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Research Article

BIOGENIC SYNTHESIS OF SILVER NANOPARTICLES USING AQUEOUS SOLUTION OF *CHERIMOYA* SEEDS EXTRACT AND ITS ANTIMICROBIAL ACTIVITY

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ABSTRACT

In this study, we report a simple and eco friendly method for synthesis of well dispersed and stable silver nanoparticles (AgNPs) using *cherimoya* seeds extract as both reducing and stabilizing agents in aqueous solution without employing any other capping and reducing agents. The arrangement of silver nanoparticles was observed by change of color from pale yellow to dark brown and the UV–vis absorption spectroscopy. The outcome of reaction time and *cherimoya* seeds amounts on the synthesis of silver nanoparticles was studied. The Size, morphology and composition of nano particles (NPs) were studied by scanning electron microscopy (SEM) and transmission electron microscopy (TEM) which showed that the silver nanoparticles were agglomerated spherical in shape with a size distribution ~ 85 nm. The crystalline structure of the silver nanoparticles was recognized by X-ray diffraction technique (XRD). The information of elemental compositions for AgNPs was carried by Energy dispersive X-ray spectroscopy (EDX). The synthesized silver nanoparticles were tested against bacteria (Gram positive such as Staphylococcus aureus and Bacillus subtilis and Gram negative such as Escherichia coli and Klebsiella pneumoniae) and fungi (Aspergillus niger and Candida albicans) and they obtained data were indicative of good antibacterial properties of the materials. The method obtainable in this paper provides a very promise move toward to synthesize other noble nanoparticles using renewable materials as capping and reducing agents.

Keywords: *Cherimoya* seeds, Silver nanoparticles, Green synthesis and antimicrobial activity.

1. INTRODUCTION

One of the most promising areas of research in modern medical science is the Nanotechnology. Nanoparticles have been used to alter and improve the Pharmacodynamic and Pharmacokinetic properties of various types of drug compounds[1-2]. In the past two decades, the noble metal nanoparticles (MNPs) were recognized to have significant applications in the fields of optoelectronics, electronic, magnetic and information storage[3-4]. Moreover, Ag NPs have been extensively studied due to their specific characteristics such as catalytic activity, optical, electronic and antimicrobial properties[5-8]. These properties were aggressively based on the size, shape, crystalline and structure of synthesized particles[9]. Hence, Ag NPs were applied in catalysis, bio sensing, imaging, drug delivery, nano device fabrication and medicine. Recently, increasing interest was observed in the synthesis of silver nanoparticles (Ag NPs) due to their different applications such as in assay as biosensors and biological tags in diagnostic applications, incorporating Ag NPs in apparel, footwear, paints, cosmetics, wound dressings and plastics. Silver nanoparticles (Ag NPs) were used in conductive inks as they can enhance thermal and electrical conductivity when incorporated in composites. Silver nanoparticles (AgNPs) exhibit metal-enhanced fluorescence (MEF) and surface-enhanced Raman scattering (SERS) thus leading to optical applications[10, 11]. In addition, silver was a safe and effective bactericidal metal which is non-toxic to animal cells and highly toxic to bacteria such as Escherichia coli and Staphylococcus aureas[12, 13].

There were several approaches in synthesis of the silver nano scale level particles such as electrochemical[14], polyol process[15, 16], sonochemical[17], electroporhoresis[18] and chemical vapor deposition method[19]. But most of these strategies consist of utilization of high energy, hazardous chemicals and complicated methods of purification. To replace these technical hitches, there were many methods on the use of naturally plentiful materials such as plants extracts[20-24]. This plant extracts function for bioreduction of silver ions to yield metallic nanoparticles.

Silver nanoparticles have arose intensive interest due to the high-efficiency, low-cost, electronic and unique optical properties which lead to potential applications in industrial fields such as catalyst[26], antimicrobial agents, conductive coating and sensors[27,28]. Silver nanoparticles with various sizes, shapes and surface properties have been synthesized by a large number of strategies such as surfactant-assisted chemical, microwave-assisted synthesis, photo reduction, radiation chemical reduction, thermal decomposition, and phase transfer method [29,30]. Among the various methods, the chemical reduction method is the most widely used due to its low cost, ease and the ease of shape and size control over silver nanoparticles[25,31,32]. Sodium boro hydride, hydrazine and ethylene glycol hydrate are frequently used as reducing agent to synthesize nano size silver particles (AgNPs)[33–35]. However, the majority of the current reducing agents cause serious environmental pollution and limit the applications of silver nanoparticles (AgNPs)[36].

A survey of literature has shown that no report on the green synthesis of Ag NPs using *Cherimoya* seeds extract. In the present study, we report the biogenic synthesis of Ag NPs using aqueous extract of *Cherimoya* seeds and to investigate the biomolecules responsible for synthesis of Ag NPs. The obtained nanoparticles were examined by using UV-Visible spectroscopy, FTIR spectroscopy, XRD, SEM, EDX and TEM. In addition, the green synthesized Ag NPs reveal admirable antibacterial (Gram positive and Gram negative) and antifungal effect against clinical isolates.

2. EXPERIMENTAL METHODS

2.1 MATERIALS

All the chemicals and reagents used in this study were of analytical grade and were used without further purification, silver nitrate (AgNO3, 99.9%) was obtained from Sigma–Aldrich Chemical Company, sodium borohydride (NaBH4, >99% pure) and sodium hydroxide be purchased from Merck (India). Ethyl alcohol and acetone were obtained from Merck (Germany) and were used at the desired dilutions. Throughout the procedures, double deionized (DI) water was used.

2.2 Preparation of seeds extract from *Cherimoya*

The *Cherimoya* seeds were collected from local market, Tirupati, Andhra Pradesh, India. The seeds were sieved to remove impurities and sun dried for a week time to remove the residual moisture. The dried *Cherimoya* seeds be powdered in burr mill and after that sieved to obtain finer powder. 10 g of the fine seeds powder was dispersed in 100 mL of sterile distilled water in 250 mL of round bottom flask and refluxed for 30 min at 60 ^{o}C until the colour of aqeous extract solution changes from watery to light yellow. Then the resultant extract was cooled to room temperature and filtered firstly through sterile muslin cloth and then through Whatmann No.1 filter paper. The filtrate was used for the preparation of Ag NPs.

2.3 Green synthesis of silver nanoparticles

50 mL of aqueous *Cherimoya* seeds extract was taken in to 250 mL round bottom flask. In the direction of this 50 mL of 1 mM $AgNO_3$ was added and stirred for 1 h. Then the reaction mixture was kept at room temperature for overnight. Finally, the colour of solution changed from pale yellow to dark brown. The Ag NPs present in the solution were confirmed by the dark brown colour. As the present method for synthesis of Ag NPs with *Cherimoya* seeds extract in aqueous solution was made without any additional hazardous chemicals, this pathway satisfies pure green eco-friendly process.

2.4 Characterization

The Ultraviolet-visible (UV-vis) absorption spectra of the apparent colloid solution were performed on UV-vis spectrometer (Shimedzu 2400 UV-vis double beam model) at a resolution of 1 nm in 200-800 nm wavelength range. The stage purities of as synthesized compounds were chequered by X-ray diffraction (XRD) technique. The XRD measurements were recorded on a Seifert 3003 TT X-ray diffract meter with Cu Kα radiation with a wavelength of 1.52 Å. The quantitative elemental analysis of the NPs were carried out an Oxford instruments Inca Penta FET × 3 energy dispersive spectrum. The Fourier transforms infrared (FT-IR) spectra of AgNPs and cherimoya seeds extract was carried out with a BRUKER-Alpha. The Size, morphology and composition of nano particles (NPs) were studied by scanning electron microscopy (SEM) and transmission electron microscopy (TEM). The information of elemental compositions for AgNPs was carried by Energy dispersive X-ray spectroscopy (EDX).

2.5 Antimicrobial Activity

The antimicrobial activity of the synthesized AgNPs against pathogenic organisms such as bacteria (Gram positive such as Staphylococcus aureus and Bacillus subtilis and Gram negative such as Escherichia coli and Klebsiella pneumoniae) and fungi (Aspergillus niger and Candida albicans) was measured using the well-diffusion method[37]. Pure cultures of bacteria and fungi were grown in Mueller-Hinton broth (Sigma, USA) for bacteria and Sabouraud broth for fungi at 35^0C and 30^0C respectively on a rotary shaker at 180 rpm. Wells were made with 6 mm in diameter on the Muller - Hinton agar and Sabouraud agar dishes using a gel puncture and each well was inoculated with individual cultures. The AgNPs in various concentrations (10, 20 and 30 μg/mL) were burdened in each well. The negative and positive controls were also maintained and the plates (triplicates) were incubated at 35^0C and 30^0C for 24 and 48 hrs. Simultaneously, the synergistic special effects of different mercantile antibiotics (Amoxicillin and Nystatin, Sigma, USA) with AgNPs against multidrug resistant pathogens were also checked in well diffusion method. After incubation, the liability pattern of the test organisms was determined by measuring the diameter of the zone of inhibition for well diffusion method.

3. RESULTS AND DISCUSSION

3.1 UV- Visible Spectral Analysis of Ag NPs

The bioreduction of pure Ag^+ ions was made with the seeds samples of *Cherimoya* was investigated by periodic sampling of the 1mL aliquots and the optical absorbance of AgNPs suspended in distilled water was characterized by UV-Vis spectroscopy. Fig. 1(a) represents the UV- vis absorption spectra for the seed extract of *Cherimoya.* It is clearly observed that there is no absorption in visible region for *Cherimoya* seed extract sample. Whereas Fig. 1(b) shows the UV- Vis absorption spectra for synthesized Ag NPs was showed absorption peak at 436 nm. However, the color of solution changed from pale yellow to dark brown depending on the extract concentration indicating silver nanoparticles formation as the color change observed is due to excitation of surface Plasmon vibration in the silver nanoparticles. A surface Plasmon resonance spectrum of silver nano particles (Ag NPs) was obtained at 436 nm. Similarly, several earlier researchers have also observed the absorption spectrum between 410 and 450 nm due to surface Plasmon resonance in AgNPs[38, 39].

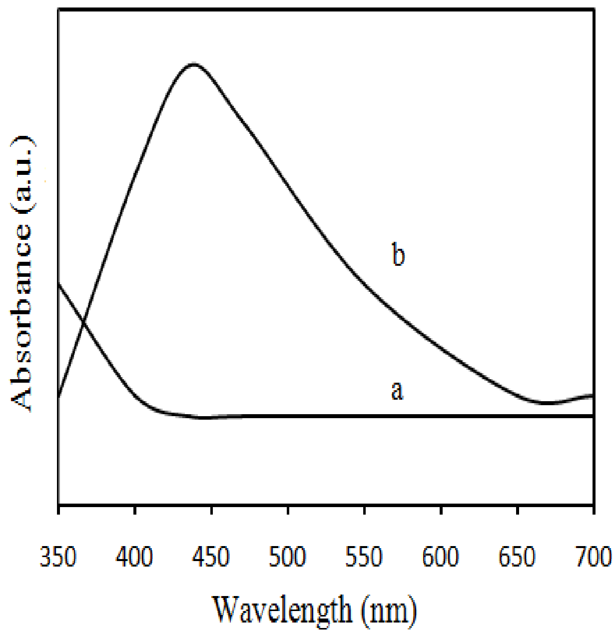

Fig. 1: UV–Visible absorption spectra of (a). Seed extract of *Cherimoya* (b). Synthesized AgNPs

3.2 FT-IR Characterization

FT-IR analysis was used to understand the existence of surface functional groups in metallic interactions. Fig. 2a shows the FT-IR spectrum of *Cherimoya* seeds extract. The bands in the region of 3580- 3250 cm^{-1} were assigned to hydroxyl stretching of poly phenols, where as carbonyl stretching frequency peak appears at 1695 cm^{-1}. Fig. 2b indicates the appearance of the new peaks at 3560-3236 cm^{-1} and 1640 cm^{-1}. The FT-IR results indicate the presence of polyphenols and other biomolecules in the *Cherimoya* seeds extract which have reduced Ag^+ to Ag as well as stabilizing Ag NPs.

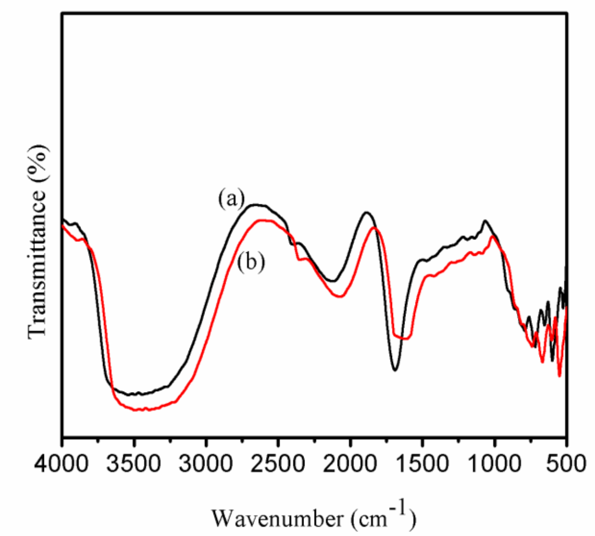

Fig. 2: FT-IR Spectrum of (a) *Cherimoya* seeds extracts and (b) synthesized AgNPs

3.3 XRD Analysis

The green synthesized Ag NPs were highly crystalline with diffraction peaks could be obviously assigned to the face-centered cubic phase of metallic silver. Fig. 3 shows five diffraction peaks present in the spectra at 2θ values of 38.1°, 44.3°, 64.5°, 77.4° and 81.6° were indexed to the (111), (200), (220), (311) and (222) reflections of the FCC structure of metallic silver. The normal grain size of the silver nanoparticles (AgNPs) formed in the bioreduction process was determined using scherr's formula D = 0.89 λ/β Cos θ where D is the average particle size, λ is the wavelength of the X-ray, β is the full breadth at half utmost intensity of the diffraction peak and θ is diffraction angle of the (111) plane of cubic silver nanoparticles and the calculated particle size is ~76 nm which is quite close to TEM result. The lattice constant calculated by the formula:$1/d^2=1/a^2 (h^2+k^2+l^2)$ where 'a' is lattice parameter, d_{hkl} is the inter planar separation corresponding to miller indices h,l,k and the designed lattice constant from this pattern was a = 4.081A, which was very close to the JCPDS card No.89-3722. It clearly exhibited the green synthesized silver nanoparticles are nano crystalline in nature.

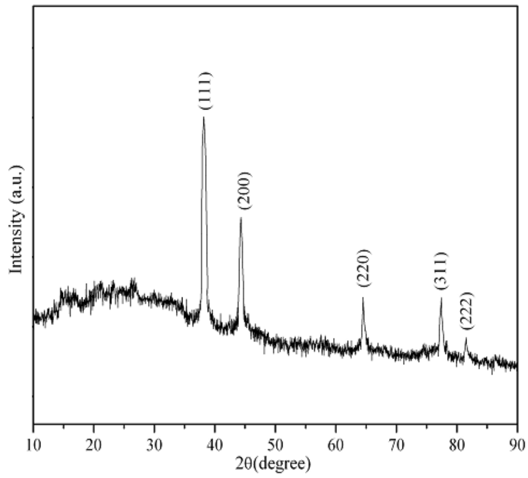

Fig. 3: XRD Pattern of synthesized AgNPs

3.4 SEM along with EDX analysis

The morphology, size and composition of NPs were studied by scanning electron microscopy (SEM). The AgNPs were observed predominately adopt a near spherical morphology with smooth surface under the Scanning Electron Microscopy in different magnifications micrometer (μm) to nano meter (nm). The SEM images of AgNPs were assembled on to the surface due to the interactions such as electrostatic and hydrogen bond interactions between the bioorganic capping molecules bound to the AgNPs[40]. A similar phenomenon has been reported previously, where the SEM micrograph shows crystalline spherical AgNPs[41]. Fig.4 shows the maximum size of the silver nano particles was obtained between 60–100 nm. The information of elemental compositions for AgNPs was carried by EDX analysis and is depicted in Fig.5. The EDX spectrum confirmed the occurrence of muscular elemental signal of the silver at 3keV which is typical for the absorption of metallic silver nano crystallites due to surface plasmon resonance[42]. Apart from silver peaks, there are weaker signals from Ag and O elements were also observed. These weaker signals are likely to be due to X-ray emission from proteins/enzymes present in the *Cherimoya* seeds extract of the biomass.

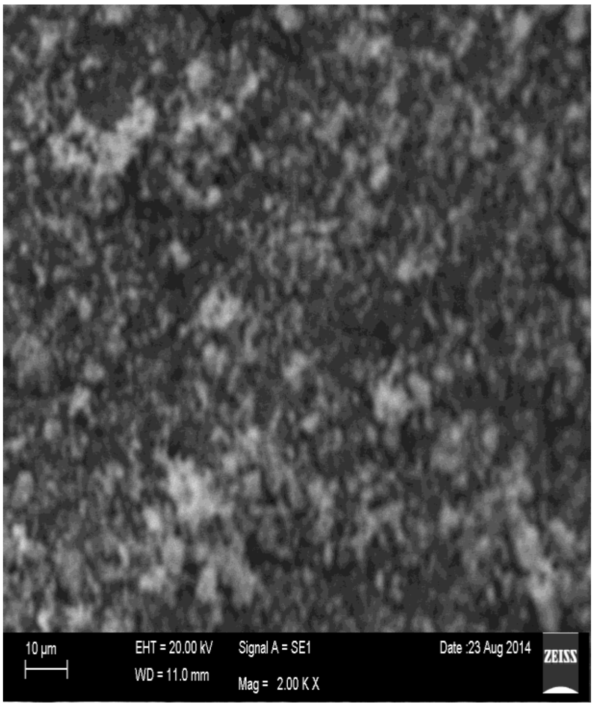

Fig. 4: Scaning electron microscope (SEM) image of synthesized silver nanoparticles

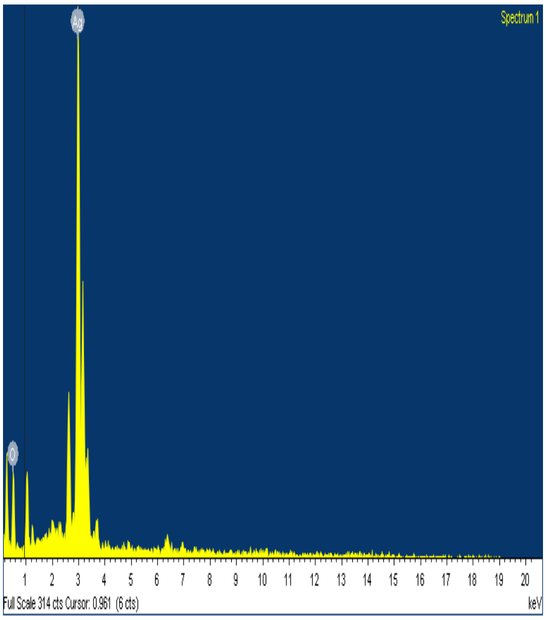

Fig. 5: Energy dispersive X-ray spectroscopy (EDX) image of synthesized AgNPs

3.5 TEM analysis

The study of the nanoparticles using TEM revealed that the Ag NPs are mono dispersed and agglomerated spherical in shape which appears to be characteristic of Ag NPs prepared from seed extract of *Cherimoya*. TEM images of the precipitated solid phase obtained after termination of the reaction between the *Cherimoya* aqueous seed extract and $AgNO_3$ solution are shown in Fig.6. The TEM image shows size range 60 nm to 100 nm and the average particles size is ~ 85 nm. The above results indicate the agglomerated spherical shape and elemental silver formed by a facile manner.

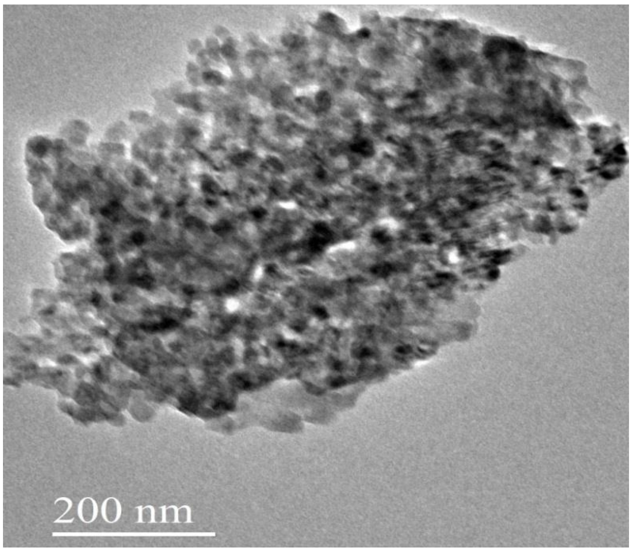

Fig. 6: Transmission electron microscope (TEM) image of synthesized silver nanoparticles

3.6 Antimicrobial Activity of the AgNPs

In this study, the antimicrobial activity of AgNPs using well-diffusion method was evaluated. The microbial activity of the AgNPs were examined against bacteria (Gram positive such as Bacillus subtilis and Staphylococcus aureus and Gram negative such as Klebsiella pneumoniae and Escherichia coli) and fungi (Aspergillus niger and Candida albicans). In this analysis, the AgNPs displayed antimicrobial activity against a range of various pathogenic microorganisms (Table 1). The signify of three replicates of the diameter of the zone of inhibition (30 μg/mL) for each microorganism was determined to be about 19.2 ± 0.20, 22.4 ± 0.11, 18.7 ± 0.31, 19.5 ± 0.22, 17.2 ± 0.25 and 22.5 ± 0.24 mm, respectively, for Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Klebsiella pneumonia, Aspergillus niger and Candida albicans. The highest antimicrobial activity was observed against *Bacillus subtilis*, Klebsiella pneumonia and *Candida albicans*. These findings are in concurrence with previous studies that examined the antimicrobial activity of AgNPs against *Bacillus subtilis* and *Candida albicans*[43]. The antimicrobial activity of silver nanoparticles due to the access into the bacteria, damage of cell membrane and release of cell contents[44]. Another possibility suggested that was the release of silver ions from the nanoparticles (NPs), which can contribute to the bactericidal properties of silver nanoparticles (AgNPs)[45, 46].

Table 1: Antimicrobial activity of the AgNPs against various pathogenic micro-organisms

Micro- organisms	Zone of inhibition (mm in diameter)			Antibiotics 30 μg/mL
	10 μg/mL	20 μg/mL	30 μg/mL	
Bacteria (Gram positive)				**Amoxicillin**
Staphylococcus aureus	7.6 ± 0.25	15.2 ± 0.32	19.2 ± 0.20	21.3 ± 0.10
Bacillus subtilis	11.1 ± 0.34	19.5 ± 0.24	22.4 ± 0.11	23.7 ± 0.24
Gram Negative				
Escherichia coli	7.4 ± 0.34	15.3 ± 0.32	18.7 ± 0.31	19.3 ± 0.10
Klebsiella pneumonia	9.2 ± 0.14	17.8 ± 0.31	19.5 ± 0.22	21.3 ± 0.30
Fungi				**Nystatin**
Aspergillus niger	6.8 ± 0.32	13.5 ± 0.23	17.2 ± 0.25	18.2 ± 0.11
Candida albicans	9.4 ± 0.21	18.2 ± 0.22	22.5 ± 0.24	24.6 ± 0.21

4. CONCLUSION

In conclusion, we have established that Ag NPs were synthesized form seeds extract of *Cherimoya* in a quick method by using eco friendly green synthesis. The synthesized silver nanoparticles were characterized by X-ray diffraction (XRD), UV-Vis absorption spectroscopy, Fourier transform infrared (FT-IR), transmission electron microscope (TEM), scanning electron microscopy (SEM) and X-ray energy dispersive spectroscopy (EDX). These nanoparticles are found to be highly crystalline as evidenced by the peaks in the XRD pattern corresponding to Bragg reflections from the (111), (200), (220), (311) and (222) planes of the FCC structure. The average size of the particle is found to be ~ 85 nm from TEM image analysis. The maximum size of the silver nano particles was obtained between 60–100 nm from SEM analysis. The EDX spectrum confirmed the presence of strong elemental signal of the silver at 3keV and there are weaker signals from Ag and O elements were also observed. The microbial evaluation of these nanoparticles indicated that they are good antimicrobial activity when compared to the standard drugs.

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