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Antibacterial Activity of Silver Nanoparticles Synthesized by using Lichens *Heterodermia boryi* and *Parmotrema stuppeum*

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Abstract
Development of eco-friendly green synthesis nanoparticles has become more interest and focus in current scenario. This study reports the synthesis of silver nanoparticles by using methanolic extracts of *Heterodermia boryi* and *Parmotrema stuppeum* lichen species collected from The Nilgiris, Tamilnadu. The physical appearance of synthesized nanoparticle was characterized by color change, UV-VIS Spectrophotometer, Electron spectroscopy, Energy dispersive spectroscopy, FTIR and X-ray diffraction techniques. The size of silver nanoparticles synthesized by *Heterodermia boryi* and *Parmotrema stuppeum* were at 27.91nm to 37.21nm and 27.69 nm to 36.00 nm respectively. The antibacterial activity of silver nanoparticle was tested against six bacterial pathogens both gram positive and gram negative. The results showed potential activity against *Acinetobacter baumanii* and *Staphylococcus aureus*.

Keywords
Silver nanoparticle, Lichen, *Heterodermia boryi*, *Parmotrema stuppeum*, antibacterial activity

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INTRODUCTION:
Lichens are symbiotic associations of a fungus (mycobiont) and either one or more algae or cyanobacterial partner (Photobiont) for photosynthesis. India is rich source of lichen diversity, occupying nearly 15 to 20% of the 20,000 species of lichens so far identified and recorded in the world's ecosystem ranging from arctic tundra to desert climates. (Negi, 2000). They are present everywhere in the barks, stems, leaves and soil but growing well in the environment unfavorable for the growth of higher plants (Bates *et al.,* 2011).
Nanoparticles are the basic essential elements in the wall of nanotechnology, and it exhibits fabulous advanced characteristic features based on their properties such as size, morphology and other size dependent properties. Currently, chemical and physical methods are mostly employed for nanoparticles synthesis at industrial level but use of toxic reducing and capping agents for synthesis, high temperature and pressure protocols, concerns for use in biomedical applications raise difficulties in utility of these methods. In view of shortcomings associated with chemical and physical methods, the interest is shifted towards utilizing potential of biological agents (living cells and their extracts) for nanoparticles production (Arun *et al*, 2013).
Synthesis of silver nanoparticles by biological method using bacteria, fungi, algae, enzymes and plant extracts has more advantages due to

environmental deterioration and ability of large-scale production over physical and chemical methods. This has raised interest over lichen adopted mode of silver nanoparticle synthesis and its application due to its rich resource of secondary metabolites (Asmita *et al*., 2012).

MATERIALS AND METHODS

Collection of Lichen Samples

Lichen samples were collected from various sites of Western Ghats, The Nilgiris, Tamil Nadu, and India. The collected samples identified as Heterodermia boryi (Fée) Kr. P. Singh & S.R. Singh, Parmotrema stuppeum (Taylor) Hale, by Dr. Snajeeva Nayaka, Principal Scientist, CSIR- NBRI, Lucknow. Samples were dried at room temperature. Herbarium was prepared and voucher specimens were deposited in JNTBGR (Voucher specimen no. 1902, 1903) for future reference.

Preparation of Lichen Crude Extracts

The dry fine grinded thalli of lichens (50g each) were subjected for methanolic extraction by soxhlet apparatus. The lichen extracts were filtered and concentrated under reduced pressure in rotary shaker evaporator and stored at 4°C for further study.

Synthesis of Silver Nanoparticles by Methanolic extracts:

Silver nitrate (Qualigens_99.8% from SD Fine-CHEM limited, Mumbai, India.) solution at concentration of 10^{-3} was prepared separately for each test extracts.

Silver nano particles synthesis was carried out using reduction method. For the synthesis of AgNPs, about 10 ml of the aqueous and other organic solvent lichen extracts were mixed with 30 ml of a 1 mM $AgNO_3$ solution in an Erlenmeyer flask and allowed to react at room temperature for 48 hours. The solution was centrifuged at 10000 rpm for 15 minutes and pellets obtained were collected. This pellet after drying is used for the antimicrobial assay as well as for the characterization of silver nano particles by various techniques (Sivalingam *et al.,* 2012).

Characterization of Silver Nanoparticles

The characterization of nanoparticle was done by checking the presence of silver nanoparticles and the size of the nanoparticles produced through SEM-EDAX, X- ray diffraction and FTIR methods respectively using standard protocols.

Colour Change

The metal processed solution appears initially in turbid white colour and then change to dark brown indicating the synthesis of silver mediated nanoparticle (Sastry *et al.,* 2002). The change in hydrogen ion concentration is important parameter for confirmation of nanoparticle synthesis.

UV-Vis Spectroscopy

The bio-reduction of silver nitrate was periodically monitored by DMB-PC based UV-Spectrometer-Systronics 2202 in the 280-760 nm range (Rajesh *et al.,* 2009). Centrifugation was carried out at 2000rpm for 30 mins. Supernatant were discarded and pellets were obtained. The semisolid pellets of extracellular filtrate were converted to dried powdered form. 2-3 drops of petroleum ether were added to each of the crude-semi solid pellet. It was further vaporized for 15 mins and stored at 4°C.

SEM- EDAX

The powdered silver nanoparticles were characterized using SEM- EDAX. The dried powder form of nanoparticles was mixed with acetone and loaded in the sample holder. The loaded samples were then dried under vacuum and subjected to Scanning Electron Microscopic (SEM) studies. It helps to study the size and the elements present in the sample. The Energy Dispersive Analysis of X- ray (EDAX) was also done along with the SEM analysis. EDAX spectrum recorded in the spot- profile mode from one of the densely populated silver nanoparticle regions on the surface of the film for the element mapping. SEM-EDAX (make-JOEL Model 6390) was carried out at 40 KV (Nithya and Ragunathan, 2009).

X- ray diffraction (XRD) patterns

X- ray patterns were carried out to check the presence of silver nanoparticles using a D-Max 3A, Rigaku X- ray diffractometer (Make-Shimadzu Model 6000). The powder form of each sample was coated as a thick smear on a glass slide. Then the sample coated slides were analysed by an X- ray diffractometer in the 2θ range from 30-80 with Cu-Kα radiation at 40 KV and 30 mA (Govindharaju *et al.,* 2010).

FTIR Analysis

The biomolecules of mediated AgNPs were studied by FTIR (Fourier transform infrared spectroscopy Shimadzu 8400S, Japan) and it was recorded in between the range of 800-4000 $cm^{-1.}$ (Nithya and Ragunathan, 2009).

Antibacterial activity of AgNPs

The antibacterial activity of the synthesized AgNPs was determined using the disc diffusion method. Different types of pathogenic bacteria, including Gram-negative and Gram-positive bacteria were tested. Pure cultures of the micro-organisms were subcultured on Mueller-Hinton agar. Each strain was swabbed uniformly onto individual agar plates using sterile cotton swabs. Sterile paper discs were placed

on the agar plates, and 50 µL of 100 mg/mL samples were applied to the discs. Various antibiotic discs (30 µl per disc) were used as the positive control and DMSO as the negative control. All the plates were incubated at 37°C for 18–24 hours. The tests were repeated three times. The zone of inhibition, which appeared as a clear area around the discs, was measured (Manojlovic *et al.,* 2002).

RESULT AND DISCUSSION:

Synthesis of lichen extract mediated silver nanoparticles

The silver nanoparticle synthesis was more focused on methanolic extracts of *Heterodermia boryi* and *Parmotrema stuppeum* demonstrating the formation of the silver nanoparticles by the reduction of the aqueous silver metal ions during exposure to the lichen extracts.

Formation of $AgNo_3$ nano particles

Formation of silver nano particles was presumed visually by observing the colour change. The methanolic extracts of both lichens were mixed with a 1 mM $AgNO_3$ solution. The mixture was kept at room temperature for 24-48 hours. The appearance of a yellowish-brown colour in the reaction vessel indicated formation of AgNPs (Figure-1). Silver nitrate nanoparticles (AgNPs) exhibited yellowish-brown colour in methanolic solution due to excitation of surface plasmon resonance in the AgNPs (Ropisah Mie *et al*., 2014).

UV-Vis spectroscopy

Ultraviolet-visible spectroscopy was used to characterize the synthesized AgNPs. The UV-Vis absorption spectra of the silver nano particles are shown in the Figure-2. The lichen extract solution exposed to $AgNO_3$ ions shows a distinct absorption peak at 412 nm. This gives the conformation of formation silver nanoparticles which had previously been presumed by the change in colour (yellowish-brown) (Nowack and Bucheli, 2007).

SEM Analysis

The SEM analysis reveals that the silver nano particles formed is cubic in nature. The size seen in the micrograph is in good agreement with the size determined from the XRD analysis. From the micrograph given in Figure 3, it was found that the particle size to be at 46.17nm & 68.06 nm, which is an agreement with the XRD analysis (Figure-3) (Basavaraja *et al.,* 2008).

FTIR Analysis

Infrared measurements were carried out to identify the possible functional groups responsible for the stabilization of the newly synthesized silver nanoparticles. The infrared spectra are recorded on Fourier Transform Spectrometer in the mid-infrared region (MIR) within the range 800-4000 cm^{-1}.

After analysis, (Figure.-4) bands are observed at 3433^{-1} , which is O-H bond stretch characteristic of phenols, 2044^{-1} that is characteristic of C-H stretch for alkanes, the absorption peak at 1589^{-1} is characteristic of N-H bond present in amines (Shahverdi *et al.,* 2007).

XRD Analysis

The structure of prepared silver nano particles has been investigated by X-ray diffraction (XRD) analysis. Typical XRD patterns of the sample prepared are shown in the (Figure-5). The XRD study indicates the formation of silver (Ag) nano particles (Roh *et al.,* 2001).

From this study, considering the peak at 46.5 degrees, average particle size has been estimated by using Debye-Scherrer formula.

$$D = \frac{0.9\lambda}{w\cos\theta} \quad \text{---------- (1)}$$

Where λ is wavelength of X- ray (0.1541 nm), "W" is FWHM (full width at half maximum), "θ" is the diffraction angle and "D" is particle diameter (size)

EDAX Analysis

This analysis was done with the SEM (scanning electron microscope). During EDAX analysis, the specimen was bombarded with an electron beam inside the scanning electron microscope. The bombarding electrons collide with the specimen atoms own electrons, knocking some of them off in the process. A position vacated by an ejected inner shell electron is eventually occupied by a higher-energy electron from an outer shell. The amount of energy released by the transferring electron depends on which shell it is transferring from, as well as which shell it is transferring to. Furthermore, the atom of every element releases X-rays with unique amounts of energy during the transferring process. Thus, by measuring the amount of energy present in the X-rays being released by a specimen during electron beam bombardment, the identity of the atom from which the X-ray was emitted can be established.

The EDAX spectrum (Figure-6) showed high for silver signals. The vertical axis shows the counts of the X-ray and the horizontal axis shows energy in keV. The strong signals of silver correspond to the peaks in the graph confirming presence of silver nanoparticles. Metallic silver nanocrystals generally show typical optical absorption peak approximately at 3 keV due to surface plasmon resonance. There were other EDAX peaks for Mg, Cl, Ca suggesting that they are mixed precipitates present in the lichen extract (Petit *et al.,* 1993).

Antibacterial activity of lichen mediated AgNPs

Antibacterial activity of methonalic lichen extract mediated AgNPs showed better inhibiting potential

than the normal methanolic crude extracts in controlling the proliferation by static or demise of bacterial population at 10 µg/ml itself. On average there was 25% increase in zone of inhibition with better results for *Acinetobacter baumanii* and *Staphylococcus aureus* (Table- 1). Similar results have been earlier proven for many bacterial and fungal mediated silvernanoparticles and few reports have been documented for lichens also (Laily *et al*., 2015 and Ropisah Mie *et al*.,2014).

Fig -1: change of colour(Formation of brownish yellow) due to silver nanoparticle synthesis by methanolic extracts of *Heterodermia boryi* and *Parmotrema stuppeum*

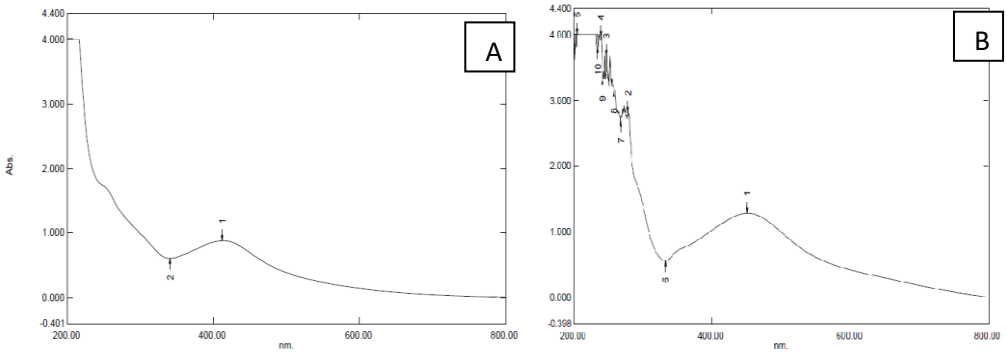


Fig.-2: UV absorption spectra for silver nanoparticle synthesis by methanolic extracts of *Heterodermia boryi* (A) and *Parmotrema stuppeum* (B)

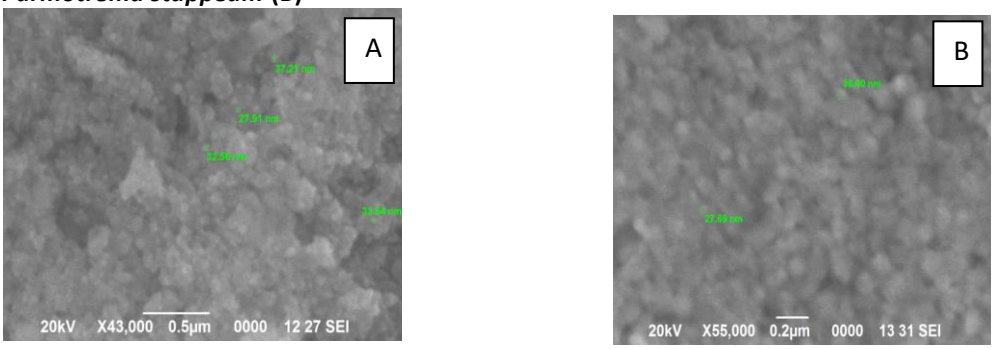
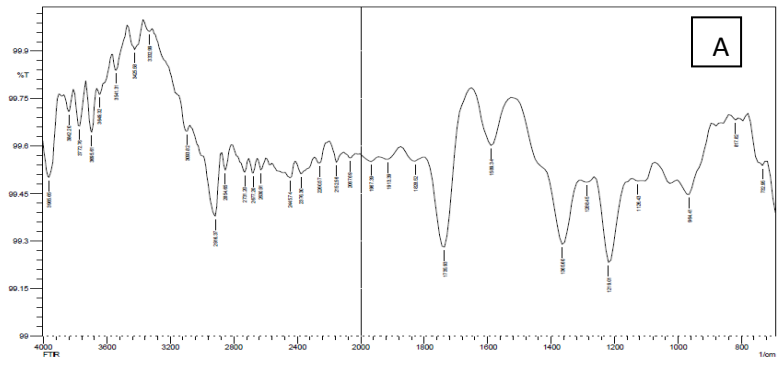


Fig-3: Scanning electron microscope for silver nanoparticles synthesised by methanolic extracts of *Heterodermia boryi* (A)and *Parmotrema stuppeum*



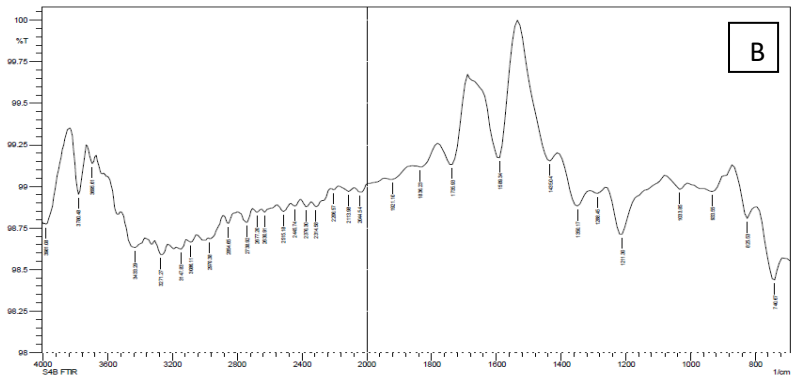


Fig.-4 FTIR spectral images for silver nanoparticles synthesised by methanolic extracts of *Heterodermia boryi* (A)and *Parmotrema stuppeum*(B)

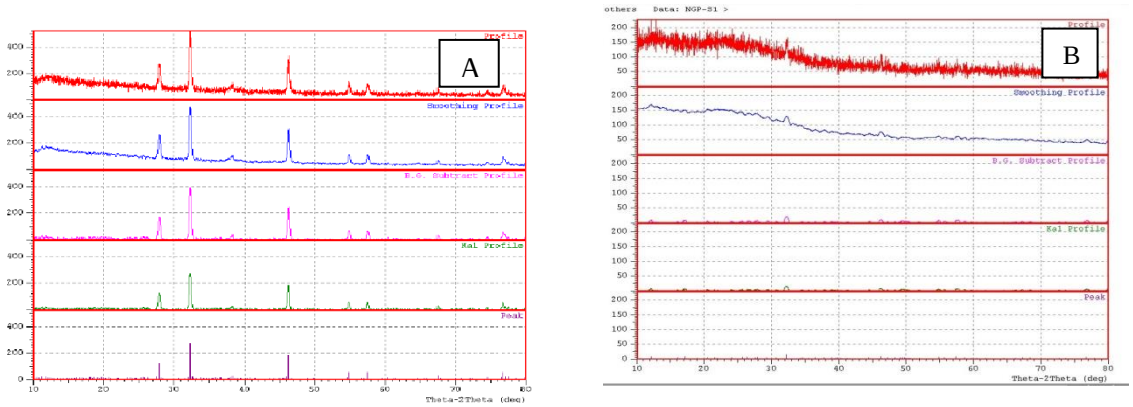


Fig 5: X-Ray Diffraction(XRD) analysis of for silver nanoparticles synthesized by methanolic extracts of *Heterodermia boryi* (A)and *Parmotrema stuppeum*(B)

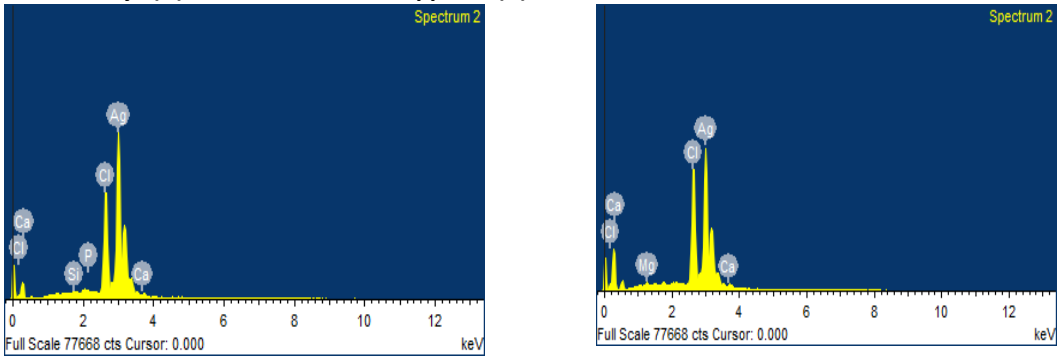


Fig 6: EDAX spectral for silver nanoparticles synthesised by methanolic extracts of *Heterodermia boryi* (A)and *Parmotrema stuppeum*(B)

Table-1: Antibacterial activity of Methanolic extracts of *Heterodermia boryi* and *Parmotrema stuppeum*

S. No	Lichens	*E. c*	*K.p*	*P.a*	*A.b*	*S.a*	*V.S*	MRSA
1	*Heterodermia boryi*	13±0.5	11±0.5	10± 0.5	22±2.0	13± 1.0	14± 0.0	11±0.0
2	*Parmotrema stuppeum*	15±0.0	19± 1.0	13± 0.5	15± 1.0	23± 2.0	21± 1.0	15± 0.5

Note: *E.C- Eschericia coli. K.p-Klebsiella pneumonia, P.a- Pseudomonas aeruginosa, A.b- Acinetobacter baumanii, S.a- Staphylococcus aureus, S.v- Viridans Streptococci and MRSA-Methicillin Resistant Staphylococcus aureus*: (* Values are mean ± by Std. deviation, n=3)

CONCLUSION

This study is ecofriendly silver naono particles synthesized by methanolic extracts of lichens *Heterodermia boryi and Parmotrema stuppeum.* The size of nanoparticle was 27.91nm to 37.21 and 27.69 to 36.00 nm and mostly formed in cubic in nature.

The antibacterial activity of this nanoparticle tested against various bacterial pathogens. The potential activirty was exhibited against *Acinetobacter baumanii* and *Staphylococcus aureus.* This study revealed green synthesis of nanopartice by using lichen and antibacterial activity against both gram positive and gram-negative bacteria. This finding leads to discovery of new drugs against drug resistant pathogens and various applications in biomedical fields.

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REFERENCES:

1. Arun, P., Shanmugaraju, V., Renga Ramanujam, J., Senthil Prabhu, S. and Kumaran. E. (2013). Biosynthesis of Silver Nanoparticles from *Corynebacterium* sp and its antimicrobial activity. *Int. J. Curr. Microbiol.App.Sci., 2*(3):57-64.
2. Asmita, J., Gavhane, P., Padmanabhan, S., Kamble, P. and Suresh, N. J. (2012), Synthesis of silver nanoparticles using extract of neem leaf and evaluation of their antimicrobial activities. *Int.J of Pharma and BioSci.,* 3(3): 88 – 100.
3. Basavaraja, S., Balaji, S. D., Lagashetty, A., Rajasab, A. H. and Venkataraman, A. (2008). Extracellular biosynthesis of silver nanoparticles using the fungus *Fusarium semitectum*. *Mater. Res. Bull.*, 43(5): 1164-1170.
4. Bates, S.T., Cropsey, G.W., Caporaso, J.G., Knoght, R. and Fierer, N. (2011). Bacterial Communities associated with the lichen symbiosis. *Appl. Environ. Microbiol.,* 77:1309-1314.
5. Govindharaju, K., Tamilselvan, S., Kiruthiga, V. and Singaravelu, G. (2010). Biogenic silver nano paricle by *Solanum torvum* and their promising antibacterial activity. *J. Biopest.,* 3(1): 394-399.
6. Manojlovic, N. T. Solujic, S.and Sukdolak, S. (2002) "Antimicrobial activity of an extract and anthraquinones from *caloplaca schaereri*," *Lichenologist*, 34(1): 83–85.
7. Negi, H.R. & Gadgil, M. (1996). Patterns of distribution of macrolichens in Western parts of Nada Devi Biosphere Reserve. *Curr. Sci*., 71(7): 568-575.
8. Nithya, R. and Ragunthan, S. (2009). Synthesis of silver nanoparticles using Pleurotus sajorcaju and its antimicrobial activity. *Dijestive J. Nanomat.Biost.,* 4(4): 623- 629.
9. Nowack, B. and Bucheli, T. D. (2007). Occurrence, behavior and effects of nanoparticles in the environment. *Environ.l Poll.*, *150*(1), 5-22.
10. Petit, C., Lixon, P. and Pileni, M. P. (1993). In situ synthesis of silver nanocluster in AOT reverse micelles. *The J. of Physical Chem.*, 97(49), 12974-12983.
11. Rajesh, W.R., Niranjan, S.K., Jaya, R.L. Vijay, D.M. and Sahebrao, B.K. (2009). Phytosynthesis of silver nanoparticle using *Gliricidia sepium. Curr. Nanosci.*, 5:117-122.
12. Roh, Y., Lauf, R. J., McMillan, A. D., Zhang, C., Rawn, C. J., Bai, J. and Phelps, T. J. (2001). Microbial synthesis and the characterization of metal-substituted magnetites. *Solid State Communications*, 118(10), 529-534.
13. Ropisah Mie, Mohd Wahid Samsudin, Laily B Din, Azizan Ahmad, Nazlina Ibrahim, and Siti Noor Adnalizawati Adnan (2014). Synthesis of silver nanoparticles with antibacterial activity using the lichen *Parmotrema praesorediosum.* Int. J Nanomedicine.; 9: 121–127.
14. Sastry, M., Patil, V. and Sainkar. (2002). Extracellular biosynthesis of silver nanoparticles using the fungus *Fusarium oxysporum*. *Trends in Biotech*, 16-29.
15. Shahverdi, A. R., Minaeian, S., Shahverdi, H. R., Jamalifar, H. and Nohi, A. A. (2007). Rapid synthesis of silver nanoparticles using culture supernatants of Enterobacteria: a novel biological approach. *Process Biochem.*, 42(5): 919-923.
16. Sivalingam, P., Antony, J.J., Siva, D, Achiraman, S. and Anbarasu, K. (2012). Mangrove Streptomyces sp. BDUKAS10 as nano factory for fabrication of bactericidal silver nanoparticles Colloids and Surfaces B: *Biointerfaces*, 98: 12-17.