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'Green' Synthesis of Silver Nanoparticles by Using Grape (*Vitis vinifera*) Fruit Extract: Characterization of the Particles and Study of Antibacterial Activity

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ABSTRACT

The synthesis of metal nanoparticles is a growing area for research due to its potentiality in the application and development of advanced technologies. In general, nanoparticles are synthesized by using chemical methods which are not eco-friendly. Here, we have used a fast, convenient and environment-friendly method for the synthesis of silver nanoparticles by reducing silver nitrate with fruit extract of grape (*Vitis vinifera*). Characterization of the metallic nanoparticles was done by UV- Vis Spectroscopy, Dynamic Light Scattering (DLS) and Energy Dispersive X-ray Spectroscopy (EDX). The particle size and lattice image of the silver nanoparticles was studied by Transmission Electron Microscopy (TEM). The antibacterial activity of these nanoparticles was studied against *Bacillus subtilis* and *Escherichia coli*. Growth curves of bacteria in presence of silver nanoparticles showed inhibition of growth suggesting antibacterial property of the nanoparticles.

Keywords: 'Green' Synthesis of silver nanoparticles, Grape (*Vitis vinifera*) fruit extract, DLS, TEM, Antibacterial activity, Bacterial Growth Curve.

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INTRODUCTION

Metal nanoparticles are now studied extensively because of their unique physicochemical characteristics including catalytic activity, optical property, electronic property, magnetic property and antimicrobial activity [1-3]. Synthesis of noble nanoparticles for applications in these areas is of current research interest [4]. Generally, metal nanoparticles are synthesized by using chemical routes such as chemical reduction [5], photochemical reactions in reverse micelles [6] and recently using routes of green chemistry [7]. Use of fungi, bacteria and plant extracts [8-15] for the synthesis of nanoparticles is quite novel leading to green chemistry which provides advantages over chemical and physical methods as it is cost-effective and environment-friendly, and can be scaled up for large scale preparation.

This article describes the 'Green' synthesis of silver nanoparticles by reducing silver nitrate solution by fruit extract of grapes (*Vitis vinifera*) along with the antibacterial activity of these silver nanoparticles.

MATERIALS AND METHODS

Materials

Silver nitrate used for the synthesis of silver nanoparticles was procured from E. Merck, Germany. Black variety of grapes (*Vitis vinifera*) used in this work were collected from the local market. Cultures of *Bacillus subtilis* and *Escherichia coli* were procured from the Department of Microbiology, Bidhannagar College, Kolkata. Dehydrated Luria broth and Nutrient agar media used for bacterial growth study were the products of HiMedia, India.

Method: Synthesis of silver nanoparticles

For the synthesis of silver nanoparticles, 20 mM stock solution of silver nitrate ($AgNO_3$) and grape fruit extract were taken. Grape fruit extract, prepared from 200 g clean and finely crushed grapes, was centrifuged at 5000 rpm for 15 minutes and the filtrate was filtered carefully. For the reduction of Ag^+ ions, 10 ml of grape fruit extract was added drop wise to 10 ml of 20 mM aqueous $AgNO_3$ solution. A distinct color change was observed after 24 hrs as the solution turned into dark yellow from normal colorless solution at room temperature suggesting formation of silver nanoparticles. The color became darker and turned into dark brown after 48 hrs. The reduction of Ag^+ was confirmed from the UV–Vis spectrum of the solution. The nanoparticles were separated out from the mixture by ultracentrifugation (at 40000 rpm for 8 hrs). The lose pellet of nanoparticles formed at the bottom of the centrifuge tube was dispersed in a small volume of deionized water after removing the liquid carefully from the centrifuge tube.

Characterization of silver nanoparticles

The following Four methods were used to characterize the 'Green' silver nanoparticles:

I. UV-Vis Spectroscopy: Formation of silver nanoparticles is easily detected by spectroscopy because the colored nanoparticle solution shows a peak ~450 nm. In this study, a Shimadzu spectrophotometer was used to measure the optical density of solutions/suspensions.

II. Dynamic Light Scattering (DLS): Dynamic light scattering (or Photon Correlation Spectroscopy) is an important technique generally used to realize the size distribution pattern of very small particles present in suspension or solution. This light scattering (Malvern) technique was used in this study to realize the size distribution profile of biologically synthesized silver nanoparticles.

III. Transmission Electron Microscopy (TEM): While DLS analysis shows the size distribution pattern of particles in a solution or suspension, TEM shows the shape and crystal structure (if any) as well as size of the particles. The grid for TEM analysis was prepared by placing a drop of the nanoparticle suspension on a carbon-coated copper grid and allowing the water to evaporate inside a vacuum dryer. The grid containing silver nanoparticles was scanned by a Transmission Electron Microscope (Philips).

IV. Energy Dispersive X-ray Spectroscopy (EDX): This technique is mainly used to determine the elemental composition of a sample. In this study it was used to confirm that the nanoparticle suspension contains nothing but silver.

Study of antibacterial activity of silver nanoparticles

Bacterial cultures (*B. subtilis* and *E. coli*) were routinely maintained on Nutrient agar medium in test tubes; for single colony isolation, cells were streaked on a Petri dish containing Nutrient agar. To study the antibacterial activity of silver nanoparticle suspension, bacteria were allowed to grow in Luria broth medium (10 ml) in its presence and absence at 37^0C with continuous shaking (150 rpm) for 20 hrs. Growth of bacteria was monitored by measuring the optical density of inoculated growth media at regular time interval (4 hrs) by an UV-Vis Spectroscope at 600 nm. In practice, a fresh colony of each strain was picked up from the Petri plate and suspended evenly in separate tubes containing 10 ml Luria broth. The tubes were incubated for an hour; then 1 ml of each culture was inoculated in separate flasks (control and experimental) containing 10 ml Luria broth. To the experimental flasks, 1 ml of nanoparticle suspension was added; in the corresponding control, deionized water was added. Aliquot from each culture was withdrawn at regular intervals and the optical density was measured. The OD values of each culture were put against time to draw the growth curves of bacterial strains.

RESULTS AND DISCUSSION

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UV-Vis Spectroscopy

The production of silver nanoparticles by reduction of silver ions due to the addition of grape fruit (*Vitis vinifera*) extract was followed by UV–Vis spectroscopy. The UV-Vis absorption spectrum of 'Green' silver nanoparticles in the presence of Grape fruit extract is shown in figure 1. The SP band in silver nanoparticles solution was found to be close to 450 nm throughout the observation period as the nanoparticles were dispersed in the solution without possibility for aggregation in UV-Vis spectrum. The high OD of the solution suggests a high conversion of Ag^+ to Ag^0 as nanoparticle.

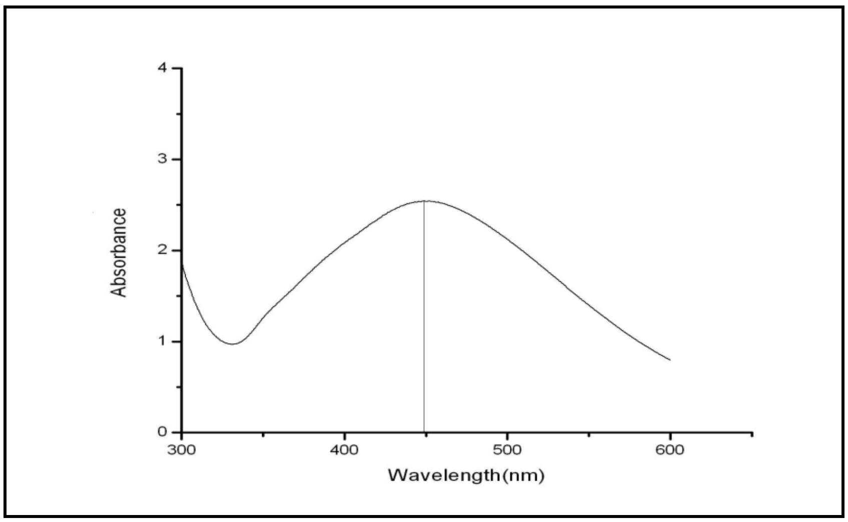

Fig.1. UV-Vis absorption spectra of 'Green' Silver nanoparticles synthesized by addition of Grape fruit extract with 10mM silver nitrate aqueous solution.

Dynamic Light Scattering (DLS) analysis

Figure 2 shows the DLS pattern of the biologically synthesized nanoparticles after separating them from the reaction mixture by ultracentrifugation. It clearly shows that the size range of nanoparticles varied within 2 to 40 nm with the average mean size (diameter) of 19 nm.

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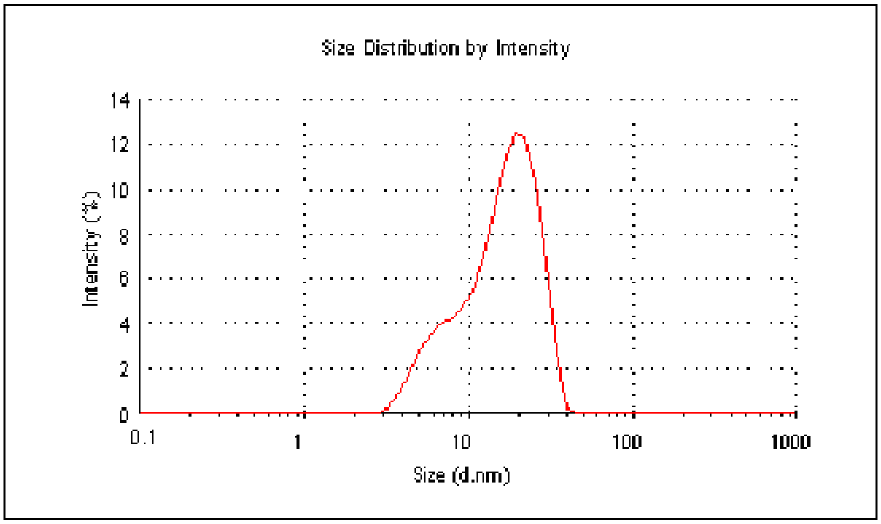

Fig.2. DLS showing mean average size of silver nanoparticles as 19 nm

TEM analysis of Silver nanoparticles

Under TEM (Fig. 3), the silver nanoparticles synthesized by grape fruit extract were observed to have an average mean size of 18-20 nm corroborating well the DLS pattern. The particles appeared to be spherical in shape with weak crystalline structure.

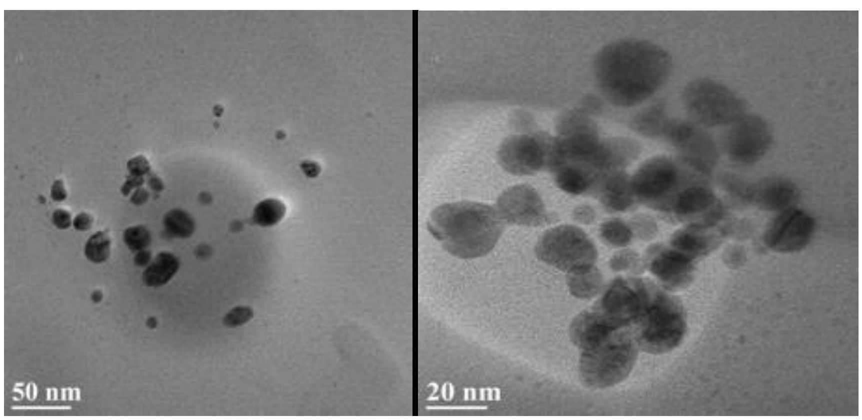

Fig.3.1. TEM images of silver nanoparticles synthesized by using Grape fruit extract

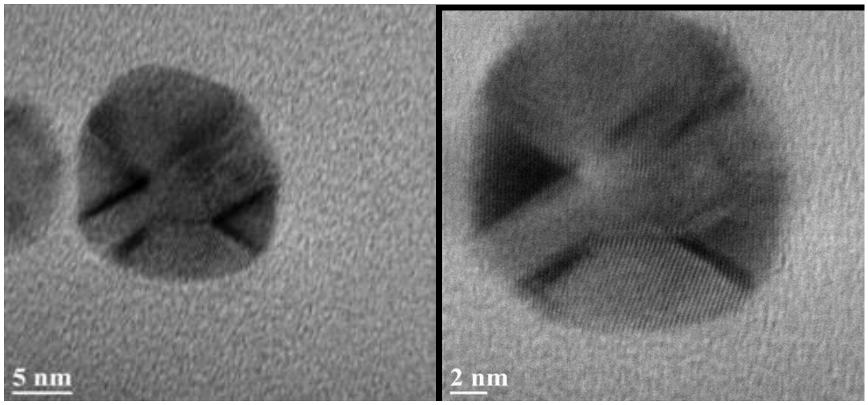

Fig.3.2. TEM images of silver nanoparticles synthesized by using Grape fruit extract

EDX analysis of Silver nanoparticles

In this study, EDX was used to verify the presence of silver in the suspension of nanoparticles purified by ultracentrifugation. The EDX result showed a small peak of silver that confirmed its presence in the suspension (figure 4).The amount (percentage) of elements present in the suspension is shown in the Table 1.

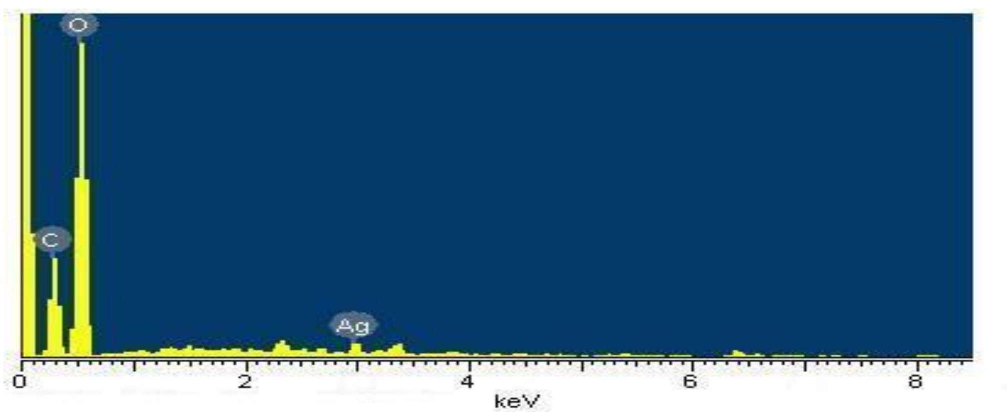

Figure.4. EDX curve with three dominant peaks for C, O and Ag respectively

Table 1: EDX result shows percentage of elements in resulting suspension

Elements	Weight%	Atomic%
C K	25.44	31.90
O K	71.98	67.74
Ag L	2.58	0.36
Total	100.00	100.00

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Study of Antibacterial activity

Figures 5 and 6 show that in absence of silver nanoparticles the optical density (at λ = 600 nm) of both bacterial cultures increased steadily up to 16 hrs indicating rapid bacterial growth, while in presence of silver nanoparticles there was a distinct reduction in the growth of both *B.subtilis* and *E. coli*. The growth reduction was more prominent in case of Gram-negative bacterium *E coli*. This confirmed the antibacterial effect of 'Green' silver nanoparticles on both Gram-positive *B. subtilis* and Gram-negative *E. coli*.

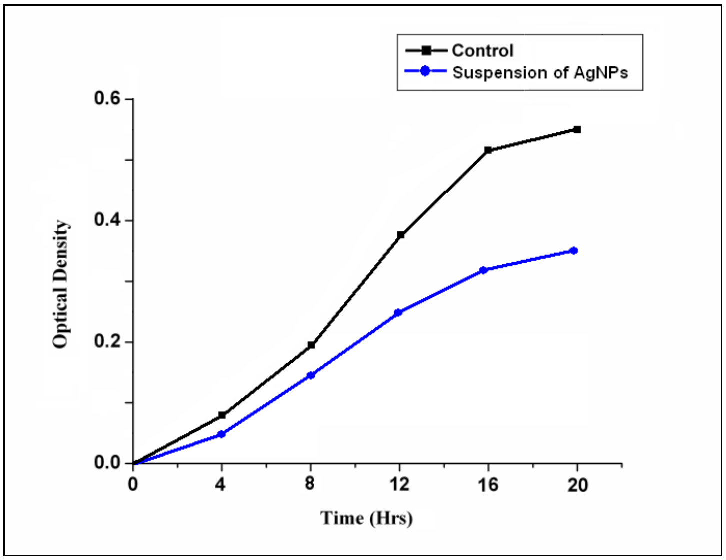

Figure.5. Effect of 'Green' silver nanoparticles on *B. subtilis*

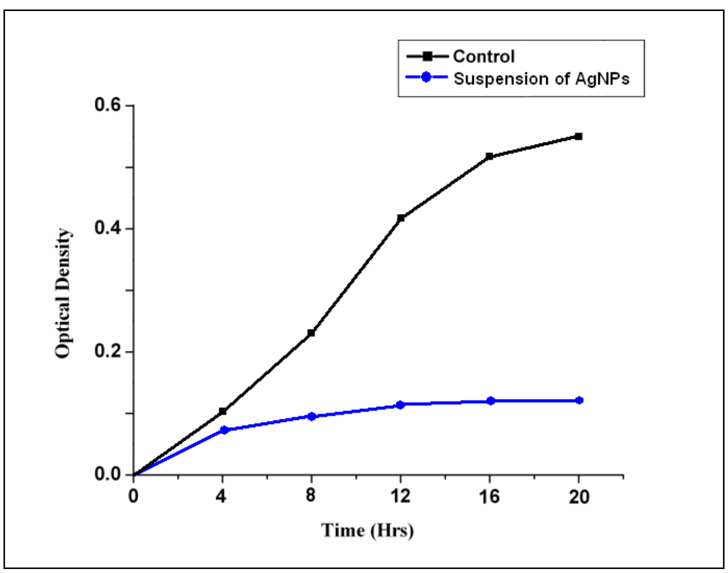

Figure.6. Effect of 'Green' silver nanoparticles on *E. coli*

CONCLUSIONS

With the objective of 'Green' synthesis of silver nanoparticles by a simple method, we screened selected fruits available in the local market, which might act as a reducing and capping agent for synthesis of silver nanoparticles from silver salts. Among the fruits tested, we found black variety of grape fruit (*Vitis vinifera*) to be very effective. Silver nanoparticles, synthesized by this method, were nearly spherical in shape with an average mean size of 18-20 nm. Many particles were found to have distinct crystalline structure. These nanoparticles showed effective antibacterial activity against *B. subtilis* and *E. coli* as well.

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