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BIOSYNTHESIS OF SILVER NANOPARTICLES FROM BANANA PEEL EXTRACT AND ITS ANTIBACTERIAL ASSAY

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ABSTRACT:
The current work describes an environmentally friendly, cost-effective, quick, and simple approach for making silver nanoparticles that uses banana peel extract (BPE) as a reducing and capping agent. Characterization of silver nanoparticles was done. X ray diffraction revealed crystalline nature of silver nanoparticles. The absorbance peak at 432 nm was detected using UV-visible spectroscopy, indicating the formation of silver nanoparticles. To determine the size of synthesized silver nanoparticles, Nanoparticles Tracking and Analysis (NTA) was used. Silver nanoparticles were tested for antibacterial activity against *E. coli* and *Staphylococcus aureus*. The activity of Tetracycline was significantly improved in combination with silver nanoparticles showing synergistic activity against both the bacteria while the maximum activity was noted against *Staphylococcus aureus.*

Key words: - Bio-synthesis, AgNPs, Banana peel, Antibacterial activity, *E. coli* and *Staphylococcus aureus*

INTRODUCTION:

The field of nanotechnology is one of the most active research areas in modern materials science. Nanotechnology has been called the next industrial revolution and is based on the manufacturing of nanomaterials or particles with at least one dimension between 1 and 100 nm (Moore, 2006). Nanotechnology is a versatile subject, which deals with biology, chemistry, physics, and engineering. The term "Nano" is derived from the Greek word which means dwarf and size of particle is around 1 to 100 nm (Singh et al. 2011). Nanotechnology involves the synthesis of nanoparticles which exhibit different sizes, shapes, and morphology (Singh et al. 2011). Bio-fabrication of nanoparticles is the most flourishing area of interest in the field of nanoscience and technology (Kalaiarasi et al. 2013).

Various chemical and physical methods are known for preparation of silver and other metal nanoparticles. These methods are very costly and toxic to the environment (Kalaiarasi et al. 2013). Different chemical approaches have been tried in the past, but they have been condemned owing to numerous biological dangers, including toxicity, which has prompted a strong desire to create some environmentally friendly technologies. The plants or plants extract, which act as reducing and capping agents for nanoparticles synthesis, are more advantageous over other biological processes (Valli & Vaseeharan, 2012), because they eliminate the elaborated process of culturing and maintaining of the cell, and can also be scaled up for large-scale nanoparticle synthesis (Saxena., et al. 2012). Moreover, plant-mediated nanoparticles synthesis is preferred because it is cost-effective, environmentally friendly, a single-step method for biosynthesis process and safe for human therapeutic use (Kumar & Yadav, 2009).

Because of their antibacterial capabilities, silver nanoparticles are gaining popularity. As per reports, silver being nontoxic, safe and antibacterial can destroy over 650 different types of disease-causing microbes. These silver nanoparticles possess activity against wide range of Gram positive and Gram-negative bacteria as well as it possesses antifungal (Gupta et al. 2014) and antiviral activity (Gaikwad et al. 2013). From the prior studies, it was reported that silver nanoparticles possess antibacterial activity hence it could be used in cosmetics, in dental materials, water treatment and coating of stainless steel which is used in medical devices.

Silver nanoparticles are fabricated by the reduction of silver ions to neutral silver atoms. Silver ions are reduced by the use of reducing agents (Kaushik et al. 2010). Biosynthesis of nanoparticles is nothing but the bottom-up approach of nanoparticles synthesis. Phytochemicals present in the plants possess anti-oxidant or reducing properties which are responsible for reduction of metal compounds. Methods used for the biosynthesis of metal nanoparticles are eco-friendly, biocompatible, nontoxic and clean (Sharma and Yangard 2009).

Bananas are consumed all over the world, after consumption of the pulp, the peels are generally discarded (Bankar et al., 2010). In addition to a few applications, banana peels, that are inherently rich in polymers such as lignin, cellulose, hemicellulose and pectin (Emaga et al, 2007) could be used in the synthesis of silver nanoparticles.

The goal of this research is to synthesise silver nanoparticles via a green biological route using an extract derived from banana peel waste, their antimicrobial activity against two human pathogenic microorganisms was explored along with the characterization of the synthesized nanoparticles using UV–visible spectroscopy, transmission electron microscope (TEM), Nanoparticles tracking and Analysis system (NTA), and Fourier transform infrared spectroscopy (FT-IR) analysis.

Preparation of Banana Peel Extract (BPE): Banana peels were washed thrice with tap water and distilled water to remove any external dirt or impurities present on it. Peel was then cut into small pieces dried in shade. The peels were dried in shade for about 5-10 days until they become dry enough to be converted into a fine powder. Domestic mixer grinder was used to powder the dried peels. Peel powder was stored in clean, sterile, airtight glass container for future use.

About 10g of peel powder was taken in a 100 ml. beaker containing 50 ml. double distilled water and then the peel was boiled at 80^{o}C for 10 min and filtered through Whatman No. 1 filter paper twice to remove insoluble fractions and macromolecules. The resultant filtrate was stored at 4^{o} C.

MATERIALS AND METHODS

Detection and characterization of Ag-NPs

Visual observation: The main evidence of silver nanoparticle synthesis was observed by visual colour changes. 8 ml of 1mM of silver nitrate solution was added to 2 ml. of banana peel extract. The colour change from yellow to brick red indicates the formation of AgNPs.

UV-Vis Spectroscopy: The synthesis of silver nanoparticles was confirmed by analysing the samples in the range of 200-800 nm range with a UV-Vis spectrophotometer (Shimadzu UV1700 Japan).

Transmission Electron Microscopic (TEM) analysis: The TEM images provided further insights into the morphology and size of the synthesized silver nanoparticles.

Nanoparticles tracking and Analysis system (NTA): To find out the average size of the particles, the Ag-NPs synthesized extract was characterized by NTA. NTA is a laser-based light scattering system during which particles are suspended within the liquid medium are injected into LM viewing unit and viewed in close proximity to the optical element. The Brownian mobility of nanoparticles is required for NTA. For the analysis, Ag-NPs samples were diluted with the distilled water, and 0.5 ml of diluted sample was injected into the sample chamber and observed through CCD camera attached to LM 20 (Nanosight Ltd).

Fourier Transform Infrared Spectroscopy (FTIR): FTIR identifies the biomolecules responsible for reducing silver ions as well as stabilizing silver nanoparticles in solution. To determine the presence of capping agents and molecules involved in Ag-NP synthesis, FTIR (Perkin-Elmer FTIR-1600, USA) analysis was conducted in the 500-4000 cm*1 range.

Assessment of antimicrobial activity of Banana peel derived Ag-NPs: Antimicrobial activity of synthesized silver nanoparticles was tested against E. coli and Staphylococcus aureus. Using the Kirby-Bauer disc diffusion method, AgNPs were evaluated for antibacterial potential alone and combined with antibiotic tetracycline against E. coli, and Staphylococcus aureus.

Standard antibiotic discs of antibiotic tetracycline were purchased from Hi-Media, Mumbai. To assess the collective effects, each standard antibiotic disc saturated with 40 ppm silver nanoparticles solution was placed on to the nutrient agar surface inoculated with test bacteria. At 37^{o}C, the plates were incubated for 24 hours. The zones of inhibition were measured in mm.

RESULT & DISCUSSION:

Banana peel extract was used for the making of silver nanoparticles. A change from yellowish to brick red color is observed after adding 1mM AgNO3 to the extract (Figure 1). The change in the colour of reaction mixture within 10 min to reddish brown after 1 h, signifying the formation of silver nano-particles, due to the reduction of silver metal ions Ag^{+} into silver nanoparticles Ag^{0} through the active molecules present in the BPE (Ahmad et al., 2003). These results support with the result obtained by researchers who worked on the synthesis of silver nanoparticles from plants (Bonde 2011; Mallikarjun et al. 2011; Gupta et al. 2014).

As a preliminary confirmation of silver nanoparticles, UV-Vis spectroscopy is the most valuable method. Absorption spectra of the synthesized silver nanoparticles showed absorbance spectra at 432 nm. (Fig. 2). The synthesized silver nanoparticles showed absorbance at specific wavelength because of the surface Plasmon resonance phenomenon of silver nanoparticles (Gaikwad et al. 2013).

Nanoparticles tracking and Analysis system (NTA): Based on measuring the Brownian motion of each nanoparticle, Nanoparticle Tracking Analysis (NTA) is used to track silver nanoparticles. The Mean for size distribution was: 24 nm, Mode: 12 nm, and SD: 25 nm.

Transmission Electron Microscopic (TEM) analysis: Spherical to angular shaped nanoparticles with the average particle size of about 25 nm was observed and the particles in monodispersed nature at different magnifications with an average diameter between 20 and 32 nm.

Fourier Transform Infrared Spectroscopy (FTIR): The primary functional groups on the Banana peel extract surface were identified using FT-IR measurements, and their likely role in the production and stability of silver nanoparticles was investigated. The broader peak at 3288 cm^{-1} represented O–H stretching vibration of phenolic compounds. A weak band was observed at 1634 cm^{-1} corresponding to N–H bending primary amines. A medium band was observed at 2105 cm^{-1} corresponding to C≡C bending alkyne. These biological compounds interact with the metal salts through their functional groups and facilitate their reduction to particles of nano-scale (Bar, 2009).

Antibacterial Activity: The in-vitro activity of nanoparticles synthesized from Banana peel extract and combination of silver nanoparticles along with antibiotics were evaluated against *E. coli* and *Staphylococcus aureus.* (Figure 6). After 48 h of incubation at 37^0C, no bacterial colonies were detected in the petri dishes. The antibiotic Tetracycline was used as standard antibacterial agent against the microbes. Synthesized silver nanoparticles alone and in combination with antibiotics showed significant activity against all tested bacteria. *Staphylococcus aureus* showed maximum antibacterial and synergistic activity as compared to other two bacteria.

Silver nanoparticles have a strong bactericidal activity due to their extraordinarily large surface area, which allows for greater interaction with pathogens. Silver nanoparticles also serve as reservoirs for the Ag+ antibacterial agent. A synergistic effect was discovered when silver nanoparticles and the antibiotic tetracycline were combined, resulting in antimicrobial action against all the three bacteria. The bacterial activity is assumed to be due to the changes caused in the membranal structure of microbial cell because of its interaction with the rooted AgNPs which leads to the increase the permeability of the cell membrane and consequently, leading to their death (Awad, 2014).

CONCLUSION:

The consistent and rapid synthesis of silver nanoparticles was achieved using banana peels which is a waste material. As an alternative to conventional microbiological, physical, or chemical methods, this green synthesis method appears to be a cheaper, non-toxic, eco-friendly solution. It is also suitable for developing a biological process for large-scale production.

Crystalline, uniform, spherical, monodisperse silver nanoparticles were synthesized from banana peel extract which had an average particle size of 23.7 nm. Antimicrobial properties were demonstrated for human pathogenic bacteria tested with the synthesized nanoparticles. Additionally, they demonstrated a synergistic effect of Tetracycline on the antimicrobial activity against the Gram-positive and Gram-negative bacteria.

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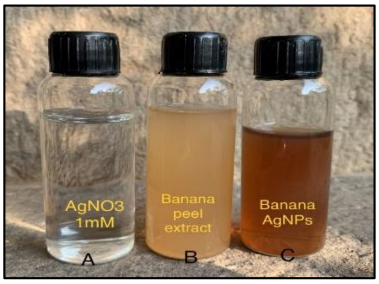


Fig 1: Synthesis of silver nanoparticles: A- 1mM $AgNO_3$, B- Banana peel extract, C- Banana peel extract after treatment of 1mM AgNO3.

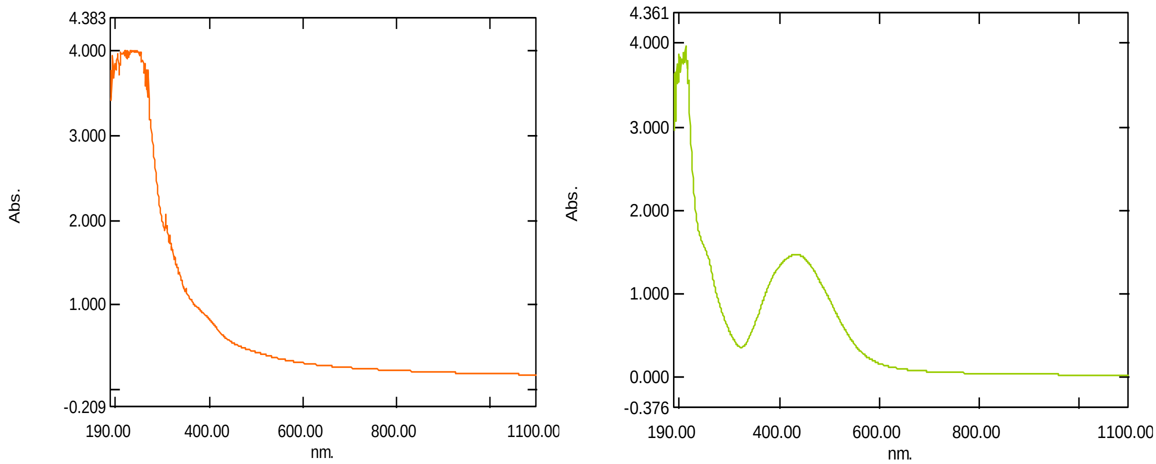


Fig 2: UV-Vis spectra of the synthesized silver nanoparticles showing absorbance at 432 nm. A- Control (banana peel extract), B- Experimental (AgNPs)

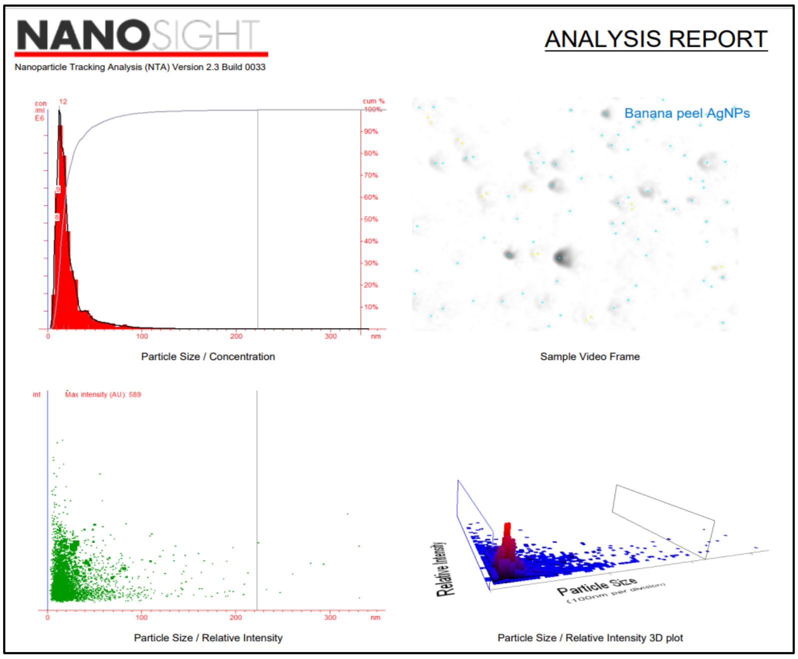
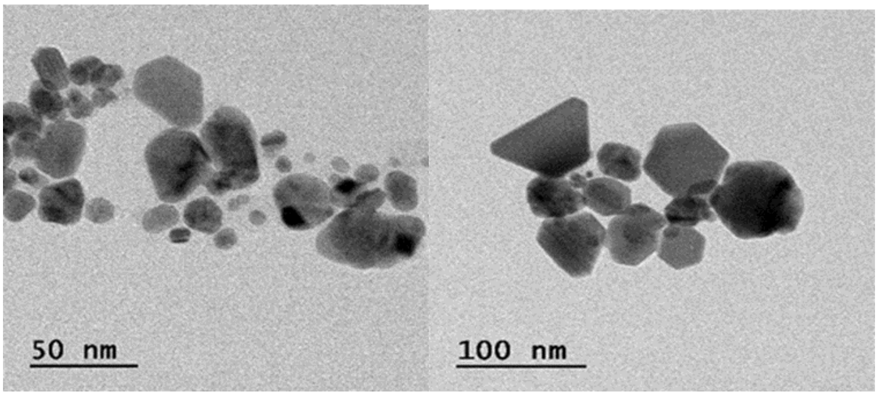


Fig. 3: Nanoparticle tracking analysis NTA (NanoSight-LM 20) histogram showing particle size distribution



Transmission Electron Microscopic (TEM) analysis

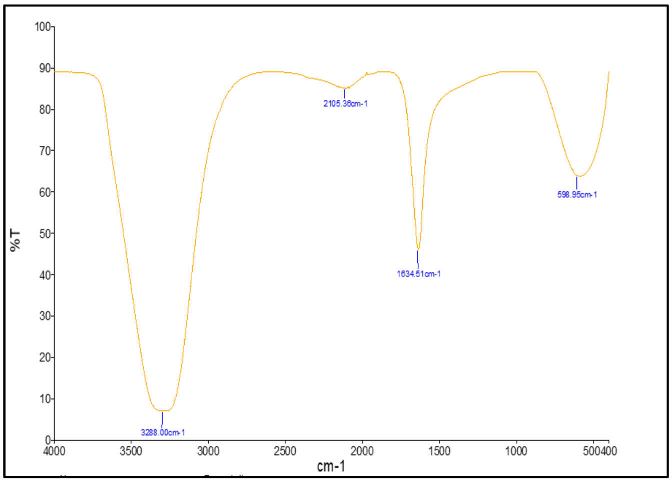


Fig. 5: FTIR spectra of banana peel extract (control) and silver nanoparticles (experimental) synthesized using Banana peel extract.

Table 1. Antibacterial activity of silver nanoparticles and its synergistic activity against two human pathogenic bacteria by disc diffusion method.

Name of test micro-organism	Diameter of inhibition zone (mm)			
	AgNPs (A)	Banana peel extract (B)	Tetracycline (C)	Tetracycline +AgNPs (D)
E. coli	14	0	19	24
Staphylococcus aureus	15	0	21	27

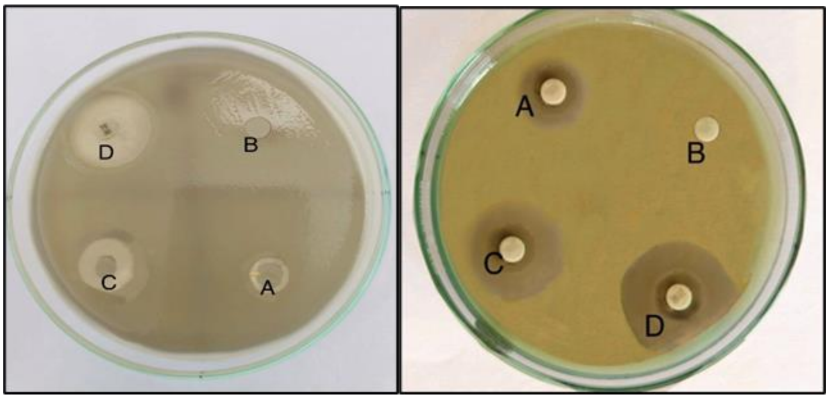


Fig 6: Antibacterial activity of silver AgNPs and its synergistic activity against *E. coli*, and *Staphylococcus aureus* where: A- AgNPs, B- Control (Banana peel extract), C- Antibiotic, D- Antibiotic + AgNPs.