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Green Synthesis, Characterization and Antimicrobial Activity of Silver Nanoparticles from *Musa paradisiaca* Flower Extract



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ABSTRACT

In this study, rapid, simple approach was applied for synthesis of silver nanoparticles using *Musa paradisiaca* aqueous flower extract. The plant extract acts as both reducing and capping agent. The extract can reduce silver ions into silver nanoparticles within 5mins after stirring the reaction mixture using magnetic bead stirrer and which was indicated forming reddish brown color. We used FTIR technique to analyze the compounds that were responsible for the reduction of silver ions and also to determine the functional groups in the Banana Plant Extract (BPE). Silver nanoparticles were characterized by many techniques like UV-Visible, SEM, TEM, EXRD, etc. The UV-Vis spectrum of silver nanoparticles revealed a characteristic Surface Plasmon Resonance (SPR) peak at 448nm. Scanning Electron Microscope (SEM) and Transmission Electron Microscope (TEM) shows spherical shaped and monodispersed nanoparticles with an average size of 47nm. Energy dispersive X-ray spectroscopy (EXRD) analysis confirmed the presence of elemental silver by showing peak in the silver region. An effective antimicrobial activity was seen for the synthesized silver nanoparticles against representative pathogens of bacteria and fungi. And we also determined the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC). The synthesized nanoparticles showed a synergistic effect with levofloxacin and amoxicillin antibiotic.



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1. INTRODUCTION

Rapid progression and technological innovations in the field of science and technology have generated immense interest in the research community across the globe to explore novel aspects of nanotechnology. Nanotechnology is making an impact in every field of life [1]. Nanomaterials are particles that are in Nano-scale size (10^{-9}), and they are very small particles with improved thermal conductivity, catalytic reactivity, nonlinear optical performance and chemical stability due to their large surface area-to-volume ratio.

Nanomaterials can be prepared by physical, chemical and biological techniques [2]. Though conventional techniques (physical and chemical methods) use less time to synthesize bulk amount of nanoparticles, they require toxic chemicals like protective agents to maintain stability, which leads to toxicity in the environment. Keeping this in focus, green technological aspects came into existence by using plants especially ornamental plants is rising as an eco-friendly, non-toxic, and safe option, since plant extract-mediated biosynthesis of nanoparticles is economically advantageous and offers natural capping agents in the form of proteins. Nanoparticle research is inevitable today not because of only application and also by the way of synthesis [3]. Nanoparticles are rapidly used nowadays for the formation of aptamer biosensor.

Noble metals like silver, gold, platinum, iron, copper etc., are known to exhibit unique optical properties due to the property of Surface Plasmon Resonance (SPR). Hence, these metals are used to prepare nanoparticles with the plant extract [4].

The main advantage of choosing silver for nanoparticle synthesis is, it is a non-toxic, safe inorganic antibacterial agent that is capable of killing about 650 types of diseases causing microorganisms [5]. And from literature review, it is clear that the silver nanoparticles have an increasing interest in the antimicrobial field [6]. They are even being projected as future generation antimicrobial agents [7]. Due to unique electrical, optical as well as biological properties of silver nanoparticles they are extensively used in all aspects and also applied in catalysis, bio-sensing, imaging, drug delivery, Nano-device fabrication and in medicine [8].

The banana plant (*Musa paradisiaca*) is the ideal to look up to when it comes to a 'no wastage' policy because almost, all parts of the banana plant can be used. The most obvious is the fruit-bananas that many of them eat almost daily. But, other parts of banana plant also offer many health benefits. People in South Asia and South-east Asia use banana flowers as a

vegetable in the daily basis either raw or steamed with dips. They also use these banana flowers in soups, curries and fried foods. The flavor resembles that of artichoke. Like artichokes, both the fleshy part of the bracts and the heart are edible. Banana flowers are called banana hearts for this reason [9]. These flowers are pretty to look, but should not dismiss them as merely as decorative elements. They have a host of health benefits like to treat infections, reduce Free Radical Activity, reduce Menstrual Bleeding, manage Diabetes & Anemia, rich source of Vitamins & Minerals, Boosts Mood & reduce Anxiety, helps Nursing Mothers etc [10].

The present study is aimed to synthesize silver nanoparticles by green synthetic approach, by using an extract derived from the flowers of *Musa paradisiaca* (Banana) and characterize the synthesized nanoparticle by using UV-Visible spectroscopy, Scanning Electron Microscope (SEM), Transmission Electron Microscope (TEM), Energy Dispersive X-ray spectroscopy (EDX), Fourier Transform Infrared Spectroscopy (FTIR) analysis and finally to screen for antimicrobial activity against the pathogens.

2. MATERIALS AND METHODS

i. Microorganisms

Representative microorganisms of Gram-positive bacteria (*Bacillus subtilis*; local isolate, *Staphylococcus aureus*; ATCC 6538) and Gram-negative bacteria (*Pseudomonas aeruginosa*; ATCC 9027, *P. aeruginosa*; local isolate, *Escherichia coli*; ATCC 8739) as well as the yeast *Candida albicans*, ATCC 120231 were used to evaluate the antimicrobial activity of prepared silver nanoparticles. Bacterial strains were maintained on nutrient agar slants and the yeast was maintained on potato dextrose agar slants at 4°C.

ii. Identification, collection and preservation of *M. accuminata*:

Musa paradisiaca flowers used for the extract preparation were procured from a local supermarket. Identification or plant authentication for this purchased flower of *Musa paradisiaca* species from various other *Musa* species was done by Dr. Noorunnisa Begum, Foundations for revitalization of local health at Yelahanka, Bangalore and then the collected flowers were stored in an air-tight container.

iii. Preparation of Plant extract

M. paradisiaca flowers were been collected and washed thoroughly in water to remove the dust and other particulate matter before the start of the process. The cleaned flowers were dried with water adsorbent paper (wet filter paper). Then, it was cut into small pieces and crushed with mortar and pestle. The fine pieces of flower in water (200 g/l) were boiled at 60°C for 20 min followed by filtering through filter paper to separate out the extract. The extract is supposed to be stored at 4°C for further experiments in a well closed container.

iv. Nanoparticles preparation:

100 ml of 1 mM of Silver nitrate (AgNO_3) metal solution was prepared. 10 ml of plant extract was added to 90 ml of metal solution for the reduction of nanoparticles. The reaction mixture was incubated in the dark at 30°C to avoid the photoactivation of silver nitrate under static conditions. The mixture was stirred with magnetic stirrer for more than 20 min. The color change is the evidence for the bio-reduction of the ions. The synthesized nanoparticles were subjected to centrifugation at 5,000 or 10,000 rpm for 15 min, which was followed by the lygtersophilisation procedures to obtain the powdered nanoparticle.

v. Characterization of synthesized silver nanoparticles

The UV-Visible spectra of silver nanoparticles were recorded as a function of wavelength using UV-Vis spectrophotometer (Shimadzu) operated at a resolution of 0.5 nm. The shape and size of silver nanoparticles were determined by SEM equipped with EDX, FESEM and TEM. For SEM and elemental analysis, the dried reaction mixtures were subjected to JEOL-JSM-5400 and SEM operating at 30 kV. The shape of nanoparticles was further characterized by FESEM (Agilent, 8500). For TEM, a drop of aqueous silver nanoparticles sample was loaded on a carbon-coated copper grid, and it was allowed to dry in room temperature, the micrographs were obtained using TEM (JEOLJEM-1200 EX) operating at 80 kV. The electron diffraction pattern for a selected area was also recorded. The average particle size and size distribution were determined by PSSNICOMP 380-ZLS particle sizing spectrophotometer. XRD pattern was carried out using X'Pert Pro X-ray diffractometer (PANalytical). The target was Cu α radiation and with $\lambda=1.54 \text{ \AA}$, the generator operated at 40 kV and 40 mA, and the scanning mode was continuous with scanning range (2θ) from $4^\circ \sim 90^\circ$. FT-IR measurements were carried out using Nicolet 6700 and by employing KBr

pellet technique. The FT-IR spectra were collected from 50 scans at a resolution of 4 cm^{-1} in the transmission mode ($4000\text{-}400\text{ cm}^{-1}$).

vi. Antimicrobial screening of synthesized silver nanoparticles

Agar well diffusion assay were used for the study of antimicrobial activity in the synthesized silver nanoparticles [11]. With the help of sterile cotton swab, the microorganisms to be tested were swabbed uniformly on the nutrient agar media and then by using sterile well bore five wells of 6mm diameter were made. Five different concentrations of 0.25, 0.50, 1.0, 1.50, and 2.0 mM synthesized silver nanoparticles of 20 μ l quantity were poured into the prepared wells. The treated wells in the plates were then incubated at $37\text{ }^{\circ}\text{C}$ for 24 or 48 h for the bacterial and yeast cultures, respectively. The diameter of inhibition zone was measured. Control sample (BPE) was used to assess the antimicrobial activity of synthesized silver nanoparticles. To determine the combined effect of silver nanoparticles and the standard antibiotic (levofloxacin), 15 ml of 0.5 mM antibiotic solution was mixed with 15 ml of silver nanoparticles solution (0.5 mM), and placed into the corresponding well of agar plate, inoculated with the tested microorganisms. The plates were incubated at $37\text{ }^{\circ}\text{C}$ for 24 h. Zone of inhibition was measured and compared with that of levofloxacin, AgNO_3 solution and silver nanoparticles individually [12].

vii. Analysis of antimicrobial activity of silver nanoparticles

The antimicrobial activity of silver nanoparticles in terms of the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) was estimated. For MIC determination, flasks containing sterile 50 ml of nutrient- or sabouraud dextrose broth media, supplemented with various silver nanoparticles concentrations (0.85, 1.70, 3.4, 5.1, 6.8 and 10.2 mg/ml), were inoculated with 0.1 ml of the microbial cultures broth (0.8 O.D 600). The flasks were incubated in shaking incubator for 24 h at $37\text{ }^{\circ}\text{C}$ and 150 rpm. Silver nanoparticles-free broth media were used as a positive control. The microbial growth was indexed by measuring the optical density (O.D 600) using UV-Vis spectrophotometer. For MBC estimation, a loopful of microbial cultures grown in nutrient broth medium supplemented with silver nanoparticles were inoculated onto nutrient agar plates or sabouraud dextrose plates free from silver nanoparticles and incubated at $37\text{ }^{\circ}\text{C}$ for 24 h. The lowest concentration of nanoparticles that prevent microbial growth was designated as the MBC.

3. RESULTS AND DISCUSSION

The criteria for selection of Noble metals were due to their unique like the property of Surface Plasmon Resonance (SPR) [13].

UV-Vis spectroscopy and color change in the reaction helped to monitor the formation of silver nanoparticles. The color of the reaction mixture started to change from greyish white to yellowish brown within 10 min and then to reddish brown after 1 h (as in figure 1a and 1b), indicating the formation of silver nanoparticles, due to the reduction reaction Ag^+ to Ag^0 i.e from silver metal ions to silver nanoparticles with the help of active molecules present in the Banana flower extract, here the flower extract acts a reducing agent or a capping agent [14]. This color is due to the excitation of SPR. As shown in Figure 2, a characteristic and well-defined SPR band for silver nanoparticles is obtained at a wavelength of 433 nm [15]. But the color change or the characteristic band feature wasn't seen in control silver nitrate solution which indicates that the abiotic reduction of silver nitrate did not occur under the used conditions.

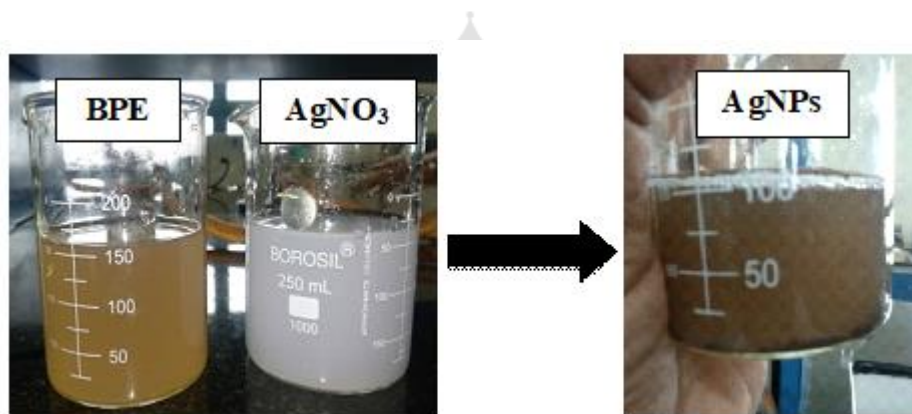


Figure No. 1a: Formation of AgNPs after addition of BPE to AgNO_3

(Note: BPE- Banana Plant Extract, AgNO_3 - Silver Nitrate and AgNPs- Silver Nanoparticles)

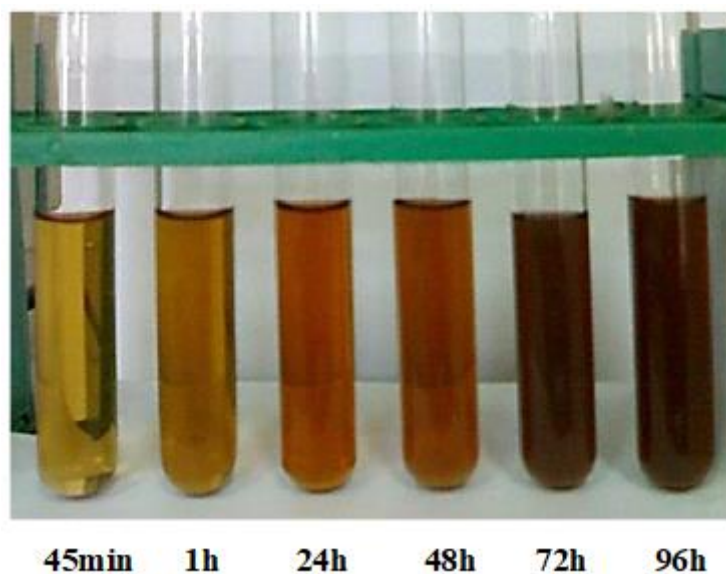


Figure No. 1b: Change in the color intensity of nanoparticle solution with respect to time

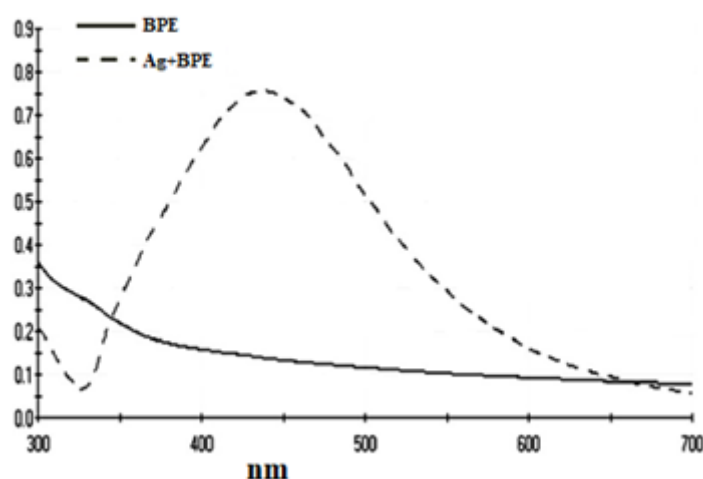


Figure No. 2: UV-Visible absorption spectra of synthesized silver nanoparticles, showing the surface plasmon resonance peak of silver nanoparticles at 448 nm

(Note: OD-Optical density, UV-Ultraviolet)

The shape of the synthesized silver nanoparticles was analyzed by SEM, representative SEM micrographs of control and treated BPE magnified at 1500 \times are shown in Figure 3a. Monodispersed spherical silver nanoparticles were formed on the surface of BPE/Banana flower extract. The image obtained by the FESEM also showed spherical nanoparticles (Figure 3b), confirming the result obtained by SEM.

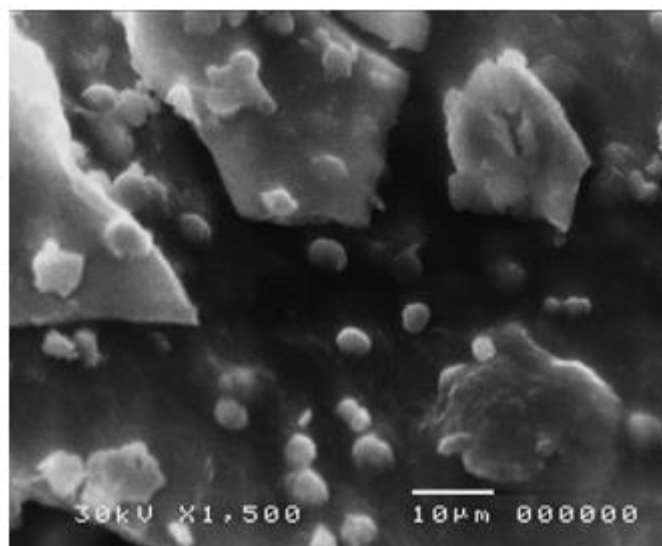


Figure No. 3a: SEM image of synthesized AgNPs

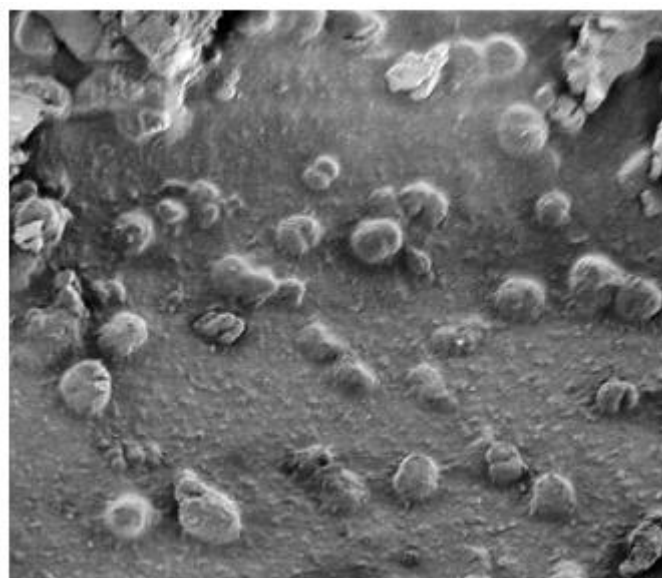


Figure No. 3b: FESEM image of synthesized AgNPs

EXRD 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 BPE shows higher counts at 3 keV due to silver, confirms the formation of silver nanoparticles (Figure 4). Generally, metallic silver nanocrystals show typical optical absorption peak approximately at 3 keV due to their SPR [16]. The elemental analysis of the silver nanoparticles shown in Figure 4 revealed highest proportion of silver followed by Cl and P.

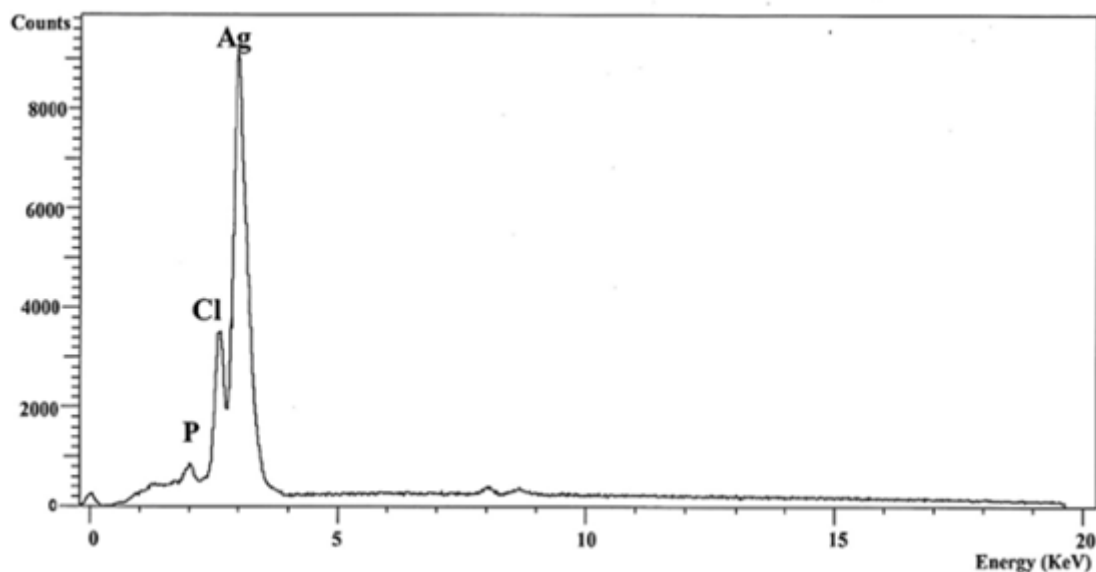


Figure No. 4: EXRD profile of the synthesized silver nanoparticles (AgNPs)

The TEM image showed monodispersed silver nanoparticles with spherical shape (Figure 5a), confirming the results obtained by SEM and FESEM. Crystalline nature of the nanoparticles is evidenced by the selected area electron diffraction patterns with bright circular spots (Figure 5a). The average particle size was determined by DLS method, and it was found to be 23.7 nm as revealed in the size distribution graph (Figure 5b).

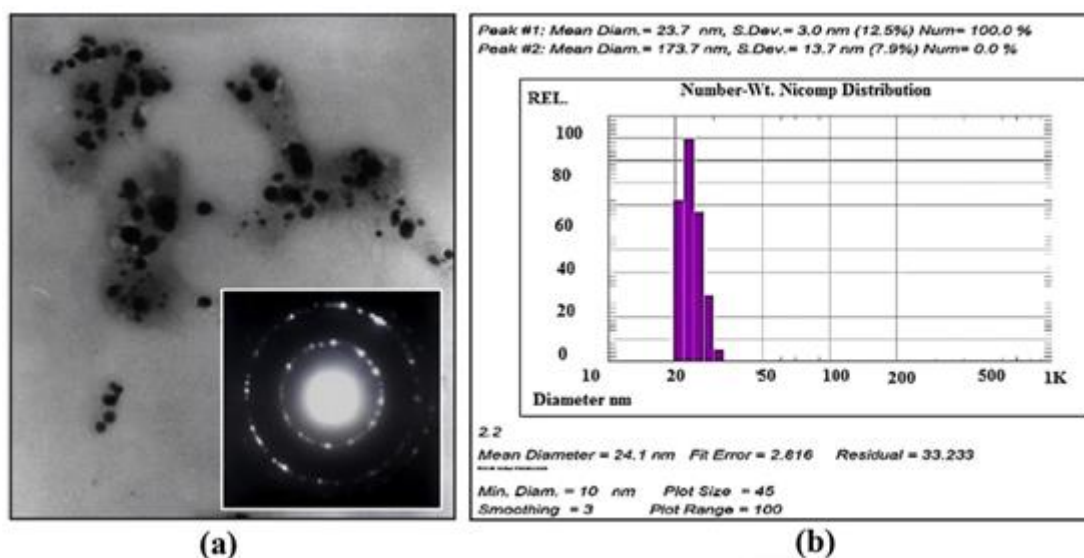


Figure No. 5: TEM micrograph of the silver nanoparticles, the scale bar corresponds to 100 nm (a) (inset: selected area electron diffraction pattern showing the characteristic crystal planes of elemental silver), and their particle size distribution histogram (b).

The crystalline nature of silver nanoparticles was confirmed by the analysis of XRD (X-Ray diffraction) pattern as shown in Figure 6. 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 20) and (3 1 1) reflection planes of face centered cubic structure of silver. In addition to the Bragg peaks representative of silver nanocrystals, additional peaks were also observed at 27.89° , 32.24° , 46.26° , and 54.79° . These peaks are due to the organic compounds which are present in the extract and responsible for silver ions reduction and stabilization of resultant nanoparticles [17]. The XRD pattern obtained is consistent with earlier reports [18].

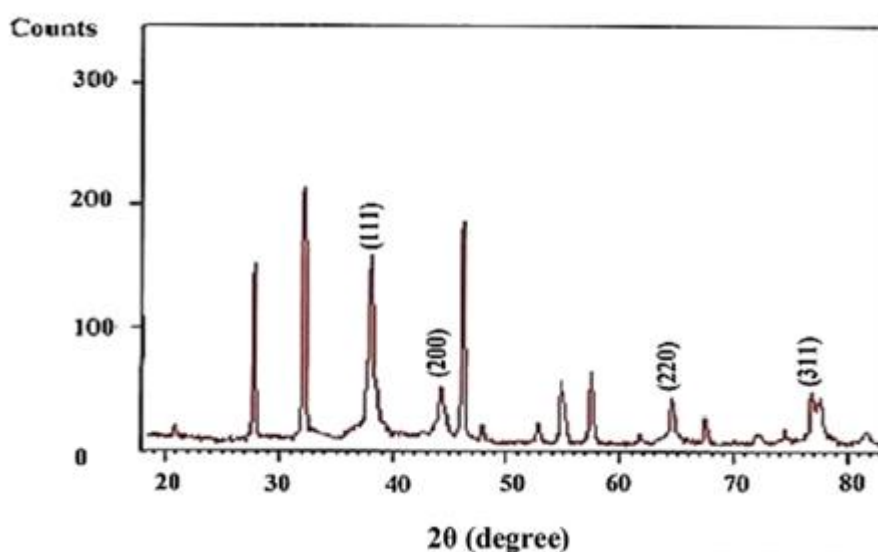


Figure No. 6: X-Ray diffraction spectrum of silver nanoparticles.

FT-IR measurements were carried out to identify the major functional groups on the BPE surface and their possible involvement in the synthesis and stabilization of silver nanoparticles. The spectra of BPE before and after reaction with silver nanoparticles are represented in Figure 7. Complex nature of biological material was confirmed from several peaks of flower extract untreated with silver nitrate (control spectrum). The bands appearing at 3411.5 , 2932.6 , 1749 , 1637.6 , 1386.5 , 1146.5 , 1077 , 829.5 and 642.4 cm^{-1} were assigned to stretching vibration of O-H of alcohol or N-H of amines, C-H of alkanes, C=O of carboxylic acid or ester, N-C=O amide I bond of proteins, CH_2 of alkanes, C-O of carboxylic acid, ester, or ether, C-N of aliphatic amines or alcohol/phenol, N-H deformation of amines, and C-C bending, respectively [19]. After reaction with AgNO_3 there was a shift in the following peaks: 3411.5 to 3420.8 , 2932.6 to 2927.7 , 1749 to 1742.9 , 1637.6 to 1626 , 1386.5 to 1383.3 , 1146.5 to 1141.1 , 1077 to 1076.3 , 829.5 to 824.5 and 642.4 to 651.3 cm^{-1} indicating that

carboxyl, hydroxyl and amide groups on the surface of BPE may be participating in the process of nanoparticle synthesis [20]. Banana flowers are mainly composed of primary metabolites especially carbohydrates like pectin, cellulose and hemicelluloses and other proteins, fats. It also has secondary metabolites like glycosides, alkaloids, tannins etc., [21] and the functional groups associated with these polymers as well as the proteinaceous matter may thus be involved in reducing the Ag^+ to Ag^0 . Biological components are known to interact with metal salts via these functional groups and mediate their reduction to nanoparticles [22].

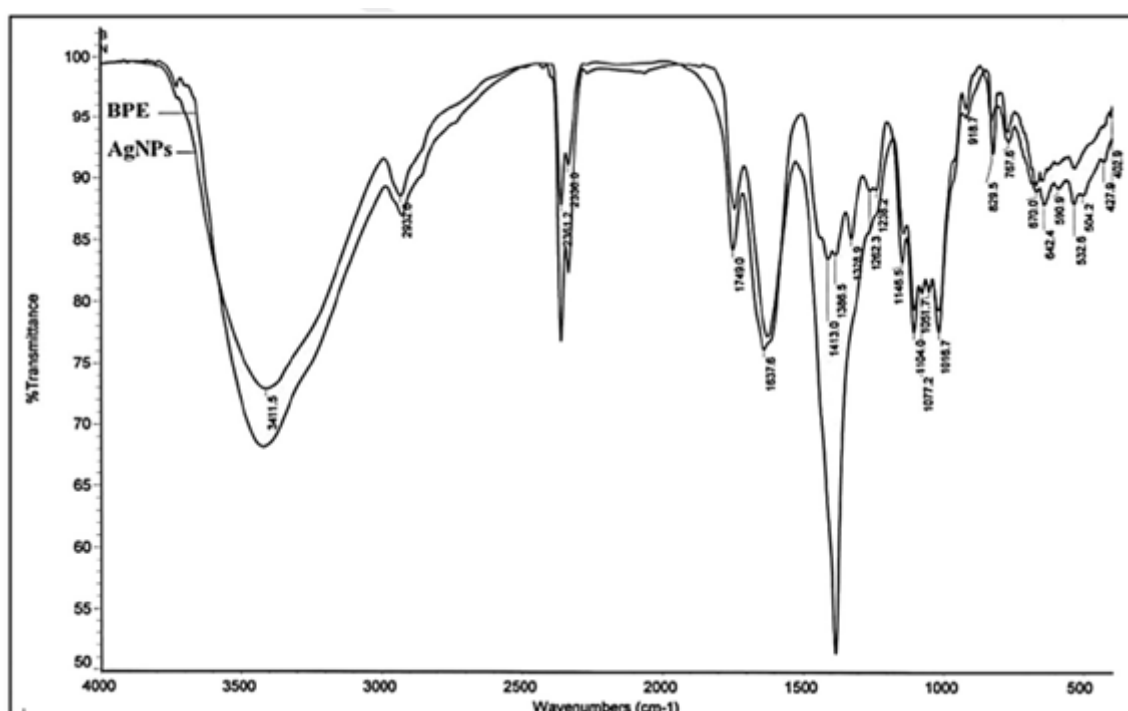


Figure No. 7: FTIR spectra of Banana Plant Extract (BPE) and silver nanoparticles (AgNPs)

AgNPs showed a very good antimicrobial activity against studied pathogenic microorganisms, with varying degrees, as proved with the diameter of inhibition zone, while flower Extract couldn't show antimicrobial activity in better way (figure 8). The Gram -ve bacteria (*E. coli* and *P. aeruginosa*) showed larger zones of inhibition with good values than compared to the Gram +ve bacteria (*B. subtilis* and *S. aureus*) (figure 9a), which is due to the variation in cell wall composition especially mycolic acid. A thick peptidoglycan layer along with a linear polysaccharide chain were found to be cross linked by short peptides in case of gram positive bacteria, this type of layer in the cell wall gives a rigid structural character to

the bacteria making the penetration of silver nanoparticles difficult. Whereas in case of gram negative bacteria this rigid structure in the cell wall is not found or it may have a thinner peptidoglycan layer [23]. AgNPs couldn't show greater antifungal activity against *C. albicans* due to smaller zone of inhibition. Various mechanisms are responsible for the bactericidal properties of AgNPs against microorganisms. First, silver nanoparticles attach to the negatively charged cell surface, alter the physical and chemical properties of the cell membranes and the cell wall and disturb important functions such as permeability, osmoregulation, electron transport and respiration [24, 25, 26, and 27]. Second, silver nanoparticles can cause further damage to bacterial cells by permeating the cell, where they interact with DNA, proteins and other phosphorus- and sulfur-containing cell constituents [28, 25]. Third, silver nanoparticles release silver ions, generating an amplified biocidal effect, which is size and dose-dependent [29, 24].



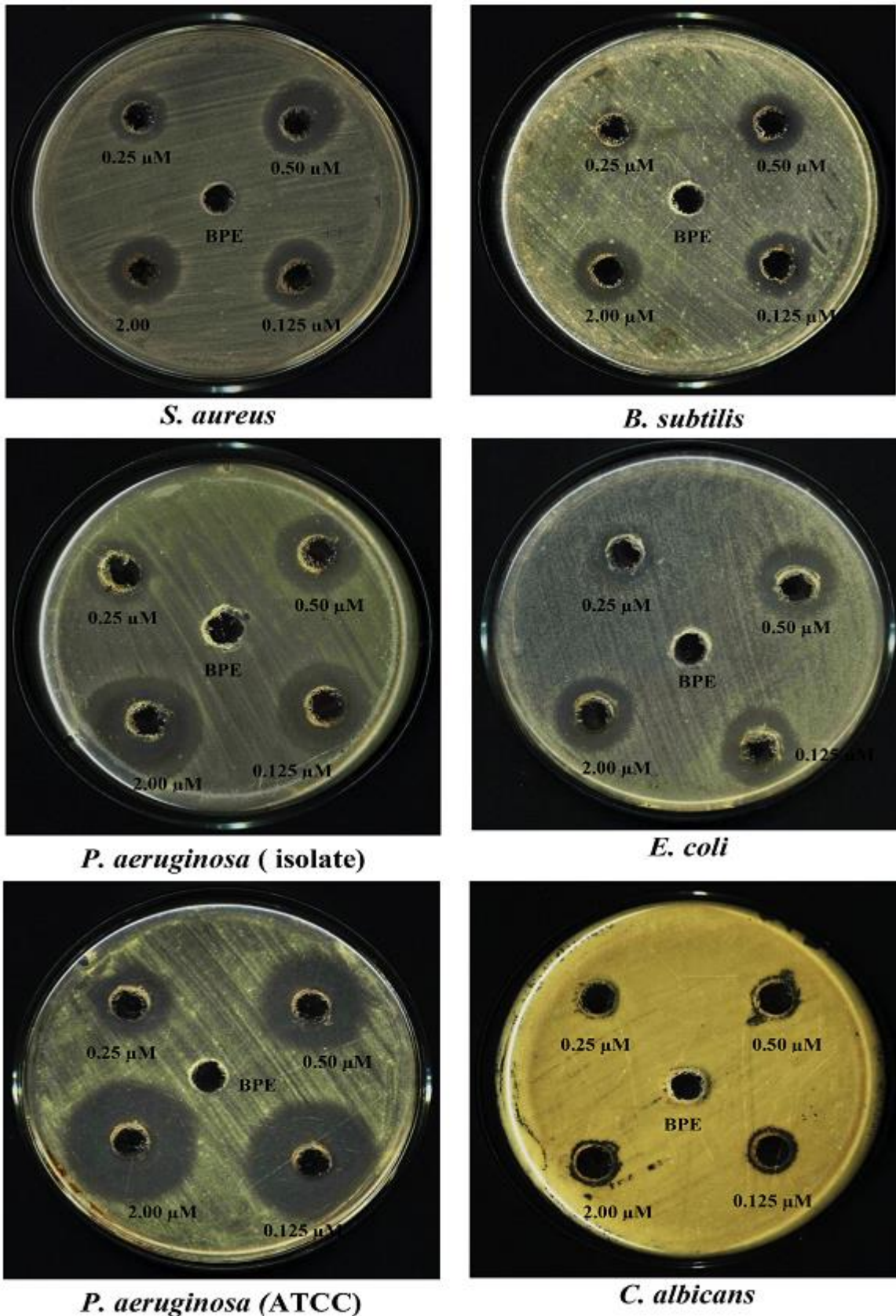


Figure No. 8: Zone of inhibition of AgNPs against various pathogenic microorganisms

MIC and MBC were used to analyze the antimicrobial activity. MIC is the minimum or lowest concentration of an antibacterial drug which is required to inhibit the visible growth of microorganisms in prescribed conditions. In general Agar dilution and broth, dilution are the most commonly used determine the MIC of antimicrobial agents, in both approaches [30]. There was no growth of *B. subtilis*, *S. aureus*, *P. aeruginosa* and *E. coli*, in the conical flasks supplemented with 6.8, 5.1, 1.70 and 3.4 mg/ml of AgNPs after 24h of incubation., and the optical density was 0.026, 0.023, 0.011 and 0.012, respectively. Therefore, the MICs were 6.8, 5.1, 1.70 and 3.4 mg/ml, respectively (Figure 9b). MBC is defined as the lowest concentration of antimicrobial agent that will prevent the growth of microorganism after subculture onto nanoparticles-free media. The MBCs of AgNPs were found to be 10.2, 10.2, 5.1 and 5.1 mg/ml, respectively. Silver nanoparticles (AgNPs) showed more bactericidal property than compared with the silver salt, the inhibition zone diameter were between 12-20mm and 10-17mm, respectively.

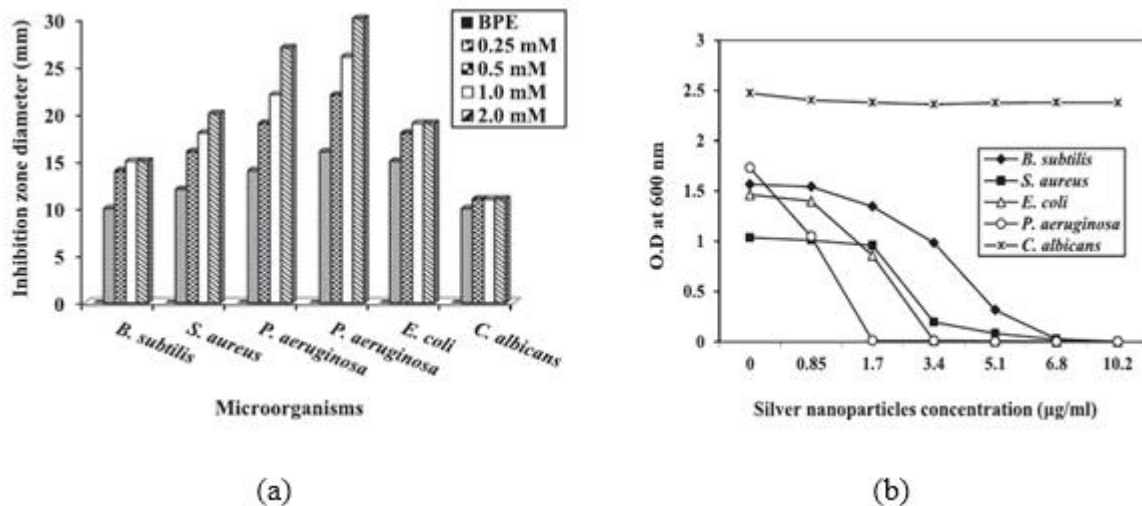


Figure No. 9: Antimicrobial activity of AgNPs against representative pathogenic microorganisms.

Table No. 1: Antibacterial activity of silver nitrate, silver nanoparticle and silver nanoparticle combined with levofloxacin against pathogenic bacteria.

Microorganisms	Diameter of inhibition zone (mm)					% of enhancement
	BPE	AgNO ₃	AgNPs	Levofloxacin	Levofloxacin+AgNPs	
<i>B. subtilis</i>	0	10	12	25	29	1.16
<i>S.aureus</i>	0.5	14	16	30	36	1.20
<i>P. aeruginosa</i> (ATCC)	0	17	20	35	40	1.32
<i>P. aeruginosa</i> (isolate)	0.5	15	18	30	38	1.27
<i>E. coli</i>	1	13	17	30	39	1.30

Note: All values represented in the table are average of results of three separately conducted experiments.

Due to the presence of large surface area on the AgNPs they provide a better contact with pathogenic microorganisms and hence AgNPs shows a higher antimicrobial. Moreover, AgNPs act as reservoirs for the Ag⁺ bactericidal agent. Combination of silver nanoparticles and antibiotic levofloxacin revealed a synergistic effect, the antimicrobial activity against *B. subtilis*, *S. aureus*, *P. aeruginosa* (ATCC), *P. aeruginosa* (isolate) and *E. coli* increased by 1.16-, 1.20-, 1.32-, 1.27- and 1.30-fold, respectively [table 1].

4. CONCLUSION

By green synthetic approach, the silver nanoparticles were synthesized from banana plant extract (flower) and the synthesized nanoparticles were characterized. Through characterization studies, it was confirmed that the formed silver nanoparticles were crystalline, uniform, spherical and monodispersed nanoparticles with average particle size of 23.7nm. The synthesized silver nanoparticles were able to possess a good antimicrobial activity against pathogenic microorganisms. AgNPs also showed a synergistic effect with levofloxacin antibiotic and proved to have broad spectrum property. Hence from this research, it is concluded that the silver nanoparticles obtained by ecofriendly and cost effective technique could be used to reduce the microbial load.

5. DECLARATION OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.





6. ACKNOWLEDGEMENT

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