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SINGLE-STEP NOVEL BIOSYNTHESIS OF SILVER NANOPARTICLES USING *CUCUMIS SATIVUS* FRUIT EXTRACT AND STUDY OF ITS PHOTCATALYTIC AND ANTIBACTERIAL ACTIVITY

K. ROY[a, b*], C.K. SARKAR[a], C.K. GHOSH[b]
[a]*Department of Electronics and Telecommunication Engineering, Jadavpur University, Kolkata-700032, India*
[b]*School of Material Science and Nanotechnology, Jadavpur University, Kolkata-700032, India*

Synthesis of metal and semiconductor nanoparticles through biological route offers a few advantages over the common chemical and physical procedures as it is an easy, fast and eco-friendly alternative that doesn't involve any costly instruments and hazardous chemicals as well. In this study, we report the biological synthesis of silver nanoparticles from silver nitrate solution using fruit extract of cucumber (*Cucumis sativus*). Formation of nano-silver was investigated at regular intervals by scanning the mixture using UV-Vis spectroscope. Crystallinity and different phases of biosynthesized Ag nanoparticles was verified by x-ray diffraction (XRD) analysis. Size, shape and morphology of silver nanoparticles were studied by transmission electron microscopy (TEM). Fourier transform infra-red spectroscopy was performed to detect the bioactive molecules liable for reduction and capping of biogenic silver nanoparticles. Photocatalytic activity of these silver nanoparticles was examined by degradation of methylene blue dye under solar irradiation. The antibacterial efficacy of these nanoparticles was tested against three bacteria- *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Escherichia coli*. These biosynthesized Ag nanoparticles showed effective photocatalytic and antibacterial property by degrading the dye and inhibiting the bacterial growth respectively.

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1. Introduction

In recent times, the research on nanomaterials flourished in all directions and influenced almost all disciplines of science and technology by unique properties that bulk materials never possess. Size, shape and crystallinity are the key parameters that control and determine the properties of nanomaterials [1]. Among various nanomaterials studied yet, nanoparticles of noble metals like silver or gold have been found to possess fascinating properties like optical [2], catalytic [3], anti-bacterial [4,5] and anti-viral [6] etc. Synthesis of these nanoparticles is the primary step for carrying out detail study of its various properties. Over the last decade, chemical [7,8] and physical routes [9] have been used redundantly for nanoparticle synthesis. Even biological routes were tried for the same purpose as they are simple, low cost and eco-friendly alternative [10,11]. Use of different fungi [12], algae [13], bacteria [14] and leaf extract of medicinal plants [15-17] for synthesis of silver nanoparticles is a well explored field now-a-days. Though the use fruit extracts of common plants have not been used extensively for praparing Ag nanoparticles from silver salts.

*Corresponding author: lordkaushikroy@gmail.com

After reviewing the literature available on this subject, it was found that the leaf extracts were used extensively because they are potential source of reducing agents that can reduce and stabilize the colloidal particles during interaction with metal ions [18]. However, fruit extract can serve this purpose very well because they also contain strong reducing and capping agents as described by Jain et al. [19]. A few reports are available as Isaac et al. prepared biogenic silver and gold nanoparticles by using *Averrhoa bilimbi* fruit extract [20]. Roy et al. synthesized Ag nanoparticles from $AgNO_3$ using fruit extract of *Vitis vinifera* [21] and *Malus domestica* [22]. Fruit extract of *Carica papaya* was used by Jain et al. for synthsis of silver nanoparticles as stated above [19].

In this paper, we report for the first time, the biological synthesis of silver nanoparticles by reduction of Ag^+ ions using fruit extract of cucumber (*Cucumis sativus*). The colloidal particles were characterized by standard techniques and its photocatalytic activity was examined by degradation of methylene blue dye under solar irradiation. In addition, the antibacterial property of these nanoparticles was later studied against three bacteria- *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Escherichia coli*.

2. Materials and Methods

2.1. Materials – For preparing fruit extract of cucumber (*Cucumis sativus*), fresh and clean cucumbers (shown in Fig.1) were collected from the nearest market. Silver nitrate ($AgNO_3$) and methylene blue (powder) required for this work were purchased from Merck India Ltd. Three bacterial strains were purchased from National Chemical Laboratory, Pune (India).Nutrient agar and normal agar powders used for antibacterial assay were the products of Himedia, India.

Fig. 1. Fresh cucumber (Cucumis sativus) fruits

2.2. Methods

2.2.1 Preparation of silver nanoparticles.

For preparing fruit extract of cucumber (*Cucumis sativus*), a fresh cucumber (wt. around 50 g) was cleaned, cut into small pieces and crushed using a grinder. It was then filtered and centrifuged at 5000 rpm for 5 minutes to obtain clear soup of cucumber extract. 0.34 g pure silver nitrate was dissolved in 100 ml de-ionized (DI) water to prepare 20 mM stock solution of $AgNO_3$. For reducing Ag^+ ions present in silver nitrate solution, 100 ml fruit extract of cucumber (*Cucumis sativus*) was added drop wise to it so that the resulting medium became half diluted (conc.10 mM). The reacting mixture was incubated at room temperature and after a couple of hours, distinct change in the color of the solution was observed (shown in Fig.2). Once reaction completed, the mixture was centrifuged at 10000 rpm for 30 minutes to separate the colloidal particles from other components of the mixture. The supernatant solution was discarded and the precipitate formed at the bottom of centrifuge tube was redispersed in small amount (10 ml) of de-ionized water. This suspension was centrifuged again at 5000 rpm for 15 minutes for removing the biomass residue completely. The pellet formed inside the centrifuge tube was then collected carefully and dried

inside a vacuum dryer to obtain dried powder of biogenic silver nanoparticles. This dry powder was used for different characterizations described in the following part.

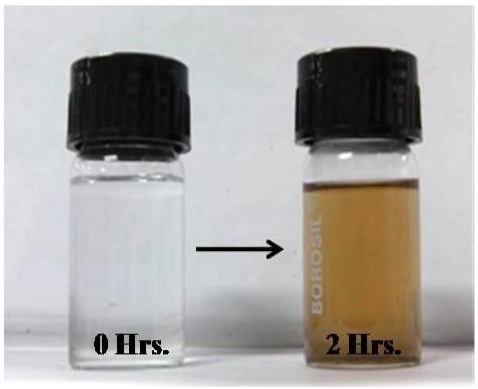

Fig. 2. Color change of the reacting mixture with time

2.2.2 Characterization of Silver nanoparticles

The absorption spectra of the reacting mixture were obtained at regular interval by scanning the mixture using Perkin Elmer UV-Vis spectrometer (USA). XRD analysis of the dry biogenic Ag nanoparticles was performed by means of Rigaku Ultima-III x-ray diffractometer (λ=0.154, operating volt.- 40kV, Japan). FTIR spectrum of these Ag nanoparticles was recorded by IR-Prestige FTIR spectrometer (Shimadzu, Japan). For preparing sample for TEM imaging, the powder of biogenic silver nanoparticles was dissolved in a small quantity of de-ionized water (maintaining concentration 50 μg/ml) and a few drops of this suspension was placed on the copper grid (carbon coated). The grid was then dried in high vacuum and scanned under JEOL-2010 high resolution TEM (operating voltage is 200 kV; USA).

2.2.3 Study of Photocatalytic activity

Photocatalytic study of these biogenic Ag nanoparticles was performed by measuring the degradation of methylene blue dye in presence of nano-silver under solar radiation. At first, 100 ml dye solution was prepared by dissolving 1 mg dye powder in 100 ml DI water keeping 10 mg/l concentration. 10 mg dry powder of biogenic Ag nanoparticles was then added to 50 ml dye solution and the suspension was stirred for 30 minutes in darkness before exposing to solar radiation. A control of dye solution was prepared without adding nanoparticles and kept under the same condition to compare any visible change with exposure time. The suspension was then exposed under sunlight with constant magnetic stirring. The ambient temperature was around 30ºC with mean shine duration of 6 hours [location of Kolkata (India): 22°32'N, 88°19'E; Date of experiment- 20th June, 2014]. 2 ml suspension was taken at regular intervals (after every two hours) and centrifuged at 4000 rpm for 10 minutes to get clear supernatant solution of the dye. The clear soup was then scanned between the wavelengths of 450 to 800 nm using UV-Vis spectrometer (Perkin Elmer, USA) for studying the percentage of dye degradation in the presence of biosynthesized silver nanoparticles.

2.4 Antibacterial activity study

The antibacterial activity of the biogenic silver nanoparticles was evaluated against one gram positive bacteria- *Staphylococcus aureus* and two gram negative bacteria- *Klebsiella pneumoniae* and *Escherichia coli* following disc diffusion method. The culture strains were maintained on suitable media prepared from nutrient agar and normal agar powder. Pure fruit extract of cucumber (*Cucumis sativus*) was taken as a control for comparing observations. The

pure extract and colloidal suspension of nano-silver (initially prepared for TEM analysis) were applied separately into two different wells created on each disc seeded with bacteria. Each disc was prepared in triplicates to obtain statistical variance of the result. The bacteria were then allowed to grow at 37ºC inside an incubator for 24 hours. The antibacterial activity was assessed by observing and measuring inhibition zone formed around the wells after incubation period.

3. Result and Discussions

3.1 UV-Vis Spectroscopy

The fruit extract of cucumber (*Cucumis sativus*) contains organic biomolecules which are supposed to be effective reducing and capping agents for synthesis of nanoparticles. After a couple of hours of extract addition, the reacting mixture turned into light brown from colorless solution (refer to Figure 2) suggesting the formation of colloidal Ag nanoparticles in the mixture. The color intensified with reaction time and became dark brown after 8 hours of incubation. The formation of silver nanoparticles was monitored gradually by scanning the mixture under UV-Vis spectrometer at regular intervals. The peak absorbance was observed at 450 nm which may correspond to the surface plasmon resonance of colloidal Ag nanoparticles [23]. The UV-Vis spectra recorded at various time intervals (1, 2, 4 and 8 hours) are presented in Figure 3. The peak absorbance increased with reaction time and its variance is shown in the inset curve of Figure 3. The peak absorbance increased linearly till 4 hours of incubation probably due to the production of more number of colloidal particles in the reacting medium. Beyond 12 hours of incubation, the formation rate saturates suggesting the end of reaction.

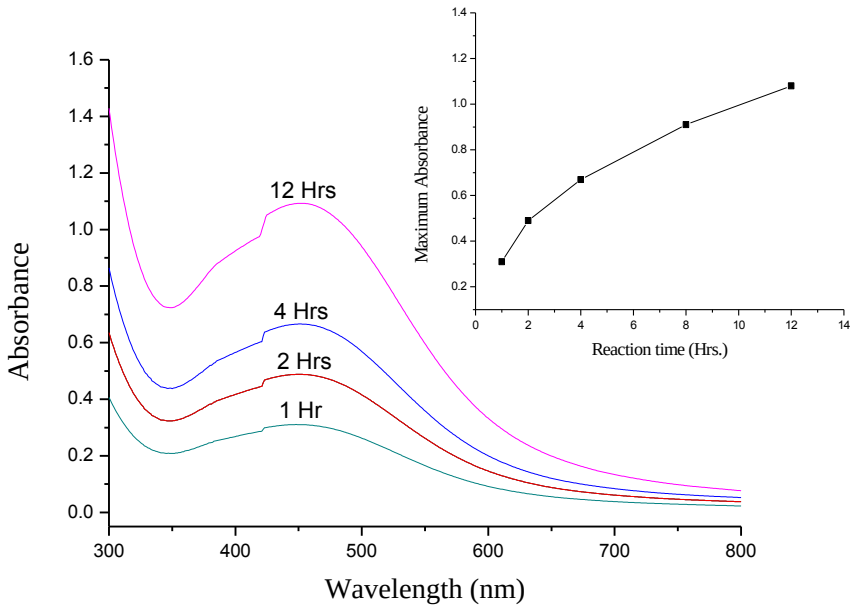

Fig. 3. UV spectra of the reacting solution at regular time intervals.
Inset curve denotes the variation of peak absorbance with incubation time

3.2 XRD Analysis

Fig. 4 shows the x-ray diffraction pattern of the biogenic silver nanoparticles. The pattern is comprised of three noticeable peaks at 2θ= 27.6°, 32.1° and 46.1° which can be attributed to (220), (122) and (231) planes of silver respectively (correleated to JCPDS: File no. 4-783).

Absence of any other peak indicates the purity and crystallinity of the biosynthesized Ag nanoparticles.

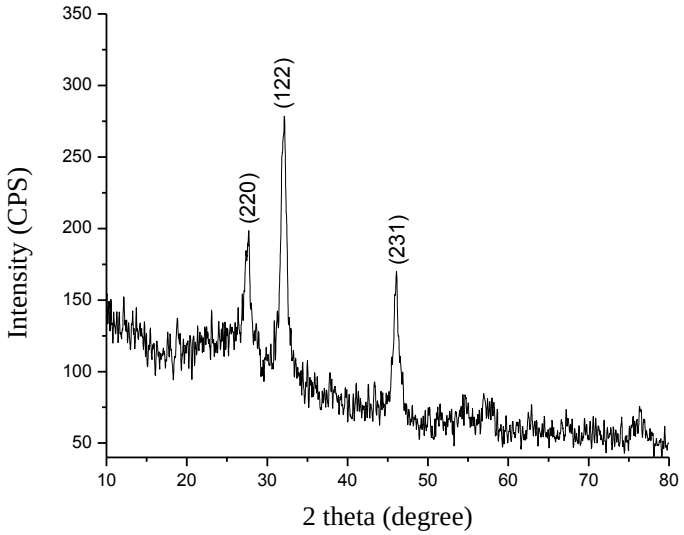

Fig. 4. XRD pattern of the biosynthesized Ag nanoparticles

3.3 TEM analysis

The morphology and microstructure of the particles were studied by high resolution transmission electron microscope. The TEM images (shown in Figure 5) denote that the silver nanoparticles are nearly spherical in shape with an average diameter around 8-10 nm. The internal structure is well crystallized with interplanar spacing of 0.282 nm that may correspond to (122) planes of the face centered cubic phase of silver nanoparticles.

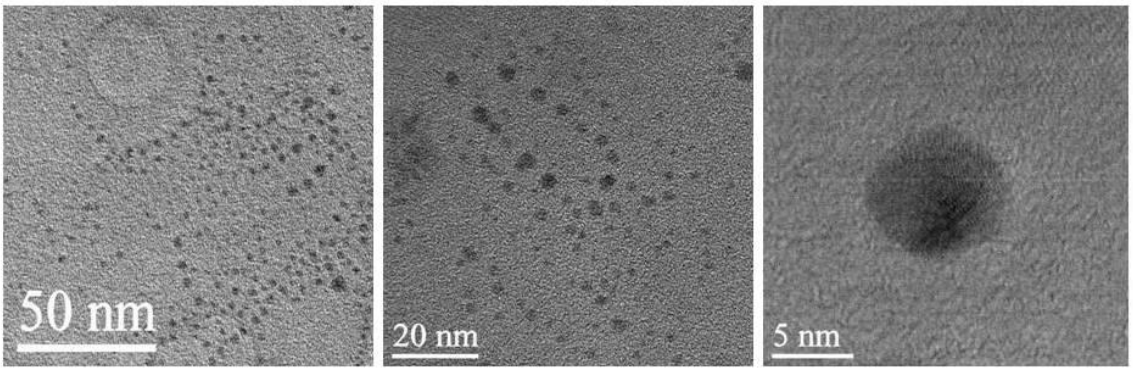

Fig. 5. TEM images of biogenic Ag nanoparticles

3.4 FTIR Spectroscopy

The recorded FTIR spectrum (in absorbance mode) of the dry Ag nanoparticles is shown in Figure 6. The spectrum consists of six distinct peaks in the entire range of recorded spectrum. Bands at 1402 and 1654 cm^{-1} can be attributed to C-H bending of alkanes and C=O stretching vibration of amides respectively [24,25]. The band at 1064 cm^{-1} may denote the stretching of C-N bonds found in amines whereas a band noticed at 3207 cm^{-1} indicates the O-H stretching of aromatic compounds (like phenol) [26]. The remaining two bands at 1240 and 1533 cm^{-1} correspond to stretching of C-O and bending of C-H bonds presnt in hydrocarbons respectively

[27]. Fron this analysis, it may be concluded that the bioactive functional molecules like phenols, amines etc. present in the fruit extract of cucumber (*Cucumis sativus*) might reduce the silver ions and stabilize the colloidal particles during interaction.

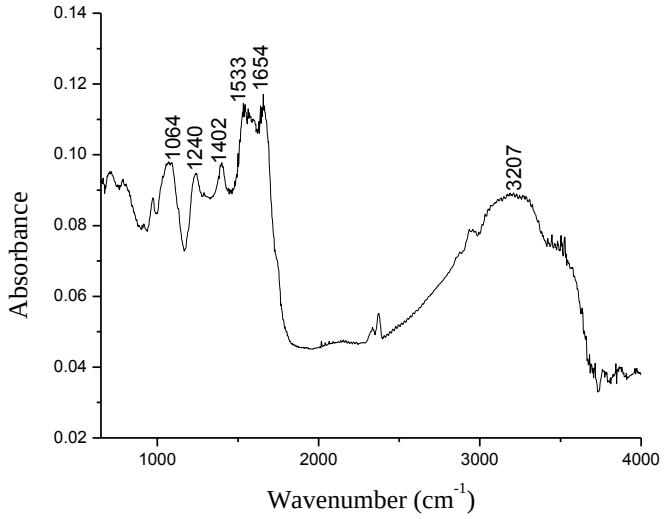

Fig. 6: FTIR spectrum of the biogenic silver nanoparticles

3.5 Photocatalytic degradation

Photocatalytic activity of these Ag nanoparticles was demonstrated by degradation of methylene blue dye in presence of colloidal particles under sunlight radiation. Degradation of the dye was visually observed by gradual change in the color of the colloidal solution from blue to colorless as shown in Figure 7.

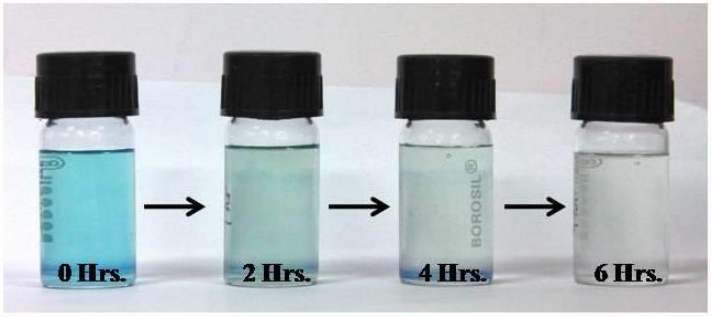

Fig. 7. Gradual change in the coloration of dye from blue to colorless

The characteristic absorption peak (noticed at 660 nm) was found to be decreasing with exposure time and approached baseline after 6 hours of exposure (shown in Figure 8). The percentage of dye degradation was manipulated by following formula and its value was found to be nearly 93% after 6 hours. Figure 9 shows the gradual degradation of dye (%) with time of solar exposure.

$$\text{Dye degradation (\%)} = [(C0 - Ct) / C0] \times 100$$

Here, C0 is the initial concentration of the dye (methylene blue) solution; C_t is the concentration of the dye solution after t hrs of exposure time in sunlight. Here, we measured the dye concentrations by taking the absorbance value at 660 nm in the recorded UV-Vis spectra as the concentration is proportional to the value of absorbance.

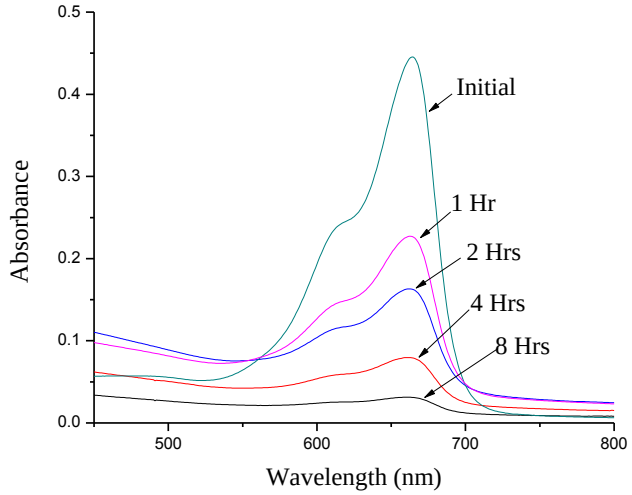

Fig. 8: UV spectra show photocatalytic degradation of methylene blue with reaction time

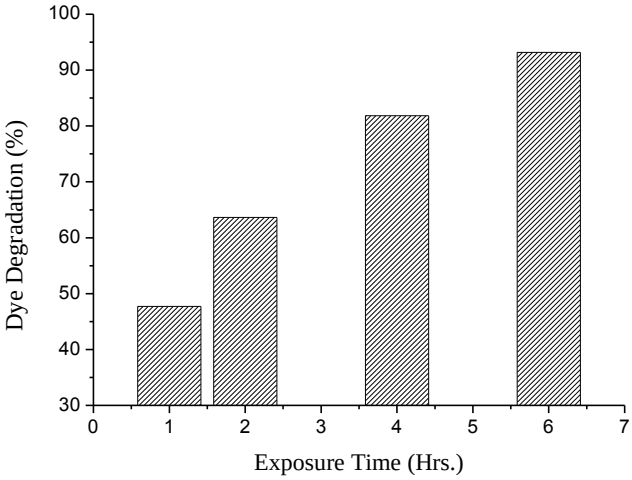

Fig. 9: The bar chart illustrates the variation of dye degradation at different time of solar exposure

The solar radiation has been proved to be an effective technique for degrading organic dyes as described by previous reports [28]. During solar exposure, the sunlight photons strike the colloidal particles present in the reacting solution causing the excitation of the electrons at particle surface [29]. The oxygen molecules dissolved in the reacting mixture can accept these energized electrons from nanoparticle surface and turned into oxygen anion radicals. These anion radicals break the molecular structure of the dye causing production of simple organic molecules and rapid degradation of the methylene blue dye [30,31]. Hence, the biogenic Ag nanoparticles can play the role of an efficient and stable photocatalyst for degradation of organic dyes under sunlight irradiation.

3.6 Antibacterial activity: Zone of Inhibition

Antibacterial activity of these biogenic silver nanoparticles was studied against three bacterial strains- *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Escherichia coli*. After 24 hours incubation in darkness (at 37°C), inhibition zones were around the wells where colloidal suspensions of Ag nanoparticles was added. This confirmed the effective antibacterial property of the biogenic silver nanoparticles against these bacterial strains. The control showed no such result. The result of antibacterial assay is shown in the Figure 10. It can be noticed from the result that the diameter of inhibition zone is higher for gram negative bacteria than that of gram positive bacteria.

This is may be due to the difference between the composition of their cell wall [32]. Cell membrane of gram negative bacteria is made of single layer peptidoglycan whereas the cell wall of gram positive bacteria comprises of multilayer peptidoglycan that makes it more rigid for penetration [33,34]. The previous studies claim that the antibacterial activity is due to the release of Ag^+ ions from silver nanoparticles when they come close to the bacterial cells [35]. The bacterial cell wall bears small negative charge, hence, attract the silver cations [36]. When the Ag^+ ions feel electrostatic attraction, they move towards the bacterial cell wall and get attached to it. As a result, the composition of cell wall varies rapidly affecting the wall permeability. This further degrades cellular transport and causes death of the cells [37].

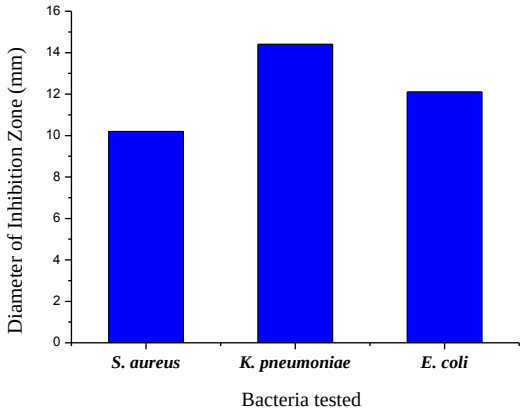

Fig. 10. Graph shows the activity of Ag-nanoparticles against three bacteria (diameters were measured as mean of triplicates)

4. Conclusion

Green synthesis procedures are gaining importance in these days because they are single-step, fast, low cost and environment friendly alternative of well known chemical and physical synthesis procedures for preparing metallic and semiconductor nanoparticles. In this study, we successfully prepared Ag nanoparticles from silver nitrate using fruit extract of cucumber (*Cucumis sativus*). These biologically synthesized silver nanoparticles were found to have spherical shape with average diameter nearly 8-10 nm. Crystalline nature of the particles was studied by XRD whereas the bioactive molecules that reduced and stabilized the colloidal particles during interaction were identified with the help of FTIR spectra. The Photocatalytic study proved the efficiency of these biogenic silver nanoparticles in degrading organic dyes under solar radiation. In addition, the result of antibacterial assay showed that these nanoparticles possess effective bactericidal property against the tested bacterial strains.

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