American Journal of Advanced Drug Delivery

www.ajadd.co.uk

Original Article

Biological Synthesis of Silver Nanoparticles from *Aspergillus fumigatus*

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Date of Receipt- 20/10/2014
Date of Revision- 06/11/2014
Date of Acceptance- 07/11/2014

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ABSTRACT

The synthesis of nanoparticles from the microbes is a boon for advance research in nanotechnology. In this study, silver nanoparticles were synthesized using the fungus *Aspergillus fumigatus* with an aqueous solution of $AgNO_3$. Synthesized silver nanoparticles (Ag-NPs) were characterized through UV-visible spectrophotometer and Fourier Transform Infrared Spectroscopy (FTIR). Maximum absorbance was observed at 420 nm in visible region. The nature of coordination between bioactive compounds secreted by fungi and silver ions were analyzed through FTIR spectroscopy. The reduction of silver ions was due to amino groups of proteins and other functional groups in the cell free filtrate of fungi. The reduction of silver ions leads to the formation of stable protein capped silver nanoparticles. The Ag-NPs and Ag-NPs + Chloramphenicol (Ab) possess potential antimicrobial activity against to *Escherichia coli, Klebsiella pneumonia, Bacillus cereus, Staphylococcus aureus* and *Streptococcus* sp.

Keywords: Ag-NPs, *A. fumigatus*, UV- visible spectrophotometer, FTIR, Antibacterial activity.

INTRODUCTION

The unusual characteristics of nanoparticles are not present in the larger sizes if they are same materials. The Professor Norio Taniguchi coined the term nanotechnology in the year 1974. One billionth is equal to one nanometer (nm). The innovations in the "Transmission electron microscopy (TEM), Scanning electron microscopy (SEM), Atomic force microscopy (AFM), Fourier Transform Infrared Spectroscopy (FTIR)"etc., nanotechnology today has reached a stage where, it is considered as the future to all technologies. Nanoparticle synthesis through biological processes is an innovative approach showing ecofriendly nature,

reliability and cost effective than present physicochemical processes. Nanotechnology is an upcoming and fast developing field with potential applications for human welfare. In context that the, nanotechnology gives a modified means of important features of various materials along metal nanoparticles.[1] Synthesis of nanoparticles, employing microorganisms has attracted great interest due to their unusual optical[2], chemical[3], photo electrochemical[4] and electronic properties[5] which enable the synthesis of nanoparticles of different chemical compositions, well-defined sizes and distinct morphologies.[6] Nanoparticles are synthesized from various microbes through extracellular or intracellular processes.[7] A large number of bacterial and fungal species have antimicrobial activity through reducing the metal ions for production of metal nanoparticles. Recent studies declaring that the fungi showed the good potential for synthesizing bio active compounds when compared with bacteria. Which indicating that the fungi was most suitable for production in large amounts.[8] In addition, the extracellular biosynthesis using fungi could also make downstream processing much easier[9], high tolerance to temperature fluctuations and show high capacity towards bioaccumulation than bacteria.[10] Synthesis of nanoparticles in higher amounts from fungal species shows beneficial aspects like environmentally friendly and amiability. Extra cellular enzymes are potentially and easily produced from fungi in large amounts. The main feasibility of using fungi in synthesis of nanoparticles include ease of handling and economically possible. For synthesis of nanoparticles filamentous fungi plays an important role. Silver ions are extracellularly reduced by filamentous fungi like *A. fumigatus* efficiently. Studies on the fungus *A. fumigatus* reports, these species produce biosynthesis of nanoparticles rapidly is a good candidate for rapid biosynthesis of silver nanoparticles.[12,13] The Ag-NPs are effective against pathogenic microbes they showed efficient antimicrobial activity.[14] The size Ag-NPs 10-15nm with increase in stability, enhanced Antimicrobial property. In order to prevent the spoilage of water, alcoholic beverages they were stored in silver containers. Silver nitrate was in treatment of chronic wounds and ulcers during 17th century. In 19th century $AgNO_3$ was used to treat burns and to prevent ophthalmic problems in young once. During beginning of the 20th century, "Barns recognized the silver nitrate was caustic for the eyes of newborns and he invented argyrol, a protein-stabilized silver colloid". Research on silver compounds grows to a wide range in order to improve antimicrobial activity, disinfection property. The Ag-NPs gained potential importance because of their salient features like antimicrobial, antisepsis activity they are widely used in medicinal applications and in to maintain the hygiene conditions.[15] The Ag-NPs have proved that they are more potential effective against bacterial species, viruses and to other eukaryotic microbes.

MATERIALS AND METHODS

Microorganism

The fungus *Aspergillus fumigatus* (MTCC-11399), used for the synthesis of Ag-NPs was isolated from paddy fields after their harvesting in Pendurthi, Vijayanagaram District of Andhra Pradesh, India. The fungal culture was maintained on Potato Dextrose Agar (PDA) slants at 30^0C for 48hrs for further use.[16]

Extracellular synthesis of Ag-NPs

The fungal strain *Aspergillus fumigatus* was freshly inoculated on a liquid media containing (g/l)" KH_2PO_4, 7.0: K_2HPO_4, 2.0; $MgSO_4.7H_2O$, 0.1; $(NH_4)_2SO_4$, 1.0; yeast extract, 0.6; and

glucose, 10; in an Erlenmeyer flask". The flak was incubated on orbital shaker at 30^0C and agitated at 150 rpm at 3days. The fungal biomass was harvested after 3 days, by sieving through Whatman No 1 filter paper, later thoroughly wash with deionized water to remove the other components in the media from the biomass. Typically 20g of fresh and clean biomass was taken into Erlenmeyer flaks containing 200 ml of deionized water and the flask was incubated at 30°C for 3 days and agitated at 150rpm. Later the cell filtrate was obtained through passage of culture media through Whatman No-1 filter paper. Fifty milliliters of cell free filtrate (CFF) was taken into 250 ml of Erlenmeyer flask and mixed with 1 mM $AgNO_3$ (0.017 g $AgNO_3$/100ml) as final concentration. The flasks were incubated at 30° C in dark room up to 3 days. Control was maintained (without addition of $AgNO_3$, only cell filtrate) with the experimental flask. In order to usage for future experiments the brownish yellow color Ag-NPs solution was stored in amber color bottles.[13]

Characterization of Ag-NP

The synthesized Ag-NPs were first characterized by UV-Visible spectrophotometer in the range of 320 - 560 nm with control as the reference. The surface plasmon resonance peaks are found noted to be reliably around 420 nm regions further the Ag-NPs kept at room temperature for three months to test their stability. Analysis of Ag-NPs by FTIR through spectrum scanning range 450- 4000 cm^{-1} at resolution of 4 cm^{-1} was carried out.[13,17]

Characterization of antibacterial activity

Antibacterial property was performed by using the "Nathan's Agar Well Diffusion" technique.[18] Eight millimeter diameter of 2 wells was made on PDA plates. These PDA plates were inoculated by swabbing the 18-24hrs isolates *Escherichia coli, Klebsiella pneumonia, Bacillus cereus, Staphylococcus aureus and Streptococcus sp.*, suspensions in order to get proper growth. The Ag-NPs (100 µl) was loaded on one well and other well with 60 µl of Ag-NPs + 40µl of Chloramphenicol (a wide range of antibacterial drug) each well. Wells without the extracts were maintained as control. After completion of incubation period at 30^0C temperature and 24hrs the susceptibility was measured by considering the inhibition zone diameter around each well to the nearest mm.

RESULTS AND DISCUSSION

Ag-NPs synthesis

The present research work was carried out in the extracellular synthesis Ag-NPs in a comprehensive manner. After 3 days of incubation, the fungal biomass was filtered, filtrate was exposed to $AgNO_3$. The reaction was started after 24 hours incubation in dark condition, the pale yellow color of the cell free filtrate (CFF) changed to dark brownish yellow color indicating the formation of Ag-NPs (Fig 1) which correlates with the results obtained by Ingle and Prameela[19,20]. There is no color change noted in the control flask incubated in the same environment.

Characterization by UV- visible spectroscopy

Synthesized Ag-NPs absorption capacity was observed at every 24hrs of incubation. Figure 2 shows the absorption maxima (0.72) band at 420nm after 3 days of incubation. Up to some extent the $AgNO_3$ intensity was increased with time was clearly recorded in the spectrum. The brownish yellow color is due to the "surface of plasmon resonance of deposited silver nanoparticles" that is, "the color of the Ag-NPs due to the coherent and collective

oscillations of the surface electrons"[21]. The peak formed at 420 nm is the characteristic indication for the presence of the proteins and enzymes. These bioactive compounds are responsible for the reduction of metal ions for synthesis of nanoparticles[22].

FTIR analysis of Ag-NPs

Silver nanoparticles were analyzed through FTIR to find out the interactions between silver and bioactive compounds produced by fungi. These bioactive compounds play major role in metal ion reduction, stabilization and synthesis of Ag-NPs. The amide linkages between amino acid residues in proteins give a important signature in the infrared region of the electromagnetic spectrum.[23] The FTIR spectrum (Figure 3) revealed a peak at 3450.41 cm^{-1} which could be attributed to strong stretching vibrations of hydroxyl functional group.[24] The peak at 1587.72 cm^{-1} indicates aromatic skeletal vibrations.[25] The peak at 1417.90 cm^{-1} may be related to COO^- symmetrical stretch from carboxyl groups of the amino acid residues.[26] The main important potentially active functional groups for Ag-NPs, Ag^+ ions, and anisotropic growth are the Tyr residues from hydroxyl groups and Asp, Glu residues from carboxyl groups.[27] The peaks 1384.47 may represent the residual nitrate (NO^-_3).[28] Peaks at 1154.88 $cm^{-1}$and 1078.10 cm^{-1}, indicates -N-H and carbonyl (C-O-) stretching vibrations respectively in amide linkages of proteins re.[29] The peak formed at 2921.20 cm^{-1} could be due to C-H stretch of methylene groups of proteins.[30]

Antibacterial activity

The Ag-NPs produced from the *Aspergillus fumigatus* were assayed for their antibacterial activity with and without the presence of standard antibiotic (Ab) chloramphenicol against the *Escherichia coli, Klebsiella pneumonia, Bacillus cereus, Staphylococcus aureus and Streptococcus sp.* The Fig 4, 5, 6, 7, 8 and 9 indicates their zones of inhibitions respectively. *A. fumigatus* is good efficient in production and green synthesis of nanoparticles. Maximum zone of inhibition was observed with *Staphylococcus aureus* (16mm), and there is no significant difference observed with Ag-NPs + Ab, remaining bacterial species showed difference in zone of inhibition formed with Ag-NPs + Ab. The diameter of zones of inhibition (mm) formed by *E. coli, K. pneumonia, B. cereus, S. aureus* and *Streptococcus sp.,* showed the similarities with[31] Manju bala and Vedpriya arya and[32] Mudasir. The unusual salient features of Ag-NPs make them being perfect model for various technologies including the antimicrobial, optical and biomedical hygiene applications, as well as use in nanotoxicology studies.[31]

CONCLUSION

In conclusion, the filamentous fungus, *A. fumigatus* has shown potential for extracellular synthesis Ag-NPs. Synthesis of Ag-NPs using the cell free filtrate is rapid. This indicates nanoparticle synthesis from biological process is quick suitable for larger scale production. The characterization of Ag-NPs was through UV visible spectrophotometer and FTIR analysis. Nanotechnology exhibits contemporary and revolutionary approach to formulate and to test the new approaches based on antimicrobial properties from the metallic nanoparticles. Ag-NPs showed remarkable antibacterial activity against *Escherichia coli, Klebsiella pneumonia, Bacillus cereus, Staphylococcus aureus and Streptococcus sp*. The bacteria which showed the resistant to antibiotics, indicates sensitivity to Abs in combination with silver nanoparticles. Synthesis of Ag-NPs using *A. fumigatus* is potential in, eliminating the problems caused by chemicals that produce negative effects

in applications, this results biological synthesis of nanoparticles are more biocompatible.

Declaration of competing and conflict of interests statement

Authors didn't have any conflicts or competing interests.

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Figure 1. Synthesized nanoparticles

Fungal cell free filtrate before and after treatment with $AgNO_3$

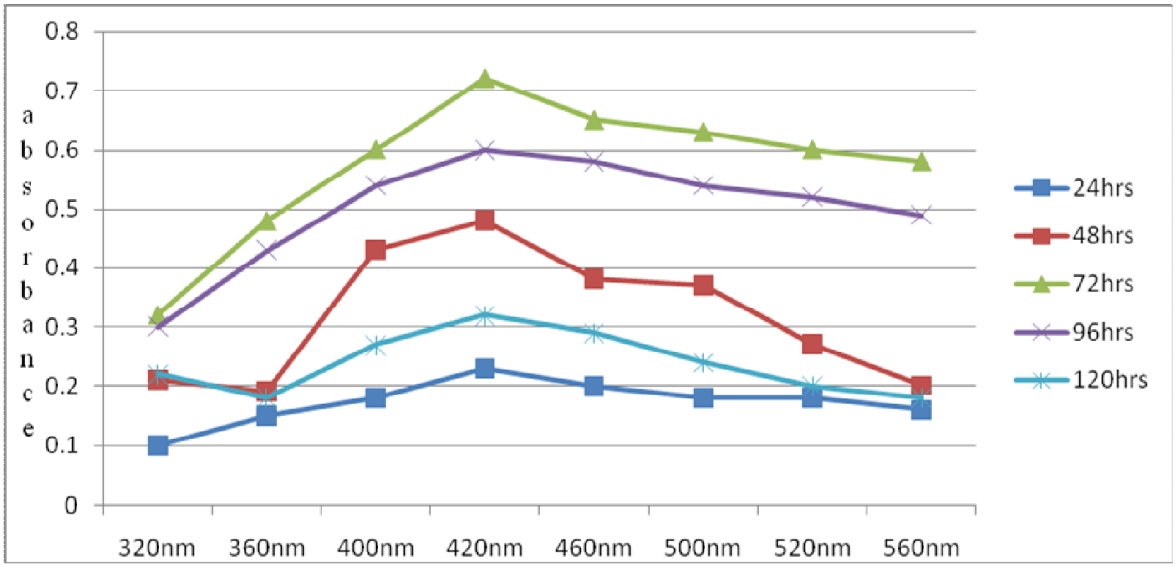

Figure 2. UV-visible spectra of Ag-NPs synthesized by *Aspergillus fumigatus*

Cell free filtrate of Ag-NPs showing maximum absorbance at 420nm at 72hrs

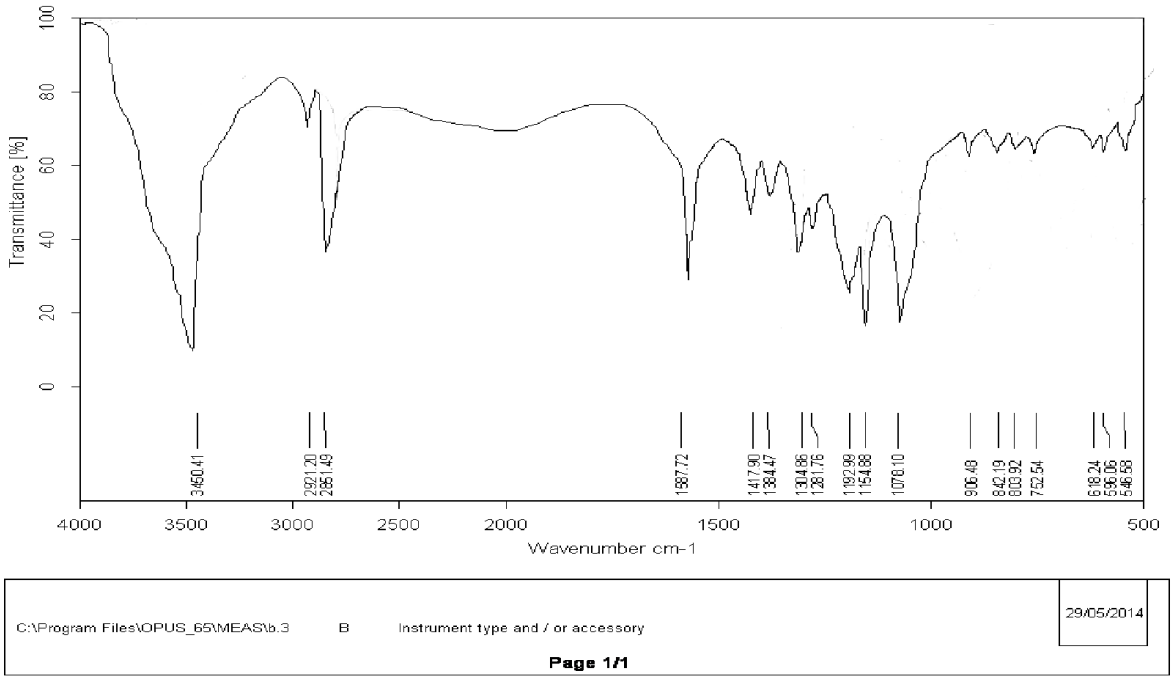

Figure 3. FTIR analysis of Ag-NPs

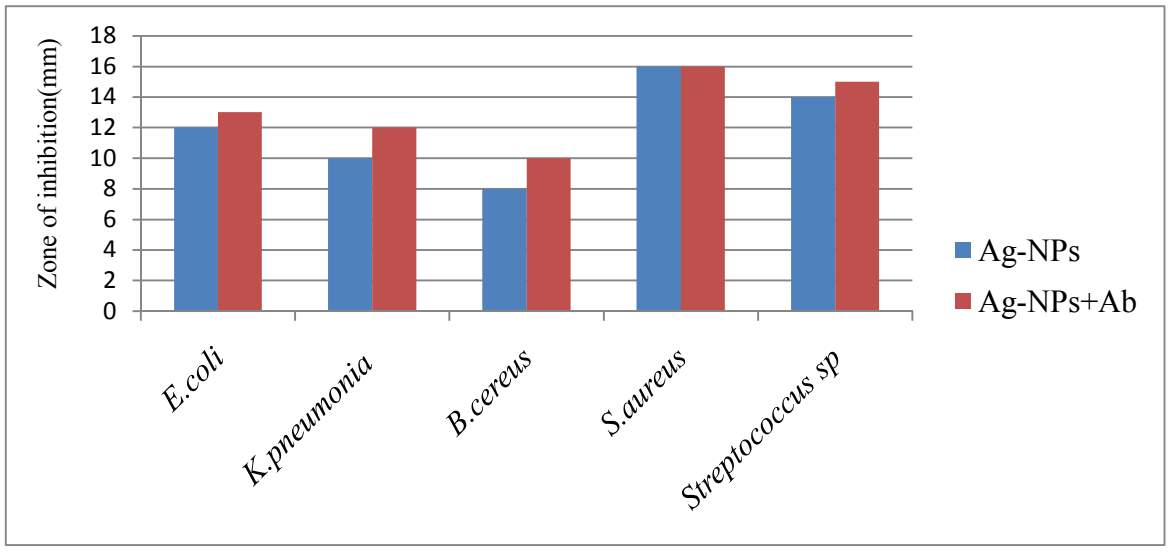

Figure 4. Antibacterial activity of Ag-NPs and Ag-NPs + Chloramphenicol (Ab)

Zone of inhibitions formed against Ag-NPs and Ag-NPs + Chloramphenicol (Ab) by bacterial species

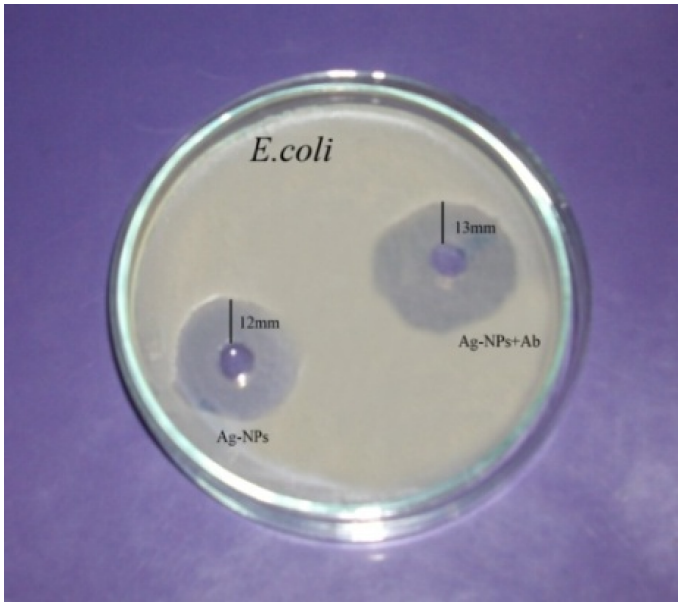

Figure 5. *E. coli*

E. coli, showing 12 mm with Ag-NPs and13 mm with Ag-NPs + Ab

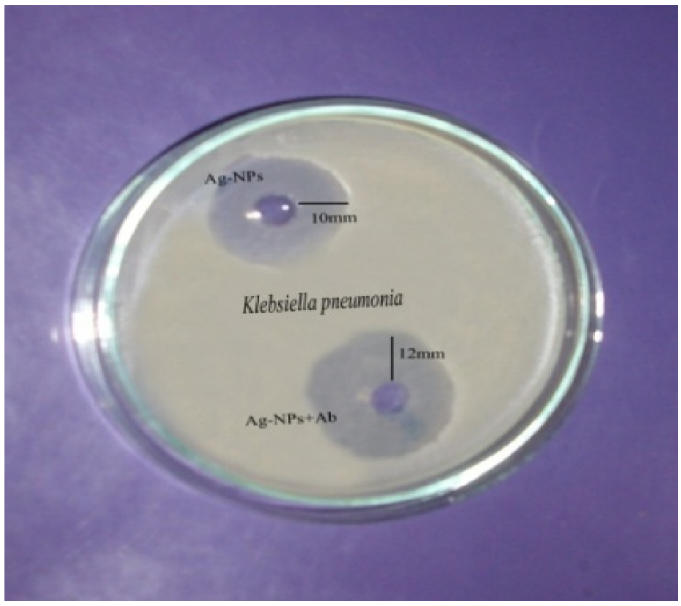

Figure 6. *K. pneumonia*

K. pneumonia showing 10 mm with Ag-NPs and12 mm with Ag-NPs + Ab

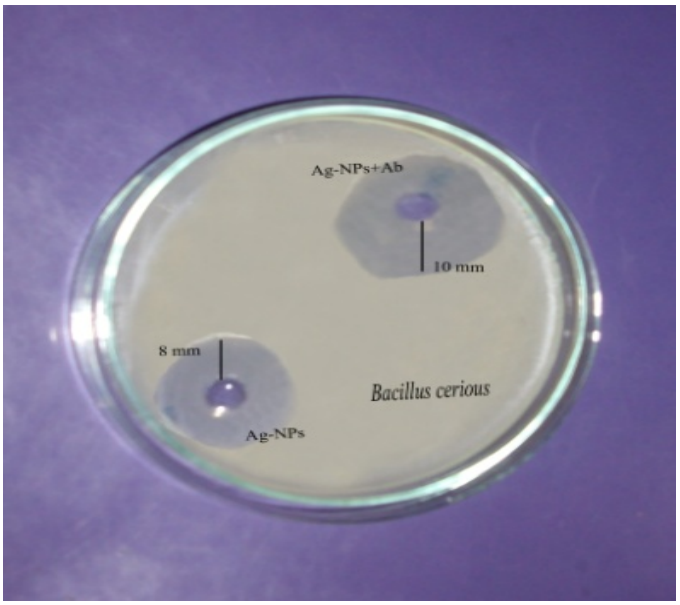

Figure 7. *Bacillus cereus*

B. cereus showing 8 mm with Ag-NPs and10 mm with Ag-NPs + Ab

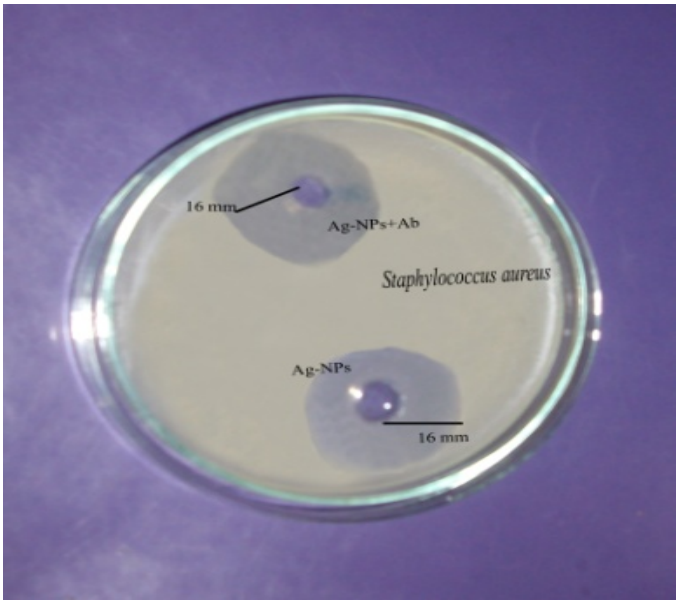

Figure 8. *Staphylococcus aureus*

S. aureus showing 16 mm with Ag-NPs and Ag-NPs + Ab

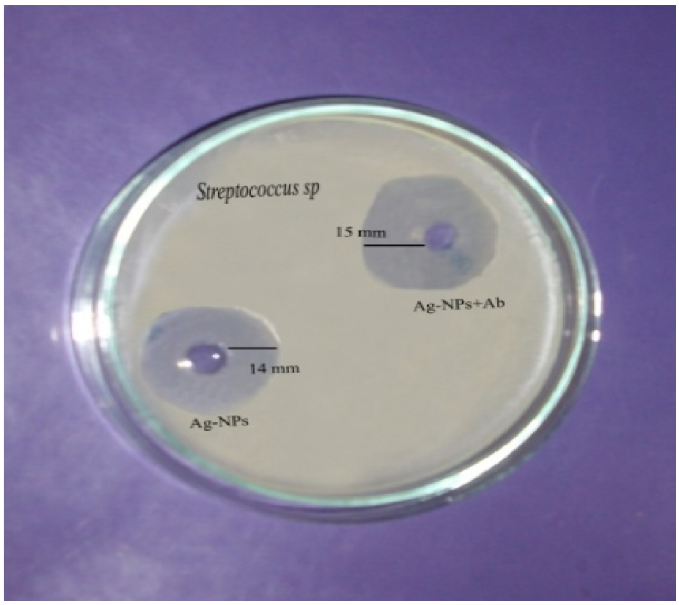

Figure 9. *Streptococcus sp*

Streptococcus sp., showing 14 mm with Ag-NPs and15 mm with Ag-NPs + Ab