Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Archives of Applied Science Research, 2012, 4 (5):2098-2104
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-508X
CODEN (USA) AASRC9

Green Synthesis of Silver Nanoparticle using *Bryophyllum pinnatum* (Lam.) and monitoring their antibacterial activities

Debabrat Baishya*, Nakul Sharma and Rituparna Bora

Department of Bioengineering and Technology, Gauhati University, Guwahati- 781 014, Assam, India

---

ABSTRACT

Synthesis and use of nanoparticles is a very expanding and a developing area. The development influenced by biological resources for nanoparticles synthesis is emerging into an exciting and the most significant bough of Nanotechnology. Conventionally, chemical reduction is the most frequently applied approach for preparation of metallic nanoparticles but in most of the synthesis protocols it cannot avoid the use of toxic chemicals. Here we report an ecofriendly, cost effective and green approach for synthesis of 1mM $AgNO_3$ solution using the aqueous leaf extracts of Bryophyllum pinnatum (Lam.) as the reducing and capping agent as well. As-synthesized nanoparticles were characterized using UV-VIS spectrophotometer, XRD, FTIR, SEM and TEM. The silver nanoparticles formed were well capped as observed through TEM and showed promising antibacterial activity against E. coli and Staphylococcus aureus.

Keywords: *Bryophyllum pinnatum*, silver nanoparticles, FTIR, TEM, antibacterial

---

INTRODUCTION

Nanotechnology provides the ability to engineer the properties of materials by controlling their size, and this has driven research toward a multitude of potential uses for nanomaterials. Because of the unique physicochemical characteristics of metal nanoparticles, including catalytic activity, optical properties, electronic properties, antibacterial properties, and magnetic properties [1-3], they are gaining the interest of scientist for their novel methods of synthesis. Silver is well known for possessing an inhibitory effect toward many bacterial strains and microorganisms commonly present in medical and industrial processes [4]. In medicines, silver and silver nanoparticles have a wide application including skin ointments and creams containing silver to prevent infection of burns and open wounds [5], medical devices and implants prepared with silver-impregnated polymers [6]. In textile industry, silver-embedded fabrics are now used in sporting equipment [7].

Current research in inorganic nanomaterials having good antimicrobial properties has opened a new era in pharmaceutical and medical industries. Silver is the metal of choice as they hold the promise to kill microbes effectively. Silver nanoparticles have been recently known to be a promising antimicrobial agent that acts on a broad range of target sites both extracellularly as well as intra-celluarly. Silver nanoparticles shows very strong bactericidal activity against gram positive as well as gram negative bacteria including multi-resistant strains [8], and also it was found to be in few studies [9].

Among the synthetic methods used for the preparation of silver nanoparticles, some toxic chemicals such as $NaBH_4$, citrate, or ascorbate are most commonly used as a reducing agent [10-12]. Considering that such reducing agents may be associated with environmental toxicity or biological hazards, the development of a green synthesis approach for silver nanoparticles is desired [13].

Plant or plant extract have been suggested as possible ecofriendly alternatives to chemical and physical methods. Using plant for nanoparticles synthesis can be advantageous over other biological processes by eliminating the elaborate process of maintaining cell cultures [14]. It can also be suitably scaled up for large-scale synthesis of nanoparticles. Green synthesis of silver nanoparticles has been reported using extracts of various plants such as *Nelumbo nucifera* [15], *Euphorbia hirta* [13], *Ocimum sanctum* [16], etc. In the present study aqueous leaf extract of *Bryophylum pinnatum* (Lam.) have been used to synthesize silver nanoparticles. *B. pinnatum* is a vegetatively propagated wild herb found in the tropical countries including India. The plant has been widely acknowledged for the treatment of bowel complaint in the traditional medicine. Neither the synthesis of silver nanoparticles nor its antibacterial activity was reported using this plant earlier.

MATERIALS AND METHODS

Plant Material and synthesis of silver nanoparticles

Bryophyllum pinnatum leaves were collected from the Botanical Garden of Gauhati University. The leaf extract was prepared by taking 20 g of thoroughly washed leaves and finely chopped into fine pieces. The chopped leaves were mixed with 100 ml distilled water in a 500 ml Erlenmeyer flask and the mixture was stirred at 60 ^{0}C for 1h .The mixture was cooled and then filtered with Whatman paper No.1. The filtrate was collected and was stored at 4 ^{0}C for further analysis. Then 5ml of leaf extract was added to 45mL of 1mM $AgNO_3$ solution for bioreduction process at 30 ^{0}C in dark and observed for change in colour.

UV-VIS spectra analysis

The bioreduction of Ag+ ions in solutions was monitored by measuring the UV-VIS spectrum of the reaction medium. The UV-VIS spectral analysis of the sample was done by using U-3200 Hitachi spectrophotometer at room temperature operated at a resolution of 1 nm between 200 and800 nm ranges.

XRD analysis

X-ray diffraction (XRD) measurements of film of the biologically synthesized silver nanoparticles solution cast onto glass slides were done on a eMMA diffractometer operating at a voltage of 40 kV and current of 20 mA with Cu K(a) radiation of 1.54187 nm wavelength. The scanning as done in the region of 2h from 20^0 to 80^0 at 0.02^0/min and the time constant was 2 seconds.

FTIR analysis

For FTIR measurements, the Ag nanoparticles solution was centrifuged at 10,000 rpm for 30 min. The pellet was washed three times with 20 ml of de-ionized water to get rid of the free proteins/ enzymes that are not capping the silver nanoparticles. The samples were dried and grinded with KBr pellets and analyzed on a Shimadzu IR-IR Affinity1 model in the diffuse reflectance mode operating at a resolution of 4 cm^{-1}.

SEM analysis of silver nanoparticles

Scanning electron microscopic analysis was done using a JSM 6360 (JEOL) machine. 25μL of sample was sputter-coated on copper stub and then observed the images of nanoparticles.

TEM analysis of silver nanoparticles

Transmission electron microscopy (TEM) was performed for characterizing size and shape of biosynthesized silver nanoparticles. The sample was first sonicated (Vibronics VS 80) for 15 min. A drop of this solution was loaded on carbon-coated copper grids, and solvent was allowed to evaporate under Infrared light for 30 min. TEM measurements were performed on JEOL model JEM 2100 instrument operated at an accelerating voltage at 200 kV.

Antibacterial activity study

The antibacterial effect of silver nanoparticles against *E. coli* and *Staphylococcus aureus* was analyzed by their growth curve. Fresh colonies from agar media were inoculated in 10ml broth (Luria Bertani) media. The media was supplemented with silver nanoparticles for one set of experiment and $AgNO_3$ for other set of experiment in order to verify their effect on the growth of these two cultures. The bacterial cultures were then incubated at 37 ^{0}C with continuous shaking at 150rpm. The growth of *E. coli* and *Staphylococcus aureus* in broth media was indexed by measuring the optical density (at λ=600nm) at regular intervals using UV-VIS spectrophotometer. Whereas control contained no exposure of silver nanoparticles synthesized from the *Bryophyllum leaf* extract.

RESULTS AND DISCUSSION

The addition of *Bryophyllum pinnatum* leaf extract to the silver nitrate solution resulted in the change in colour and silver particles exhibit brown colour in the aqueous solution because of the excitation of surface plasmon vibrations with the silver nanoparticles (Fig 1).

Fig1a: *Bryophyllum pinnatum* (Lam.), b: Colour change of leaf extracts containing silver before and after synthesis of silver nanoparticles

Silver nanostructure exhibit interesting optical properties directly related to surface plasmon resonance (SPR), which is highly dependent on the morphology of the samples. The SPR band in nanoparticles solution remain close to 418 nm, suggesting that the nanoparticles were dispersed in the aqueous solution with no evidence for aggregation in UV-Vis absorption spectrum (Fig 2).

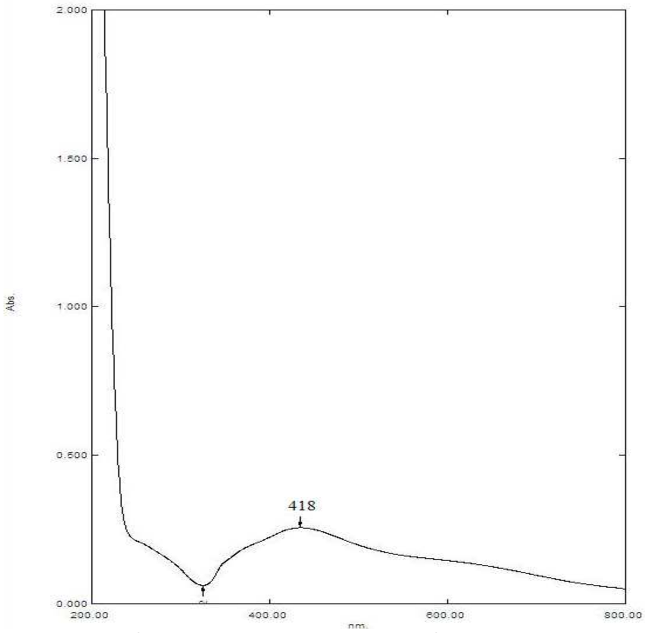

Fig 2: UV-VIS absorbtion spectra of silver nanoparticle synthesized from *Bryophyllum pinnatum* (Lam.) leaves at 1mM silver nitrate

XRD pattern obtained for silver nanoparticles (Fig.3) showed number of Braggs reflections that may be indexed on the basis of the face centered cubic structure of silver. The XRD pattern thus clearly showed that the silver nanoparticles formed by the reduction of Ag+ ions by the *Bryophyllum* leaf extract are crystalline in nature [17].

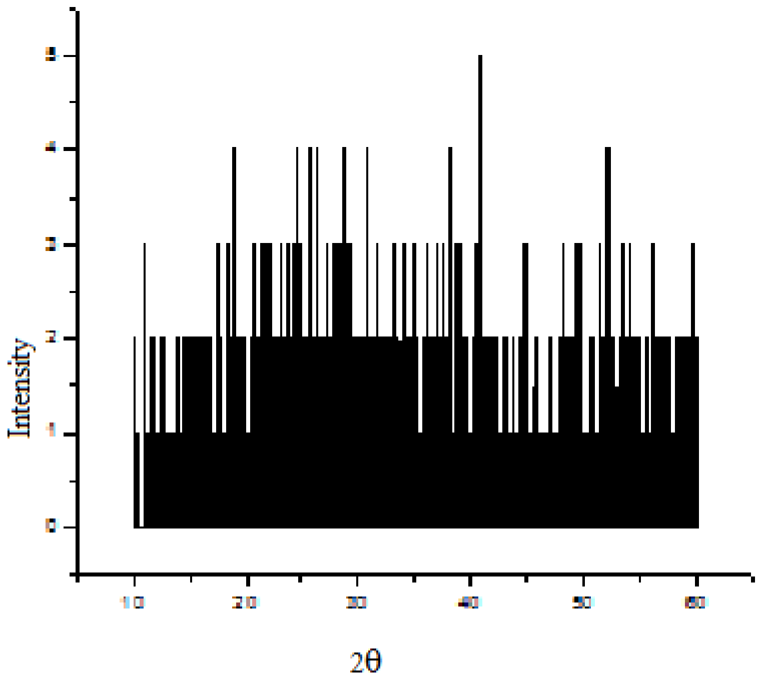

Fig.3: XRD pattern of silver nanoparticles synthesized by *Bryophyllum pinnatum* leaf extracts

The FTIR spectrum (Fig.4) of silver nanoparticles showed strong IR bands characteristic of hydroxyl (3437.15 cm^{-1}), alkanes (2,357.01 2,927.94 and 2,854.60 cm^{-1}), C=C of benzene (1,604.71 cm^{-1}), aromatic amines (1,381.03 cm^{-1}) and aliphatic amines (1,095.75 cm^{-1}) functional groups. The FTIR analysis strongly supported the capping behaviour of bioreduced silver nanoparticles synthesized by *Bryophyllum pinnatum* leaf extract which in turn imparted the high stability of the synthesized silver nanoparticles. The optical properties of the long stored (4 months) silver nanoparticles were reinvestigated and found satisfactory (data not shown).

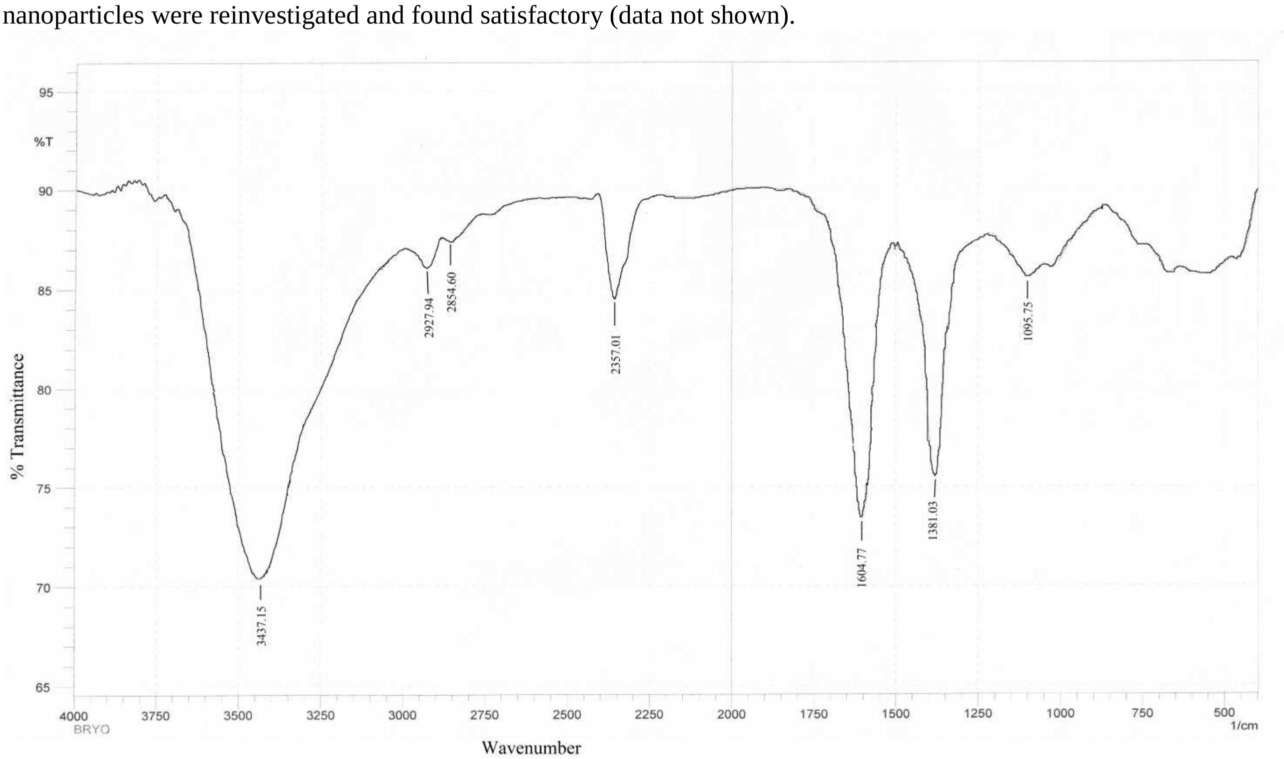

Fig.3: FTIR spectrum of silver nanoparticle synthesized by *Bryophyllum pinnatum* leaf extracts

The SEM image showed relatively spherical shape nanoparticle formed with diameter range 70-90 nm (Fig.4). Similar phenomenon was reported by Chandran *et al* [18].

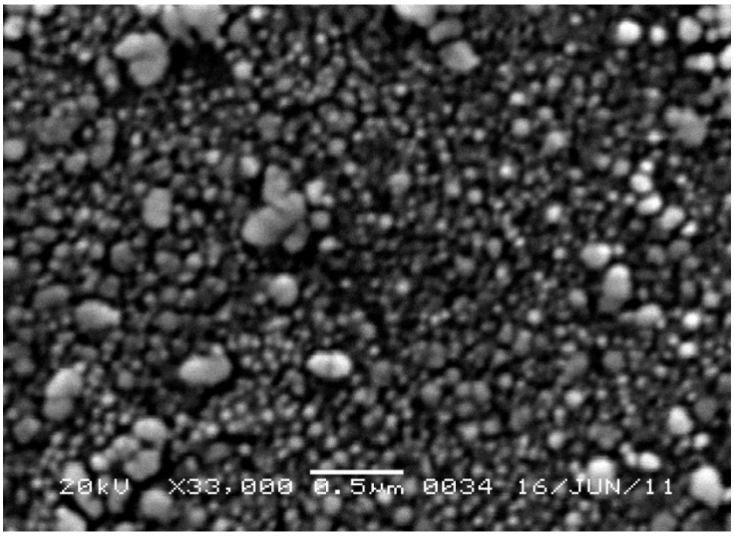

Fig.4: SEM image of the silver nanoparticles synthesized by *Bryophyllum pinnatum* leaf extracts

TEM analysis reveals that the Ag nanoparticles are predominantly spherical (Fig.5). The overall morphology of the silver nanoparticles produced by reduction of Ag+ ions with 1mM $AgNO_3$ is composed of almost uniform nanoparticles. Further, the capping ability of *Bryophyllum pinnatum* leaf extracts was also observed.

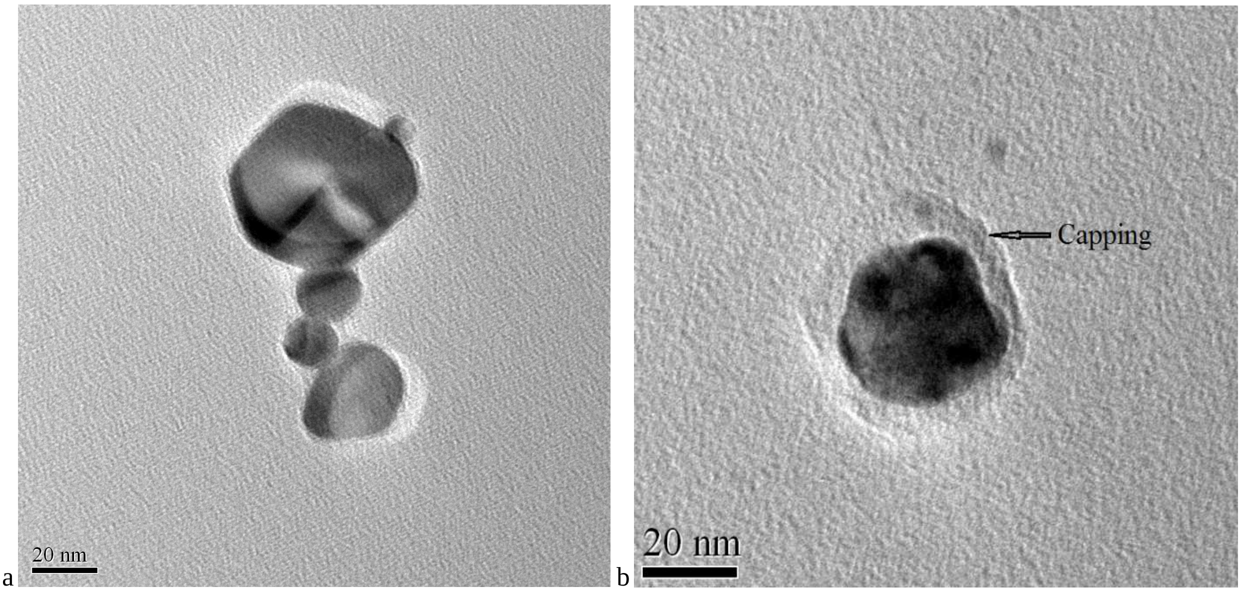

Fig.5 a: TEM images of silver nanoparticles, b: Nanoparticle showing capping

It was well known that silver nanoparticles exhibits strong antibacterial activity due to their well-developed surface which provides maximum contact with the environment. Here, antibacterial effect of silver nanoparticles and $AgNO_3$ solution were studied by using optical intensity as function of time for 25 hours. Fig.6 and Fig.7 showed that in the absence of silver nanoparticles as in the case of control as well as in presence of $AgNO_3$ there was increase in optical density showing bacterial growth but in presence of silver nanoparticles , there was reduction in the growth of *E. coli* and *S. aureus*.

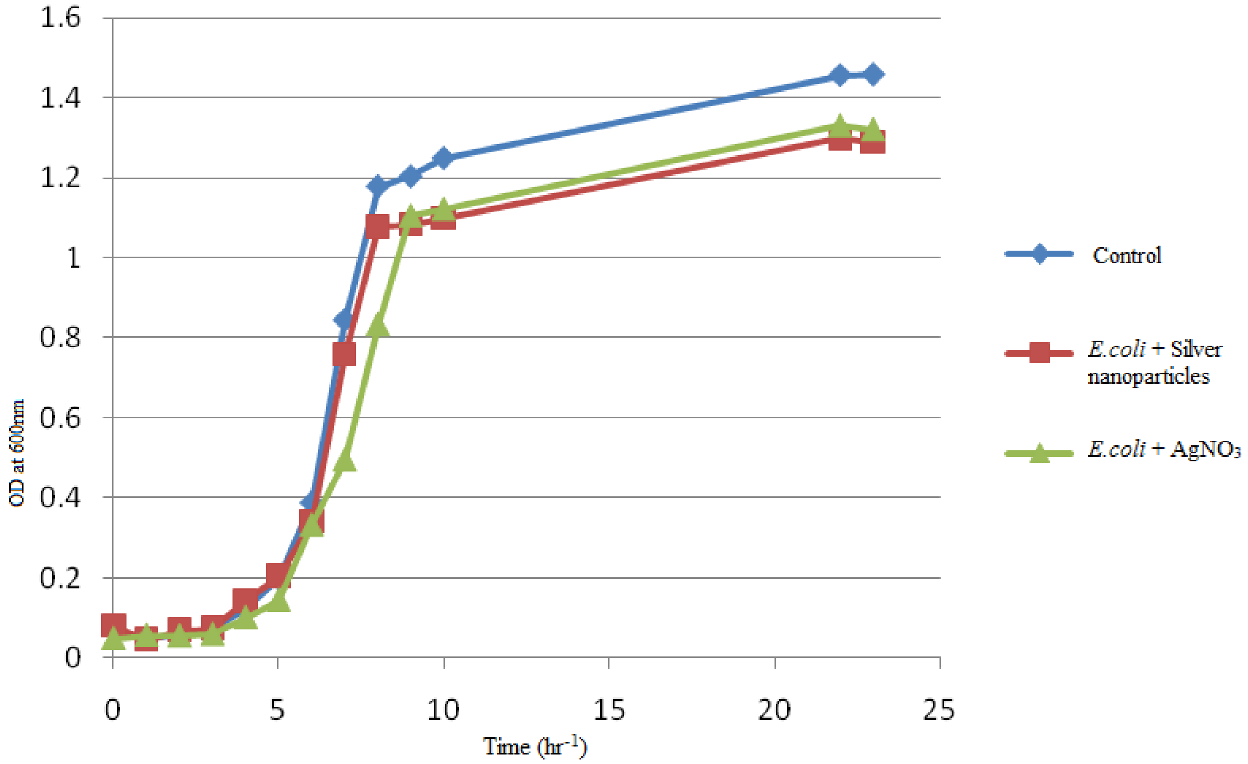

Fig.6: Effect of silver nanoparticles on *E. coli* growth rate

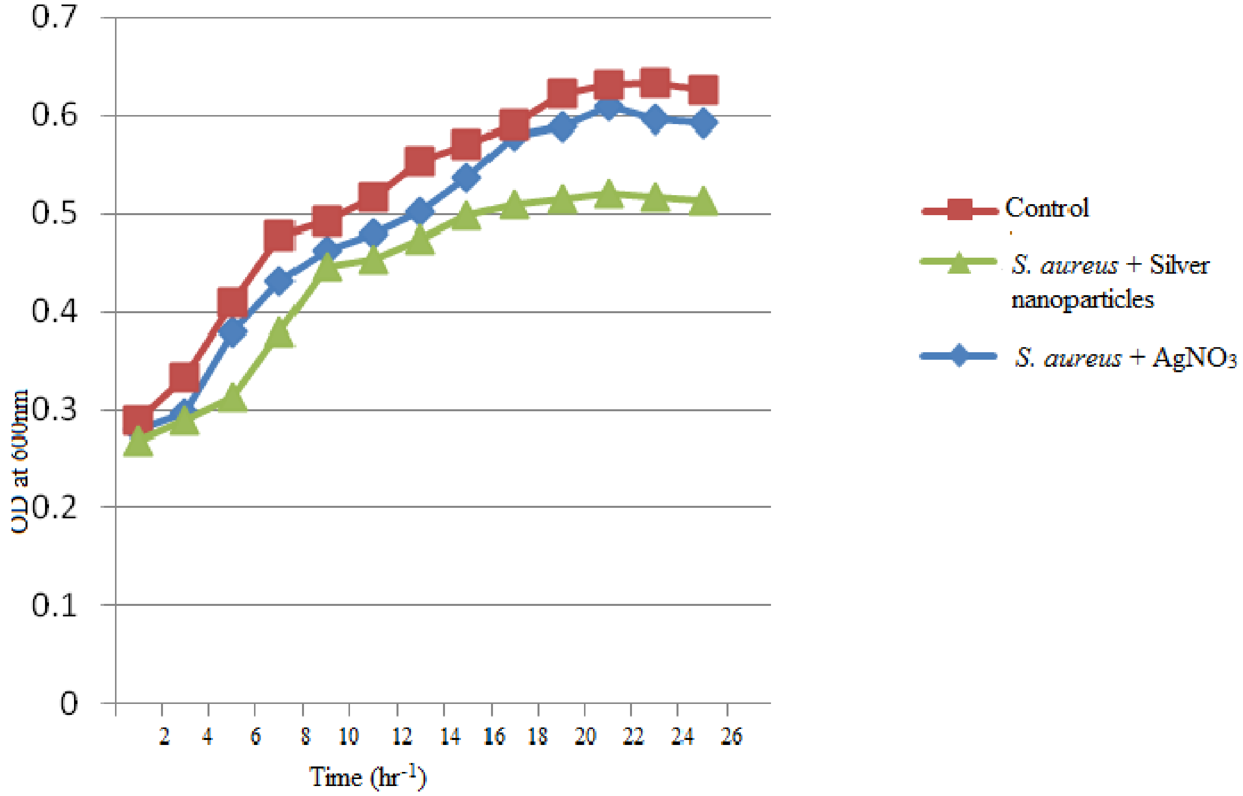

Fig.7: Effect of silver nanoparticles on *S. aureus*

CONCLUSION

The present investigation has evaluated a well known plant not exploited for silver nanoparticles synthesis earlier. The method described a novel application of leaf extract of *Bryophyllum pinnatum* (Lam.) and fortifies the screening of a new plant as a potential source of reducing and capping agent for the synthesis of silver nanoparticles. The synthesized silver nanoparticles were well capped and showed a strong antibacterial activity which are very important from the aspects of its bio-medical application.

REFERENCES

[1] M Catauro; MG Raucci; FD De Gaaetano; A Marotta. *J Mater Sci Mater Med*, **2005**, 16, 3, 261–265.
[2] JH Crabtree; RJ Brruchette; Ra Siddiqi; IT Huen; LL Handott ; A Fishman. *Perit Dial Int*, **2003**, 23, 368–374.
[3] A Krolikowska; A Kudelski; A Michota; J Bukowska. *Surf Sci*, **2003**, 532, 227–232.
[4] H Jiang; S Manolache; ACL Wong; FS Denes. *J Appl Polym Sci*, **2004**, 93, 1411–1422.
[5] N Duran; PD Marcato; OL Alves; GIH De Souza; E Esposito. *J Nanobiotechnol*, **2005**, 3, 8–14.
[6] RO Becker. *Met Based Drugs*, **1999**, 6, 297–300.
[7] T Klaus; R Joerger; E Olsson; CG Granqvist. *Proc Natl Acad Sci* USA, **1999**, 96, 13611–13614.
[8] S Shrivastava; T Bera; A Roy; G Singh; P Ramachandrarao; D Dash. *Nanotechnology*, **2007**, 18, 9.
[9] DW Zeng; CS Xie; BL Zhu. *Material science and Engneering*, **2003**, 10, 468-72.
[10] M Chen, LY Wang, JT Han; JY Zhang; ZY Li; DJ Qian. *J. Phys. Chem. B*,**2006,** 110, 11224.
[11] XW Lou; CL Yuan; LA Archer. *Chem. Mater*,**2006,** 18, 3921.
[12] PL Kuo; WF Chen. *J. Phys. Chem. B*,**2003,** 107, 11267.
[13] EK Elumalai; TNVKV Prasad; V Kambala; PC Nagajyothi; E David. *Arch Appl Sci Res*, 2010, 2, 76-81.
[14] SS Shankar; A Rai; A Ahmad; M Sastry. *J Colloid Interface Sci*, **2004**, 275, 496–502.
[15] T Santoshkumar; AA Rahuman; G Rajakumar; S Marimuthu; A Bagavan; C Jayaseelan; AA Zahir; G Elango; C Kamaraj. *Parasitol Res*, **2010**, 108, 693-702.
[16] G Singhal; R Bhavesh; K Kasariya; AR Sharma; RP Singh. *J Nanopart Res*, **2011**, 13, 2981-2988.
[17] J Huang; Q Li; D Sun; Y Lu; Y Su; X Yang; H Wang; Y Wang; W Shao; N He; J Hong; C Chen. *Nanotechnology*, **2007**, 18, 105104-105114.
[18] SP Chandran; M Chaudhary; R Pasricha; A Ahmad; M Sastry. *Biotechnol Prog*, **2006**, 22, 577.