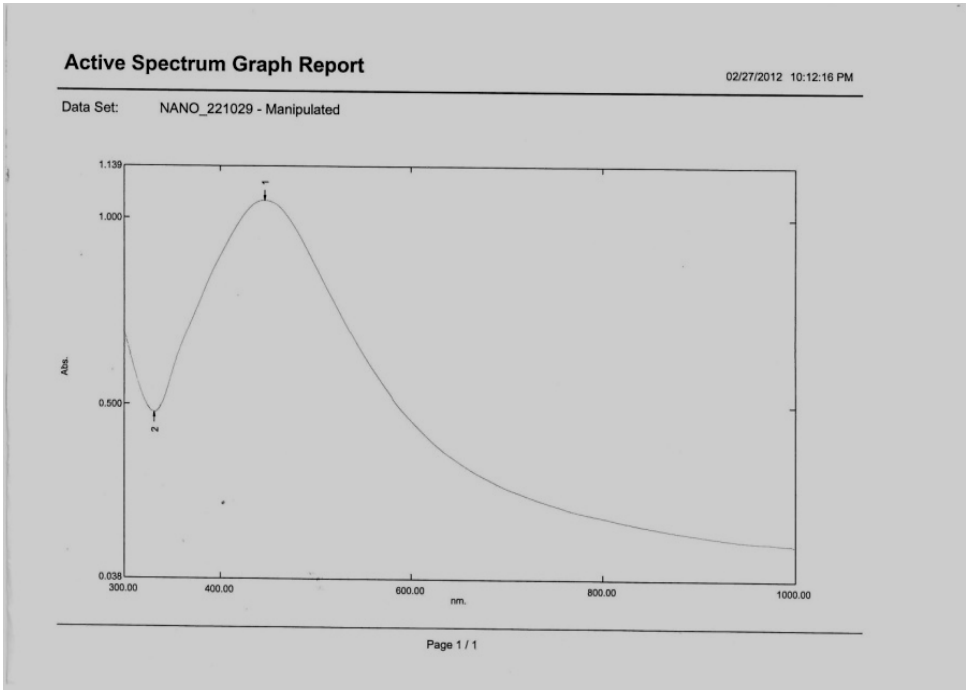
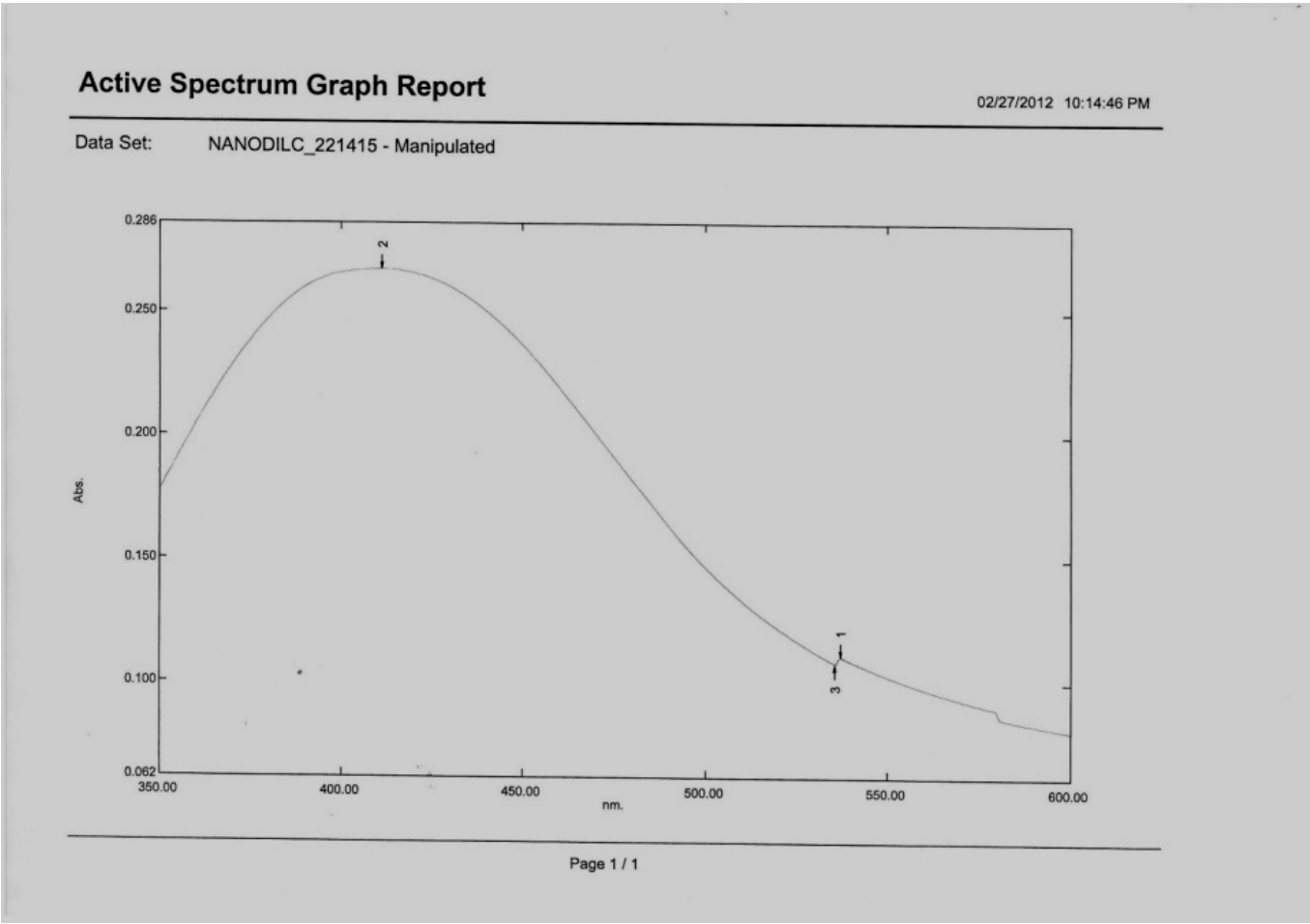


Figure 4
UV spectra of silver nanoparticles synthesised by chemical method

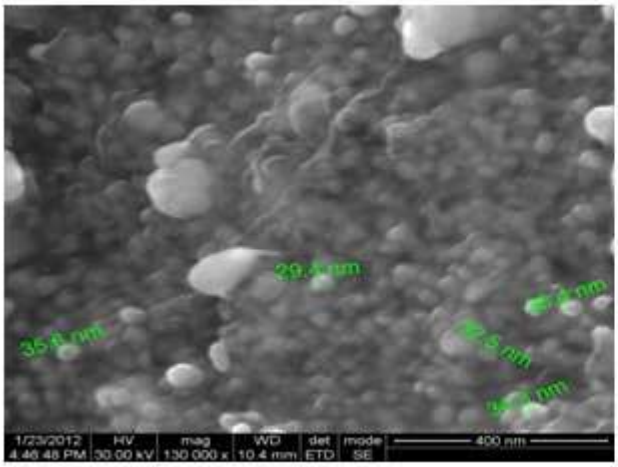


SEM ANALYSIS

The morphological charaterization of SNP was studied by HR-FEG SEM.The images obtained for the samples confirmed the presence of silver nanoparticles. Scanning electron microscopy studies revealed that the size of silver nanoparticles were ranging from 29-36 nm for plant mediated method.(fig:5) and 26-149 nm for chemical method.(fig:6)

Figure:5

SEM image and EDAX data of silver nanoparticles synthesised by plant mediated method



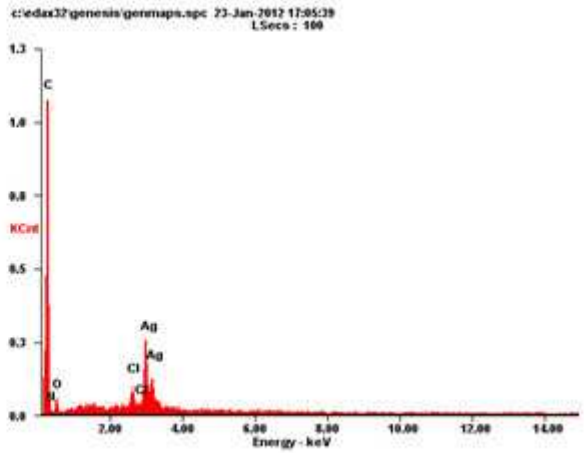
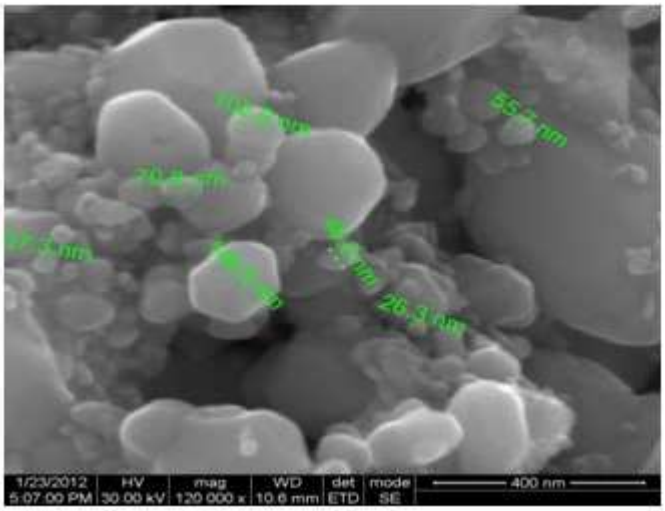
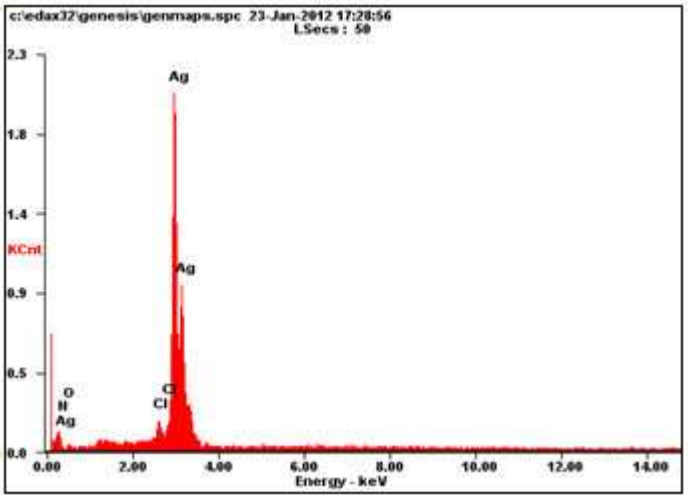


Figure:6

SEM image and EDAX data of silver nanoparticles synthesised by chemical method

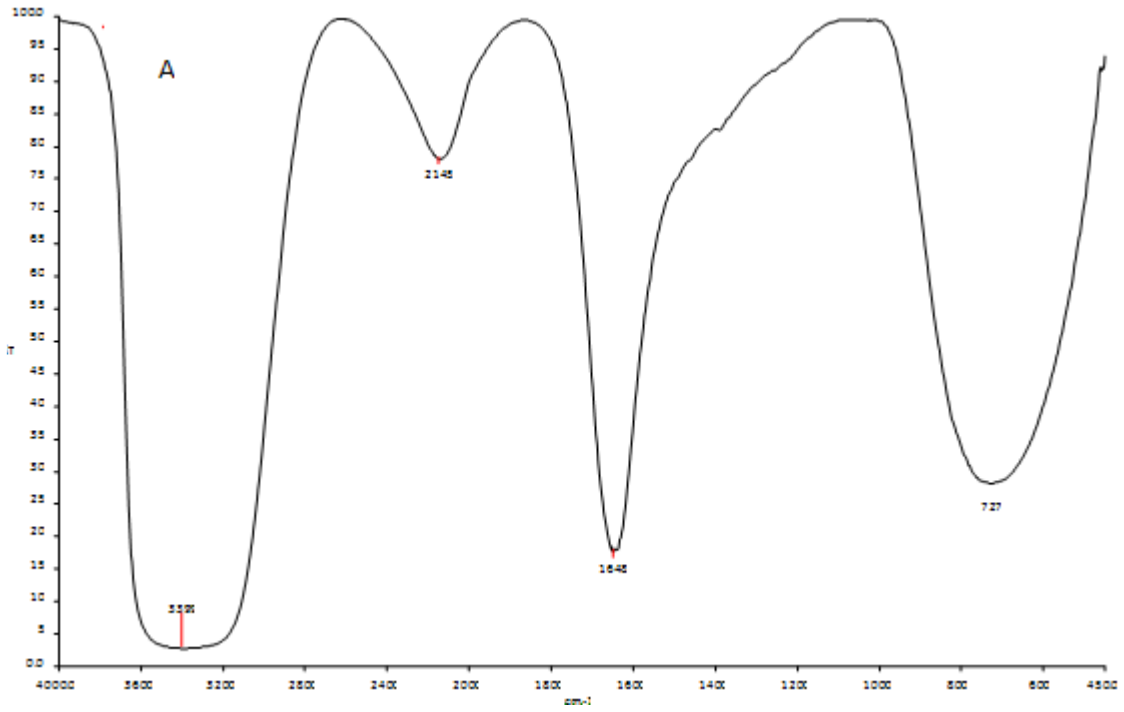




FTIR ANALYSIS:

The peaks were obtained at 3399 cm-^{1} correspond to N-H amide group. The absorption peak at 2145 cm-^{1} correspond to C=C group. The peak at 1648 cm-^{1} correspond to C=O functional group with a stretched type of vibration and the intensity is strong. The peak at 727cm-^{1} correspond to C-Cl functional group(Fig. 7)

Figure :7
FTIR analysis of silvernanoparticles produced by plant mediated method



ANTIMICROBIAL ACTIVITY

The strong antimicrobial activity of synthesised silver nanoparticles was evident from the clear zone of inhibition which may be attributed to the high affinity of silver towards sulphur or phosphorus. The bacterial cell membrane contains abundance of sulphur containing proteins, SNP can react with these proteins, leading to the inhibition of enzyme functions or interact with phosphorous moieties in DNA , resulting in inactivation of DNA replication .The bactericidal effect of silver causes the zone of clearance.(fig:8 - fig:12 & Table 1)

Figure 8
Antibacterial activity of silver nanoparticles against Gram positive bacteria - Staphylococcus aureus

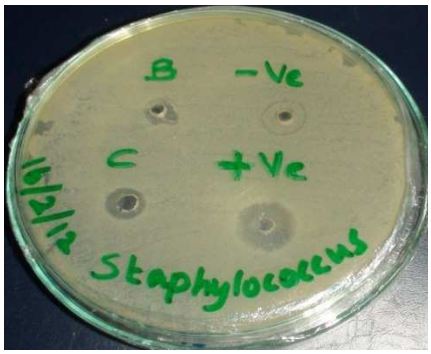


Figure 9
Antibacterial activity of silver nanoparticles against Gram positive bacteria - Bacillus subtilis

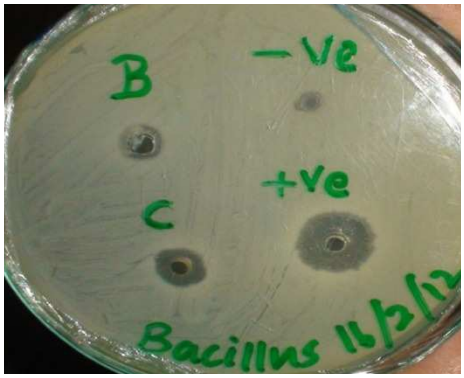


Figure :10
Antibacterial activity of silver nanoparticles against Gram negative bacteria - Pseudomonas aeruginosa

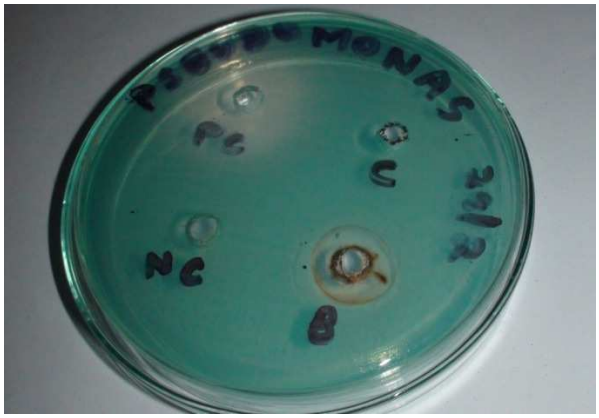


Figure :11
Antibacterial activity of silver nanoparticles against Gram negative bacteria – Klebsiella sp.

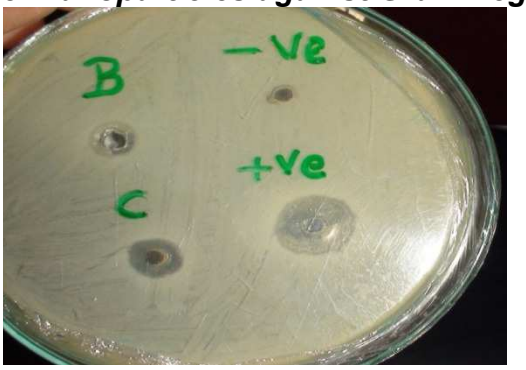


Figure 12
Antibacterial activity of silver nanoparticles against Gram negative bacteria – E.coli.

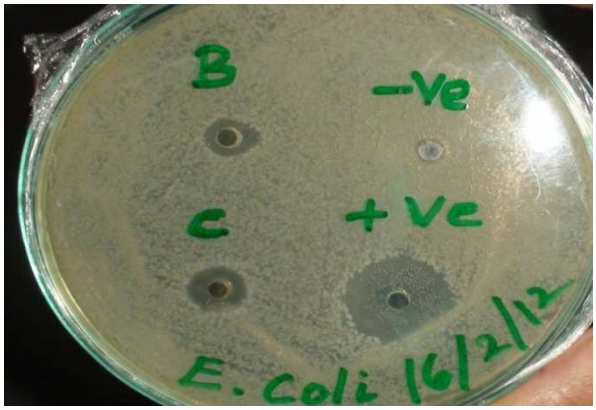


Table 1
The antibacterial activity of SNP synthesized by bio and chemoreductive methods

	Zone of Inhibition in mm			
Organism	Antibiotic streptomycin(positive control)	Neem leaf extract (negative control)	SNP sythesized by Chemical method	SNP sythesized by plant mediated method
Staphylococcus aureus	13± 0.04	-	9 ±0.02	10±0.03
Bacillus subtilis	9 ±0.02	-	8 ±0.03	8±0.04
Pseudomonas aeruginosa	17±0.15	-	7 ±0.04	10±0.05
Klebsiella	15±0.03	-	7 ±0.04	7±0.02
E.coli	12±0.04	-	6 ±0.05	9±0.15

CONCLUSION

In this study, bioreductive and chemoreductive approaches for synthesis of silver nanoparticles have been demonstrated and morphological characterization and antibacterial activity of silver nanoparticles synthesised by both the methods were performed. In UV-Visible spectra, the Plasmon resonance peaks were observed at 411 nm for chemical method and 445nm for plant mediated method which is an indication of reduction of silver ion to silver nanoparticles. Scanning electron microscopy studies revealed that the size of silver nanoparticles were ranging from 29-36 nm for plant mediatedmethod and 26-149 nm for chemical method. Through Fourier transform infrared (FT-IR) spectroscopy ,the attachment of biocomponents present in neem leaf extract to the silver nanoparticles was confirmed. The silver nanoparticles synthesised by both the methods showed strong antibacterial activity against both gram positive and gram negative bacteria.

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