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Green Synthesis, Catalytic and Antibacterial Activity of Silver Nanoparticles Synthesized from *Olax acuminata*

ALAKESH PHUKAN and BOLIN CHETIA*

Department of Chemistry, Dibrugarh University, Dibrugarh-786 004, India

*Corresponding author: Fax: +91 373 2370323; Tel: +91 373 2370210; E-mail: bolinchetia@dibru.ac.in

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Silver nanoparticles were successfully synthesized by a simple green and eco-friendly route, using *Olax acuminata* leaf extract for the first time. Preliminary confirmation for the formation of silver nanoparticles was done visually by colour change from light yellow to dark brown and by UV-visible spectrophotometer. Further the synthesized nanoparticles were characterized by FTIR, XRD, TEM and SEM. The catalytic activity of the synthesized silver nanoparticles was investigated by the degradation of methylene blue and photocatalytic activity by the degradation of methyl orange. The synthesized silver nanoparticles are found to have a good catalytic effect on the reduction of both the dyes which is confirmed by the decrease in absorbance maximum values with time using UV-visible spectrophotometer. Further the antibacterial activity was evaluated against four bacterial species *i.e., Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Escherichia coli* at four different concentrations (5, 10, 15 and 20 µg/mL) and was assessed by the zone of inhibition (mm). The highest activity was found against *Staphylococcus aureus* (11 mm) and it was found that bacterial growth significantly reduced in a dose dependent manner. Antibiotic ciprofloxacin was used as the standard in the antibacterial assay.

Keywords: Nanoparticles, Catalytic, Photocatalytic, Degradation, Antibacterial activity.

INTRODUCTION

In recent years, biosynthesis of metal nanoparticles by using plant extracts have received significant research interest due to its rapid, economical and eco-friendly protocol[1]. It lacks the drawbacks of the existing chemical and physical methods such as the use of hazardous chemical, low material conversion and high energy requirement[2] and thereby it provides a suitable alternative for large scale synthesis of metal nanoparticles[3]. Among diverse metal nanoparticles particularly silver nanoparticles have drawn attention due to its extensive application in areas such as cell electrodes[4], optics, as antimicrobials[5], as sanitization[6] and also in accelerating some chemical reaction such as the oxidation of styrene.[7] Besides that silver nanoparticles and their composites show catalytic activity in the degradation and removal of dyes[8], which are released by various industries such as paper, plastic, leather, food, textile, cosmetic and pharmaceutical industries[9]. These dye effluents result in significant environmental pollution[10] and therefore their abatement have become very important. However, due to the increase in resistance of these dye effluents to microorganisms, their reduction by conventional biological methods has become ineffective. These are also resistant to physical-chemical treatment in a highly effluent concentration. Silver nanoparticles in this regard due to their high surface area exhibit an enhanced reactivity[11].

In this paper, we report the green synthesis of silver nanoparticles using aqueous extract of *Olax acuminata* which is a traditionally used ethno-medicinal plant distributed in North-East region of India. The biosynthesized silver nanoparticles were further evaluated for their catalytic activity in the degradation of methylene blue and methyl orange and also for antibacterial activity.

EXPERIMENTAL

Silver nitrate was obtained from Sigma Aldrich. Methylene blue and methyl orange were obtained from Merck. Leaves of *Olax acuminata* were collected from Karbi Anglong, Diphu, Assam. Bacterial species of *Staphylococcus aureus* (MTCC 10619), *Bacillus subtilis* (MTCC 10619), *Pseudomonas aeruginosa* (MTCC 424), *Escherichia coli* (MTCC 443) procured from MTCC, Chandigarh.

Synthesis of silver nanoparticles: For the synthesis of silver nanoparticles, 2 mL of *Olax acuminata* leaf extract was added to 30 mL of 1 mM aqueous $AgNO_3$ and stirred at room temperature for 3 h. The colour of the reaction product turned yellowish brown due to the reduction of Ag^+.

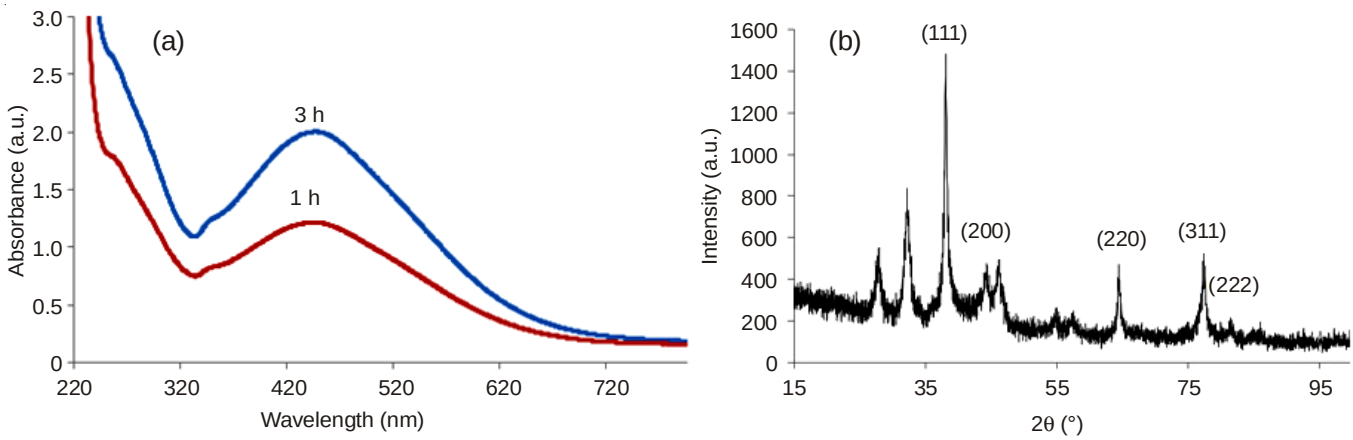

Fig. 1. (a) UV-visible spectra recorded as a function of time of reaction of 10^{-3} M aqueous solution of $AgNO_3$ with *Olax acuminata* leaf extract and (b) XRD pattern of silver nanoparticles

Characterization of silver nanoparticles: The formation of silver nanoparticles was monitored by UV-visible spectroscopy using UV-1700 (Shimadzu) spectrophotometer. X-ray diffraction (XRD) measurement were carried out by Rigaku X-ray diffractometer (Model: ULTIMA IV, Rigaku, Japan) with CuK_α X-ray source ($\lambda = 1.54056$ Å) at a generator voltage 40 kV, a generator current 40 mA with the scanning rate 2° min^{-1}. FTIR spectra was recorded using KBr plates on a spectrophotometer (Model: Prestige-21, Shimadzu) in the range of 4000-650 cm^{-1}. The SEM analysis was done using Scanning Electron Microscope (SEM) (Model 6390 LV JEOL Asia PTE Ltd., Singapore/JEOL JSM). The high resolution transmission electron microscopy (HRTEM) images were recorded by a JEOL Model 2100 EX, Japan operated at an accelerating voltage of 200 kV.

Catalytic activity on degradation of methylene blue: To evaluate the catalytic activity of the silver nanoparticles, degradation of methylene blue in aqueous solution was investigated as a model system. For this, 1 mL of methylene blue (1×10^{-4} M) was mixed with 0.2 mL of aqueous extract and 1.8 mL of water and the reaction was monitored after 1h using UV-visible spectrophotometer. The same procedure was performed by using 1.8 mL of silver nanoparticles instead of water and the reactions were monitored after 20 min and 1 h. A control was also set up without using plant extract and silver nanoparticles.

Photocatalytic activity on degradation of methyl orange: Photocatalytic activity of the silver nanoparticles were tested by investigating the degradation of methyl orange (MO) under sunlight. For this 4 mL of the silver nanoparticles were added to 20 mL of methyl orange solution and stirred for about some time followed by irradiation under sunlight. The absorption spectrum of the reaction mixture was recorded with time using UV-visible spectrophotometer.

Antimicrobial activity of the synthesized silver nanoparticles: The antibacterial activity of silver nanoparticles was investigated by using standard antimicrobial assay[12].

RESULTS AND DISCUSSION

Characterization of the silver nanoparticles: The formation of silver nanoparticles was visually confirmed by colour change from light yellow to dark brown. Further UV-visible spectral analysis showed Surface Plasmon Resonance at 444 nm, which increased in intensity as a function of time without any shift in the peak. Fig. 1a shows the UV-visible spectra of silver nanoparticles as a function of time which confirms the fairly rapid bio-reduction of Ag^+ to Ag^0.

The formation of the silver nanoparticles was further confirmed by the XRD analysis. The XRD patterns of silver nanoparticles using *Olax acuminata* leaf extract are shown in Fig. 1b. A number of Bragg reflections are observed with 2θ values of 38.36°, 44.74°, 64.85°, 77.40° and 81.85 corresponding to the (111), (200), (220), (311) and (222) set of lattice planes and this may be indexed as the band for face centered cubic (fcc) structure of silver. The unassigned peaks may be due to the capping agent stabilizing the nanoparticles.[13]

FTIR measurements were carried out to identify the possible biomolecules responsible for the reduction of silver nanoparticles and capping of the bioreduced silver nanoparticles synthesized by using *Olax acuminata* leaf extract. Fig. 2 shows the FTIR spectra of Ag sample synthesized by using *Olax acuminata* leaf extract. The synthesized silver nanoparticles showed peaks at 3258 cm^{-1} (O-H vibration), 1612 cm^{-1} (C=C groups or from aromatic rings), 1370 cm^{-1} (*geminal* methyls) and 1074 cm^{-1} (ether linkages) suggesting the presence of flavonoid on the surface of silver nanoparticles.

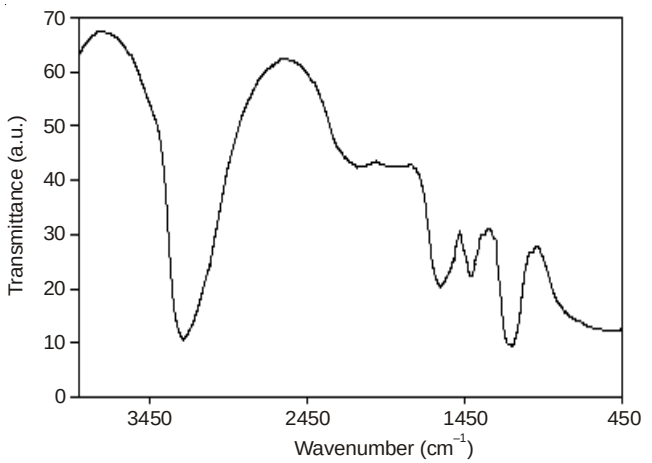

Fig. 2. FTIR of silver nanoparticles synthesized by using *Olax acuminata* leaf extract

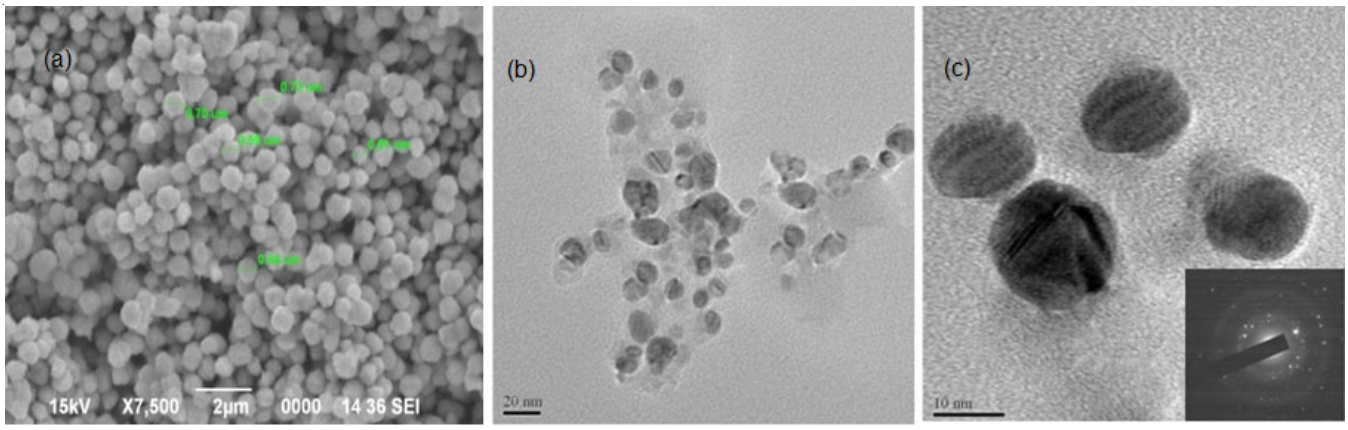

Fig. 3. (a) SEM and (b) TEM images of the silver nanoparticles at different magnification where inset in (c) is the SAED pattern of the silver nanoparticles synthesized by using the aqueous solution of $AgNO_3$ with *Olax acuminata* leaf extract

To understand the topology of silver nanoparticles, SEM analysis was carried out which showed the synthesis of monodisperse spherical nanoparticles with almost same size (Fig. 3a). Further TEM analysis also showed formation of spherical nanoparticles predominantly having average size of 10 nm (Fig. 3b and 3c).

Catalytic activity of silver nanoparticles on reduction of methylene blue: The catalytic activity of the silver nanoparticles was investigated by choosing the catalytic degradation of methylene blue as a model system (Fig. 4a). It was found that on adding the extract, the main characteristic absorption peak of pure methylene blue at 664 nm decreased and shifted to higher wavelength which indicates the ability of the extract to degrade methylene blue. However, marked decrease in the absorbance of methylene blue was observed for a system containing methylene blue, plant extract and silver nanoparticles revealing that silver nanoparticles act as an electron transfer mediator between methylene blue and extract.

Photocatalytic activity on degradation of methyl orange: On addition of silver nanoparticles followed by exposure to sunlight, the absorption peak of methyl orange at 460 nm decreased with time without any shift in peak position (Fig. 4b). However addition of only plant extract or silver nanoparticles to methyl orange had no effect on the degradation. It was also reported that compared to other irradiation technique, sun light was found to be faster in decolourising methyl orange in the presence of metal catalyst[14].

Antibacterial activity: The antibacterial effect of silver nanoparticles were assessed by the presence/absence of zone of inhibition against *B. subtilis*, *S. aureus, P. aeruginosa* and *E. coli* by agar well method at four different concentrations (Table-1). The highest activity was found against *S. aureus*. This observed difference in the diameter of zone of inhibition could be due to the difference in the susceptibility of different bacteria to the synthesized silver nanoparticles. Also the Gram-positive bacterial species are found to have better activity and this could be due to their effective interaction which may be due to the absence of outer membrane in the cell wall[15].

Conclusion

To conclude, silver nanoparticles from leaf extract of *Olax acuminata* were synthesized for the first time and was characterized by UV-visible, XRD, SEM and TEM. We also showed the use of the synthesized silver nanoparticles for the

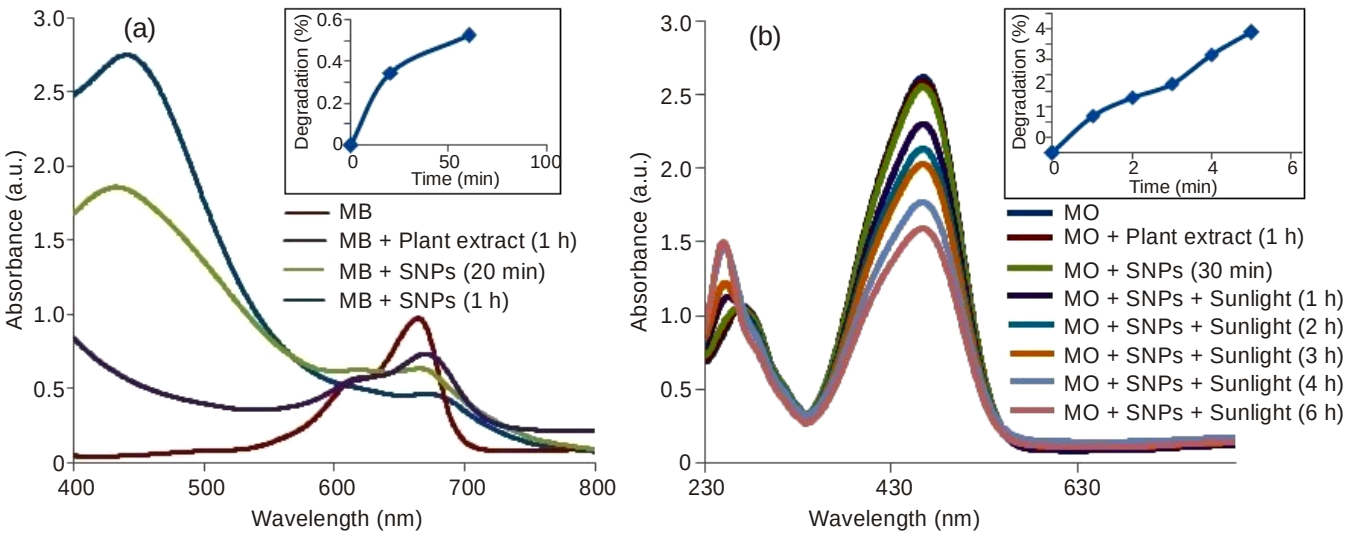

Fig. 4. UV-visible spectra of the degradation of (a) methylene blue (MB) and (b) methyl orange (MO) where insets in (a) and (b) are the plots of % degradation of methylene blue and methyl orange as a function of time

TABLE-1
ANTIBACTERIAL ACTIVITY OF THE SILVER NANOPARTICLES SYNTHESIZED BY USING *Olax acuminata* LEAF EXTRACT

Sample	Conc. (µg/mL)	Zone of inhibition (mm)			
		S. aureus	*B. subtilis*	*P. aeruginosa*	*E. coli*
Silver nanoparticle	5	6	6	6	6
	10	8	6	6	6
	15	9	9	7	7
	20	11	10	7	8
Ciprofloxacin	10	30	22	26	22

catalytic degradation of methylene blue and photocatalytic degradation of methyl orange. The synthesized silver nanoparticles were also able to inhibit the growth of both Gram-positive and Gram-negative bacteria. Thus the utilization of bioreductive procedures opens new possibilities in the area of dye reduction and removal.

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