



ANTIBACTERIAL EFFECT OF SILVER NANOPARTICLES OBTAINED BY GREEN SYNTHESIS METHOD USING MILLETIA PINNATA AQUEOUS EXTRACT

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ABSTRACT

In this work, we have investigated the use of Millettia Pinnata leaf extract as a powerful reducing as well as capping agent for the synthesis of silver nanoparticles (AgNPs) at room temperature. The synthesized samples were characterized by Powder X-ray diffraction, UV analysis, FTIR analysis, Cyclic voltammetric studies, Impedance studies and Fluorescence studies. From powder-XRD studies, the particle size was calculated. The formation of silver nanoparticles was confirmed by observing the colour change from pale yellow to dark brown colour and an intense peak was observed in the UV-Spectrophotometer thereby the energy band gap is calculated. FTIR spectrum reveals the various functional groups present in the sample. The electrochemical redox properties of silver nanoparticles were carried out using Cyclic voltammetry and the scan rate defines the behavior of the electroactive species on the electrode surface. Impedance studies investigated the impedance changes at the electrode surface. The properties of emission and excitation of silver nanoparticles were studied in Fluorescence studies.

KEY WORDS: *Green synthesis, cyclic Voltammetry, silver nanoparticles, Impedance, Fluorescence.*

1. INTRODUCTION

Nano science has been established recently as a new interdisciplinary science. It can be defined as a whole knowledge on fundamental properties of nano-size objects. Nanoparticles, generally considered as particles with a size up to 100 nm, exhibit completely new properties or improved properties as compared to the bulk material that they are collected based on particular characteristics such as size, distribution and morphology [1]. Recent developments in nanoscience and nanotechnology have brought potential building blocks for electronics, optoelectronics, medicines and solar cells [2]. Nanoparticles of noble metals, such as gold, silver and platinum are broadly applied in many fields and also directly come in contact with the human body, such as shampoos, soaps, detergents, shoes, cosmetic products and tooth paste, besides medical and pharmaceutical applications [3]. Nowadays, nanoparticles based on their electrical, optical, magnetic, chemical and mechanical properties are used in various areas, such as the medical sector for diagnosis, antimicrobial, drug delivery and also used in the chemical sector for catalysis for environmental protection and energy conversion.

Nowadays green synthesis procedure are generally used in various biological systems such as yeast, fungi, bacteria and plant extract for synthesis of silver nanoparticles (Ag NPs) [4]. The main reason for selection of the green synthesis method is, due its low cost, non-toxic and eco-friendly.

Milletia Pinnata is a species of tree in the pea family, Fabaceae, native in tropical and temperate Asia including parts of Indian subcontinent, China, Japan, Malaysia, Australia and Pacific islands[5]. It is often known by the synonym Pongamia Pinnata as it was moved to genus Milletia only recently. Common names include Indian beech and Pongam oiltree[6]. It has been applied as crude drug for the treatment of tumors, piles skin diseases and ulcers. The root is effective for treating gonorrhoea, cleaning gums, teeth, in ulcers and is used in vaginal and skin diseases[7].

2. OBJECTIVES

1. To develop environmentally beneficial technologies in material synthesis.
2. To utilize the large variety of active functional groups of plants in the process reduction of silver ions.
3. To produce silver nanoparticles with different morphologies.
4. To eliminate expensive and toxic chemicals and to use clean and eco-friendly method.

3. METHOD OF PREPARATION

3.1 Preparation of leaves Extract:

Milletia Pinnata leaves were thoroughly washed with deionised water for several times to remove dust particles and dried at room temperature for one day to remove all the moisture content. The dried and purified leaves was mixed with deionised water in a beaker and heated with 60°C for 5 hours. Later, an obtained leaf extracts were vacuum filtered through whatmann filter paper.

3.2 Synthesis of Silver nanoparticles:

The prepared Milletia Pinnata leaf extract was mixed with 20 ml of 1 M aqueous Silver Nitrate ($AgNO_3$) solution for the reduction of Silver (Ag). This solution was stirred in a magnetic stirrer and after 10 minutes the solution was turned into brown colour by continuous stirring for 6 hours at 50°C. After completion of 6 hours, reduction of nitrogen takes place and the dark brown colour powder was observed [8]. The precipitate of silver nanoparticles obtained was used for further purification.

4. CHARACTERIZATION

Silver nanoparticles were examined using Powder XRD, FTIR, UV-Visible spectroscopy, Cyclic voltammetry, Impedance and Fluorescence.

4.1 Powder XRD:

X-ray diffraction was carried out for the synthesized Silver nanoparticles as shown in Fig. 1. It was done to determine the crystalline nature of Ag nanoparticles. All major peaks are indexed for the face centered cubic structure of silver. The average crystal size of the silver nanoparticles was calculated from the diffraction peaks using Scherrer's formula.

$$D = \frac{k\lambda}{\beta \cos \theta}$$

Where, D is the mean crystalline size, β is the full width of the peak at half maximum intensity of a specific phase in radians, λ is the wavelength of incident rays and θ is the Bragg diffraction angle. The resulted peaks were found at 35.7°, 43.5°, 62.45° which corresponds to *hkl* planes of (111), (200) and (220) [9]. They were compared with the standard powder diffraction card of JCPDS file. A few intense unassigned peaks were also noticed which was raised at 29.89°, 32.1°, 49.6° respectively. These Bragg peaks might have resulted from some bioorganic compounds/proteins present in the Milletia Pinnata leaf extract. The average particle size estimated was approximately 17.44 nm [10]. The dislocation density of the nanoparticles were calculated from particle size by the relation,

$$\delta = \frac{1}{D^2}$$

4.2 Fourier Transform Infrared Spectroscopy:

The FTIR spectrum of Silver nanoparticles is shown in Fig.2. In this study, the FT-IR spectrum is recorded in the range of 400 – 4000 cm^{-1} . The broad and sharp peaks situated at 3442.04 cm^{-1} and 1631.94 cm^{-1} indicates N - H stretching and C = C Symmetry Stretching[9], 2927.75 cm^{-1} peak represents the presence of alkanes in lipids, 2364.35 cm^{-1} peak indicates the presence of symmetric stretching of COO^- just in the biosynthesized solution, 1416.17 cm^{-1} peak indicates C-C-C bending [11], 1416.17 cm^{-1} indicates S = O (sulfate ester) group and 560.64 cm^{-1} peak indicates N - H bending. FTIR study indicates that the carboxyl (-C=O), hydroxyl (-OH) and amine (N-H) groups of milletia pinnata leaves extract are mainly involved in reduction of Ag^+ to Ag nanoparticles. These structural changes indicates that the reduction and stabilization of silver nanoparticles proceeds via the coordinates between N of the amide group and silver ions [12]. This study confirms the fact that the amide group from proteins has the stronger stability to bind metal indicating that the protein could possibly form a layer covering the metal nanoparticles to prevent agglomeration and thereby stabilize the medium.

4.3 UV – Visible Spectroscopy:

The reacted mixture of Milletia Pinnata leaf extract and silver nitrate solution changes the colour from transparent to light brown indicating the formation of Ag nanoparticles. After 6 hours there was a significant colour change to dark brown due to increase in reaction time which enhances the growth of Ag nanoparticles. The peak shown in Fig. 3 indicates the absorption peak of Ag nanoparticles. The characteristic peak of Ag nanoparticles was observed with a maximum at 303 nm [12].

The energy band gap value of Ag nanoparticles was found to be 4.10 eV using the formula ($E_g = \frac{hc}{\lambda}$). The distinctive colours of colloidal silver were due to a phenomenon known as Plasmon absorbance. It indicates that the particles are isotropic in shape and uniform in size.

4.4 Electrochemical Studies:

The cyclic voltammogram demonstrated the electroactivity of the nanoparticles. The biosynthesized Ag nanoparticles were characterized for comparison with the Glassy Carbon Electrode (GCE). The cyclic voltammetric of cold water extract of silver nanoparticle was carried out at pH 5.0 in aqueous media. Fig. 4 shows typical cyclic voltammogram evaluated in 0.1 molar HCl as well as a bare Glassy Carbon Electrode. One cathodic peak and one anodic peak were observed. The voltammetric curves were scanned around in the potential range -1.8 to 1.8

V at a scan rate of 100 mV/s [13]. During forward cycling oxidation takes place and backward cycling reduction takes place, finally silver nanoparticles were formed on working electrode surface. The cathodic peak is around the potential -1.548 V. But peak response of particles was decreased as increase of pHs, it may be due to less interaction of silver nanoparticles. Moreover, peak current is also a function of crystallographic orientation meaning at least that the electrocatalytic activity of carbon modified with Ag nanoparticles that depends on the plane orientation of Ag nanoparticles.

4.5 Impedance Studies:

The electrochemical impedance spectroscopy (EIS) was carried out to investigate the impedance changes at the electrode surface. The Nyquist plot of Ag nanoparticles is shown in Fig. 5. The impedance can be presented as a sum of the real (Z') and imaginary (Z'') components that arises from resistance to capacitance. The shape impedance spectrum and the electron-transfer kinetics can be evaluated. The semicircle parameter corresponds to the electron transfer resistance (R_{et}) and the double layer capacity (C_{dl}) nature of the AgNPs / GCE electrode [13]. Figure shows that it is clear that the peak of the semicircles appears at the low frequency region. In this region, the long range conduction mechanism contributes more to the conductivity.

According to the curve of Z_{re} and Z_{im} values increases at pH 5.0 as the value of R_o and R_i . This causes the shrinking of impedance plot. The electrical resistivity of deposited silver nanoparticles is higher at pH 5.0. It may be due to the nanocrystalline nature of silver nanoparticles, crystalline boundary discontinuities, presence of surface states and small thickness of the nanoparticles [14,15].

4.6 Fluorescence Studies:

The properties of emission and excitation of silver nanoparticles synthesized by Milletia Pinnata leaf extract were investigated by fluorescence studies with wavelength from 310 nm to 800 nm. The fluorescence emissions were greatly enhanced with the observation of new emission peaks. The excitation wavelength showed to slightly affect the fluorescence emission. In the case of silver nanoparticles, the emission of plasma light during the explosion process is a clear indication to the generation of ions. Some of these ions may get adsorbed to the surface of the formed nanoparticles and they became the emissive centers. Similar proposal was put to explain fluorescence emission from silver nanoparticles in solution exposed to silver ions. The interaction between the emissive centers and the interface environment also has its signature on the observed fluorescence. Indeed, the explosions were carried out in water and the fluorescence was recorded

in air. These may give rise to partial surface oxidation. The partial oxidations of metal nanoparticles lead to the formations of metal oxide clusters on the metal nanoparticle surface. The fluorescence of metallic nanoparticles should be extended to other applicable systems. The fluorescence of silver nanoparticles more or less agrees with literatures [16]. The possible explanation of these emissions is the creation of surface/interface energy bands where the probability of some allowed transitions is increased. The metal nanoparticles obtained may find applications in the field of water purifications and biomedicines. The prominent peaks of the fluorescence-emission and fluorescence-excitation spectrum of silver nanoparticles were observed [17,18].

5. CONCLUSION

The green synthesis of Ag nanoparticles using *Milletia Pinnata* leaf extract was shown to be rapid, eco-friendly and fairly uniform in size and shape. Silver nanoparticles shown by the UV-visible spectrum was found to be at 303 nm. It showed that the formation of Ag nanoparticles was increased with time. The XRD peaks explain the FCC structure of Ag nanoparticles. The average size of the particle diameter was approximately 17.44 nm. The FTIR spectrum explains the biological molecules which performs the dual function of formation and stabilization of Ag nanoparticles in aqueous medium. The electrochemical characterization of biosynthesized silver nanoparticles is studied by cyclic voltammetry and electrochemical impedance spectroscopy exhibits good electrochemical activity. Therefore, biosynthesized silver nanoparticles can be used as sensors for nucleophilic and electrophilic species present in the environment.

6. FIGURES AND TABLES

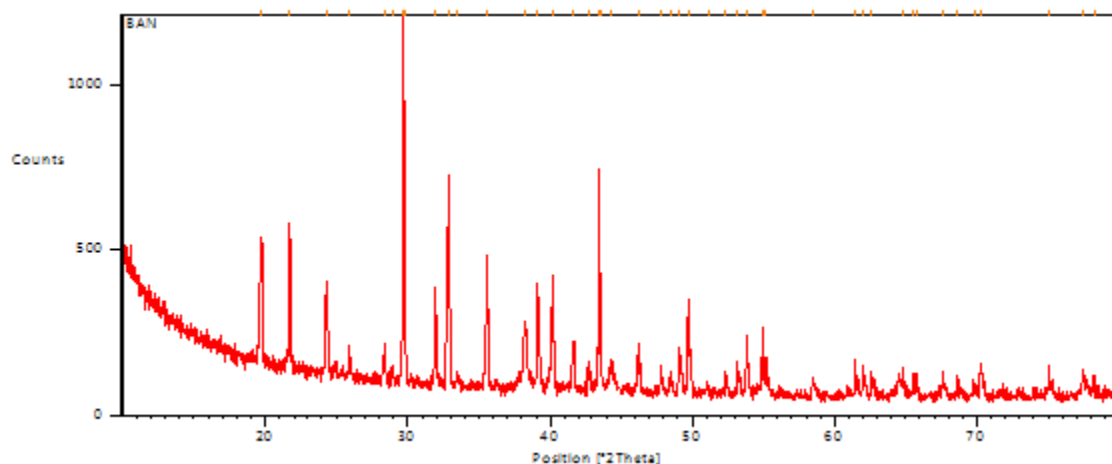


Fig.1. XRD spectrum of Silver Nanoparticles

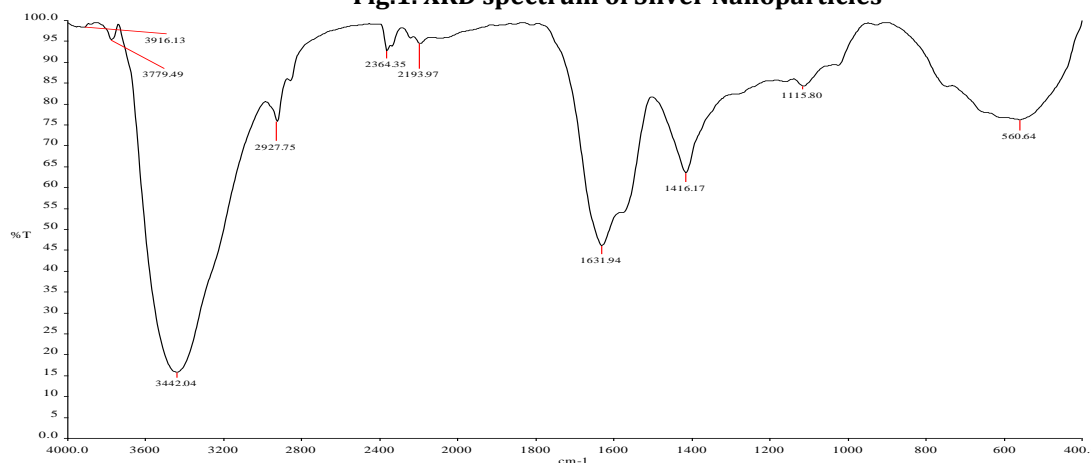


Fig. 2. FTIR spectrum of Silver Nanoparticles

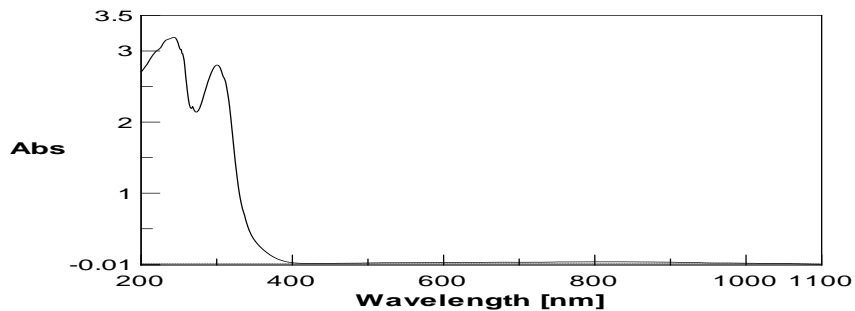


Fig. 3. UV - Visible Absorption spectrum of Silver Nanoparticles

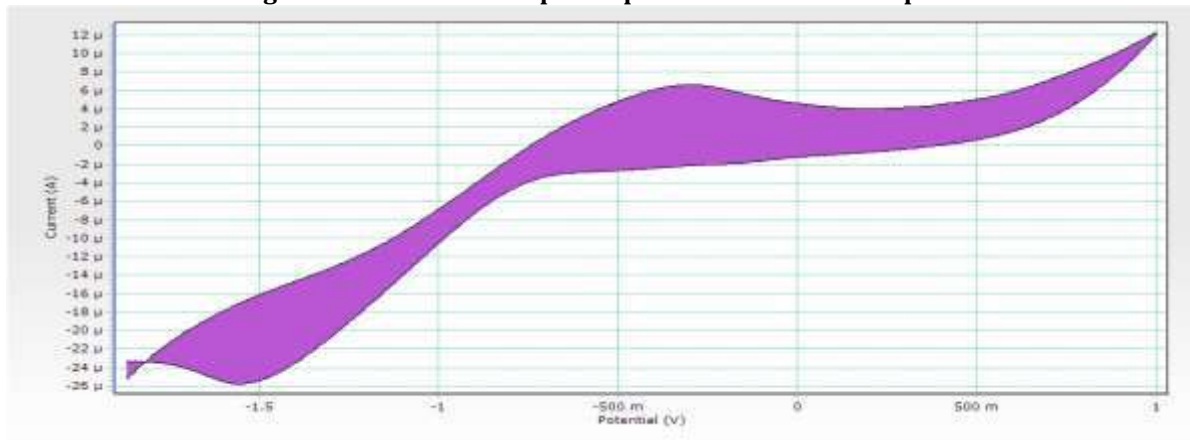


Fig. 4. Cyclic voltammetric behaviour of Silver Nanoparticles

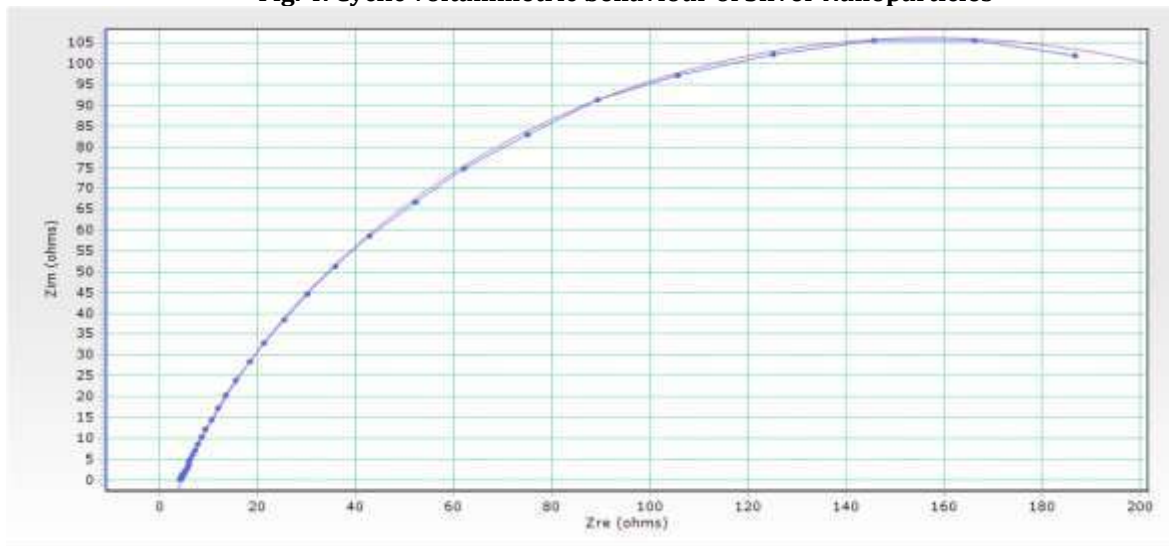


Fig. 5. Electrochemical impedance spectroscopy of Silver Nanoparticles

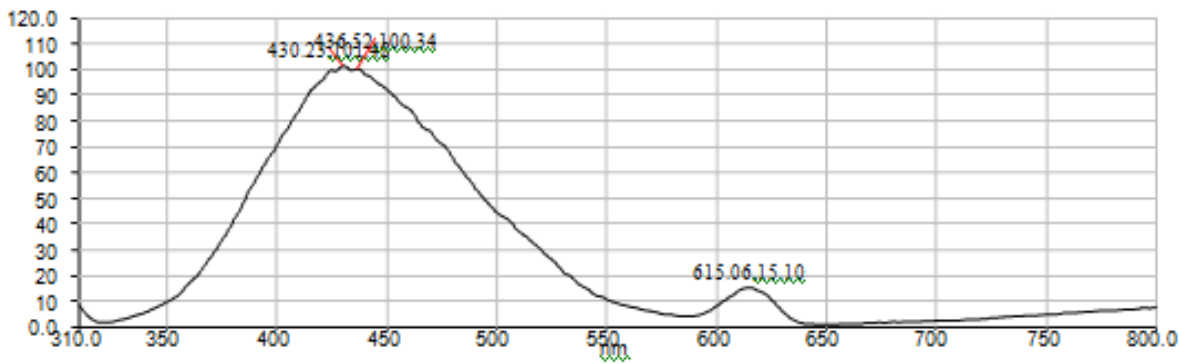


Fig. 6. Fluorescence - Emission spectrum of Silver Nanoparticles

Wave Length (nm)	Emission
430.23	101.45
436.52	100.34
615.06	15.10

Table 1. Fluorescence - Emission

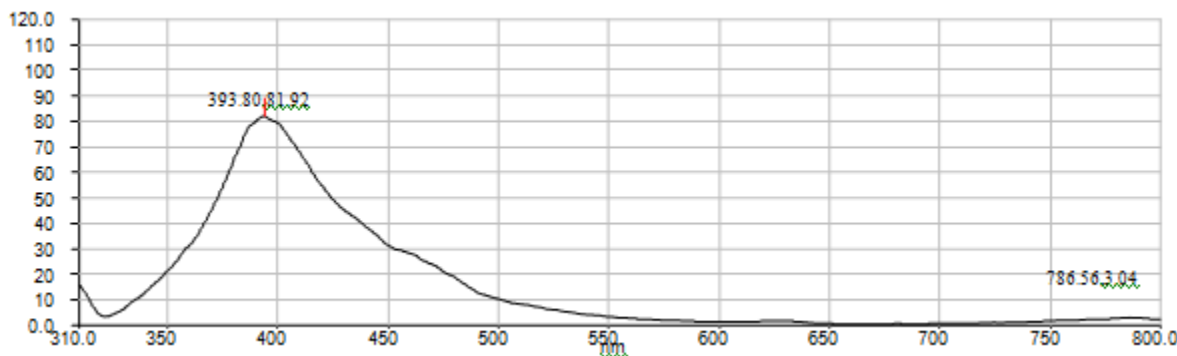


Fig. 7. Fluorescence - Excitation spectrum of Silver Nanoparticles

Wave Length (nm)	Excitation
393.80	81.92
786.56	3.04

Table 2. Fluorescent - Excitation

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