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Research article

BIOSYNTHESIS AND ASSESSMENT OF SILVER NANOPARTICLES WITH SPARFLOXACIN AND OFLOXACIN SYNTHESIZED FROM *PENICILLIUM SP.* ON SOME BACTERIAL PATHOGENS

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ABSTRACT: Here in this paper we have reported the extracellular biosynthesis of silver nanoparticles by using filamentous fungi *Penicillium sp*. The *Penicillium sp.* was isolated from the soil sample collected from vegetable market in Chennai, Tamil Nadu, India. The purified fungal culture was subjected for the biosynthesis of silver nanoparticles. The color change of the solution in the conical flask into dark brown suggests the formation of silver nanoparticles(AgNPs). These Silver nanoparticles have been further characterized by UV-vis spectrophotometer which showed the absorption peak at 416nm which confirms the nanoparticles synthesis, Fourier Transform Infrared (FTIR) Spectroscopy showed proteins and functional groups which stabilizes the nanoparticles as capping agent , X-ray diffraction (XRD) analysis determine the crystalline nature of silver nanoparticles and Field emission scanning electron microscopy (FESEM) analysis which showed the particle size was around 35 to 67 nm and. These biologically synthesized silver nanoparticles showed good antibacterial activity against the selected bacterial pathogens and also these nanoparticles enhanced the antibacterial property of Sparfloxacin and Ofloxacin.

Key words: Silver nanoparticles, *Penicillium* sp., FESEM, UV- vis Spectrophotometery, XRD, FTIR.

INTRODUCTION

The increase in the infectious diseases caused due to the bacteria and fungi, which are now becoming resistant to different available antibiotics is the matter of concern. Therefore present situation demand to find a newd new antibacterial material to control the bacterial and fungal infections. Due to the advancement in the field of nanotechnology and nanoscience which involves with the use of metallic nanoparticles have increased their application in the field of medicine, biotechnology, ceramics and food packing (Gajjar *et al* ., 2009). In the ancient times silver and copper were used to treat the bacterial infections (Moghimi 2005) These metallic nanoparticles have attracted the attention of many researchers, Pharmaceutical companies due to its antibacterial property, small size and surface effect (Nanda *et al* ., 2009) Among the all metallic Nanoparticles silver Nanoparticles posses high antibacterial activity and can be used for various medical products, textile products, water filtration, coatings etc due to high thermal stability and less volatility(Rai *et al* 2009, Gong *et al*., 2007). Biological method for the synthesis of Silver nanoparticle is the method of choice as it is simple and free from toxic chemicals,easy to handle and ecofriendly. Silver nanoparticles can be synthesized from bacteria,plants and fungi but fungi has the advantage over bacteria and plants because they are very tolerant towards the metals,secretes large amount of enzymes, high capacity to bind with the cell wall and production of mass is quite simple for the synthesis of silver Nan particles (Chen *et al* ., 2003, Dias *et al*., 2002). Here we reported the extracellular biosynthesis of sliver Nanoparticles from *Penicillium species* which is followed by characterization of Nanoparticles using UV-vis spectrophotometry, FTIR, XRD and FESEM. These biologically synthesized nanoparticles have been checked for its antibacterial activity against various bacterial pathogens and evaluate the synergistic effect of these Nanoparticles with Sparfloxacin and Ofloxacin.

MATERIALS AND METHODS

Sample collection

Soil sample was collected from the different areas of Vegetable market of Chennai Tamil Nadu India. Soil Sample was collected from 4 to 5cm depth with the help of sterile spatula. Samples were transferred in to sterile plastic bags and brought to the Biomedical and Research laboratory and stored in a refrigerator at 4^0c up to further process.

Isolation of fungal culture.

Isolation of soil fungi was performed by serial dilution and spread plate method. one gram of soil sample was serially diluted in sterilized distilled water to get the concentration which ranges from10^{-1} to 10^{-6}. A volume of 0.1 ml of each dilution was transferred aseptically to SDA plates. The sample was uniformly distributed by using sterile glass spreader. The plates were incubated at room temperature for 5 days. The fungal isolates were sub cultured on SDA plates in order to segregate the isolated fungi in to pure culture. Pure isolated fungal culture were maintained at 4^{o}c for further studies.

Microscopic and colony characterization

The fungal isolate of *Penicillium species* was observed by the author expertise using hand lens and the colony morphology was recorded with respect to color, shape, size and nature of colony and also by using laboratory manuals.

Synthesis of silver Nanoparticles

Penicillium species was utilized for the extracellular synthesis of silver nanoparticles. Fungal biomass was grown aerobically in a liquid medium containing (g/L): KH_2PO_4 7.0; 2.0 K_2HPO_4 $MgSO_4$. $7H_2O$ 0.1; $(NH_4)2SO_4$ 1.0; yeast extract 0.6; glucose 10.0 at 25 ± 3^{0}c. After incubation, the biomass was filtered using Whatman filter paper No.1 and extensively washed with distilled water to remove residual parts. The fresh and clean biomass was taken into an Erlenmeyer flask, containing 100ml of deionized Milli-Q water. The flask was incubated at 25 c in a shaker incubator at 140 rpm for 72 hours. The biomass was filtered again with Whatmann filter paper No.1 and the cell free extract was used further. 1mM $AgNO_3$ was repared and 50ml was added to the cell-free extract and kept further in the incubator at 25 c, 140rpm for 72hours in dark condition.

Characterization of silver nanoparticles

The samples were observed for alteration of solution color and maximum absorbance was analyzed using UV-spectrophotometer. 1ml of sample supernatant was taken after 72hours and absorbance was measured by using UV-visible spectrophotometer between 300-600nm analysis. The powder form of the sample was subjected to FTIR spectroscopy analysis. Two milligram of sample was taken and press it to form pellet .The sample was placed into the sample holder and FTIR graph was taken. After the synthesis, these silver nanoparticles were further characterized by X ray diffraction analyses which determine the crystalline nature of silver nanoparticles .For XRD sample is prepared by the centrifugation of the silver nanoparticle solution, for 10 minutes at 15000 rpm. The pellet is then dried and makes it in to powder form and subjected for the XRD analysis. FESEM is used to determine the surface morphology and size of the nanoparticles. For FESEM sample has been prepared by centrifugation and then dried into powder form and subjected to FESEM analysis

Evaluation of antibacterial Activity

Biologically synthesized silver nanoparticles were checked for its antibacterial activity by using disc diffusion method. The antibacterial activity of silver nanoparticles from *Penicillium sp.* was tested against the pathogenic bacteria such as *Staphylococcus aureus, Bacillus cereus, Escherichia coli* and *Proteus vulgaris*. The combined formulation of silver nanoparticles with standard antibiotic disc Ofloxacin and Sparfloxacin were used to find out the synergistic activity against the above bacterial pathogens. The zone of inhibition was measured after overnight incubation at 37^{0}c (Bauer *et al*.,1996).

Increase in fold area

The increase in mean fold area can be calculated by the mean surface area for the zone of inhibition of each antibiotics that has been used alone and antibiotic + AgNPs. The increase in fold area of different pathogens for antibiotics and antibiotics + AgNPs can be calculated by using this equation $(B^2 – A^2)/A^2$, where A is the antibiotic alone and B is the antibiotic + AgNPs respectively (Birla *et al*., 2009).

RESULTS AND DISCUSSION

The *Penicillium species* were used for the biosynthesis of Silver nanoparticles(Fig 1).The change in the color of solution in to the dark brown by the addition of Silver nitrate (AgNO3) results in the formation of silver nanoparticles due to the reduction of Ag^{+} to Ag^{0} (Fig 2). These reports have been shown many researchers in the past (Gardea.*et al* 2003, Gade *et al* 2008, Ingle *et al* 2009). After the formation of silver nanoparticles from the fungal filtrate which have been further characterized by using UV-Vis spectrophotomer which shows the absorption peak at 416nm due to surface Plasmon resonance (Mulvaney 1996, Ingle *et al* 2008) (Fig 3) which reports the synthesis of silver Nanoparticles and peak is specific for silver Nan particles(Sastry *et al* 2003).

FTIR analysis were used to identify the molecules , proteins and functional groups involved in the reduction of silver ions in to silver nanoparticles and stabilizes them as capping agent (Fig 4).

The FTIR analysis obtained for the Nanoparticles shows the absorption peaks located at 3396.3 cm^{-1}(O-H stretch), 2924.8 cm^{-1}(C-H stretch alkanes), 1648.9 cm^{-1} (NH stretch of amide), 1546.2 cm^{-1} (N-H(1^0) bend of amide),1382cm^{-1} (NO_2 aliphatic) ,1236 cm^{-1} (C-O stretch of carboxylic acid),1070.2cm^{-1} (C-N Stretch of amines) and also some small peaks are there.

XRD is used to determine the crystalline metallic nature of silver Nanoparticlesby using X ray diffractometer. X- ray diffraction shows the theta value peaks at 38,44 and 66 of silver obtained from *Penicillium species* (Fig 5). The XRD analysis shows that silver Nanoparticles from *Penicillium species* are face centered cubic structures.(Mohsen *et al* 2012, and also shows that silver Nanoparticlesare crystalline in nature.(Dubey et al., 2009). These Nanoparticles have been further characterized by Field emission Scanning electron (FESEM) which has been used to understand the surface topology and the size of silver nanoparticles and also shows silver Nanoparticles are spherical in shape with the diameter ranges between 35 nm and 67nm from (Fig 6).

Invitro antibacterial activity of Silver nanoparticles synthesized from *Penicillium species* were carried out and also in combination with the Sparfloxacin and Ofloxacin antibiotics available in the market against clinically isolated pathogens viz., *Staphylococcus aureus, Bacillus cereus, E. coli,* and *Proteus vulgaris* were satisfactory in the present study (Fig 7). The combined formulation of silver nanoparticles with different available antibiotics in the market like Sparfloxacin (5mcg disc) and Ofloxacin (5mcg disc) showed remarkable results against all the pathogens studied (Table 1) when subjected to antibacterial analysis. Each disc is impregnated with 30µg silver nanoparticle solution .The antibacterial activity of Ofloxacin and Sparfloxacin were amplified in the presence of Ag-NPs against the bacterial strains. The highest increase in fold area was observed for Ofloxacin and Sparfloxacin were against *E. coli* also at (1.4%) & (0.73%) respectively (Table 1). Silver Nanoparticles showed good antimicrobial activity alone. It was found that the silver nanoparticles produced from *Penicillium species* enhanced the reaction rates of the antibiotics in a synergistic mode as well as in its own way on these clinically isolated pathogens. Fig (8) and Fig (9) shows the graphical representation of the combination effect of Ofloxacin and Sparfloxacin with Ag-NPs against these clinically isolated pathogens.

Table 1: Zone of inhibition (mm) of Ofloxacin and Sparfloxacin against test pathogens in the presence and absence of silver nanoparticle

S.No.	Pathogenic Bacteria	Fungal fitrate 25µl/disc	Ag NPs 25µg/disc	Ofloxacin (A)	Ofloxacin + AgNPs (B)	Increase in fold area (%)	Sparfloxacin (A)	Sparfloxacin + AgNPs (B)	Increase in fold area (%)
1	*Staphylococcus aureus*	11	24	24	28	0.29	24	29	0.46
2	*Bacillus cereus*	13	18	21	25	0.41	21	25	0.41
3	*Eshcheria. coli*	12	20	19	30	1.49	22	29	0.73
4	*Proteus vulgaris*	10	20	23	29	0.58	24	31	0.66

Increase in fold area was calculated by using the equation $(B^2-A^2)/A^2$ where *A* is the zone of inhibition of Antibiotic and *B* is the zone of inhibition of antibiotic +AgNPs respectively

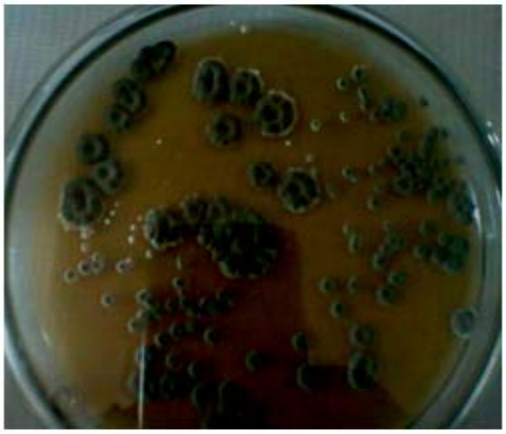

Fig 1.*Penicillium species* on SDA plate

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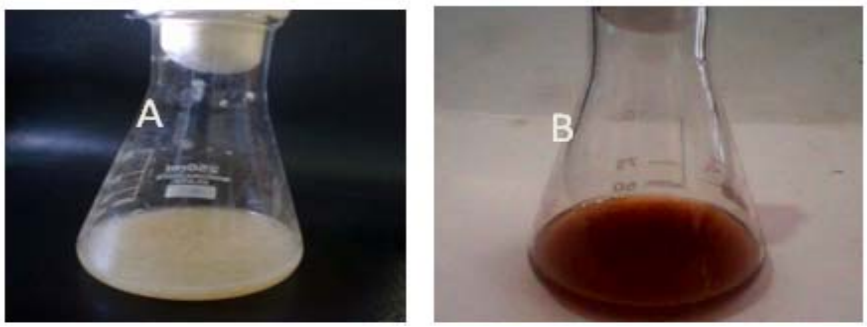

Fig 2:BioSynthesis of silver Nanoparticles from *Penicillium species*before treatment (B) after treatment of silver nitrate. Color change

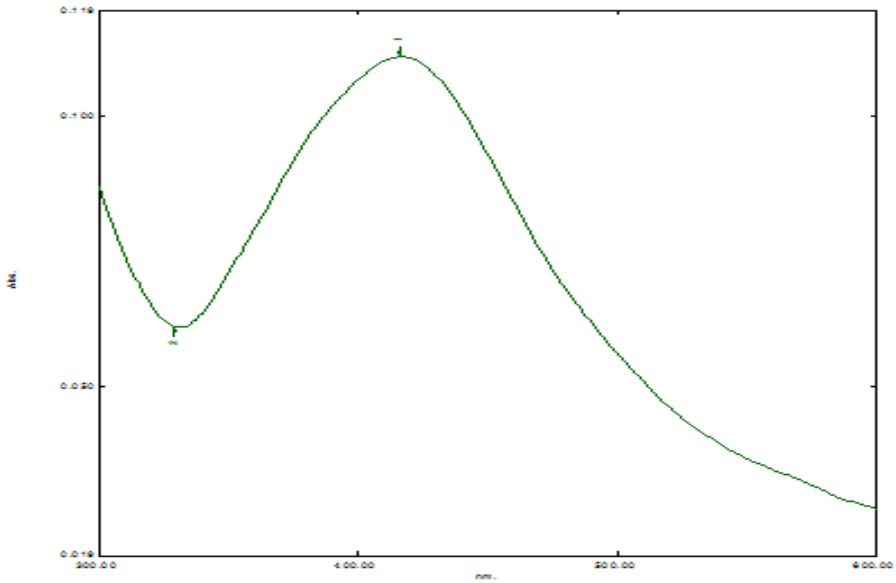

Fig 3; UV-visible spectrum of Silver nanoparticles synthesized from *Penicillium Species.*

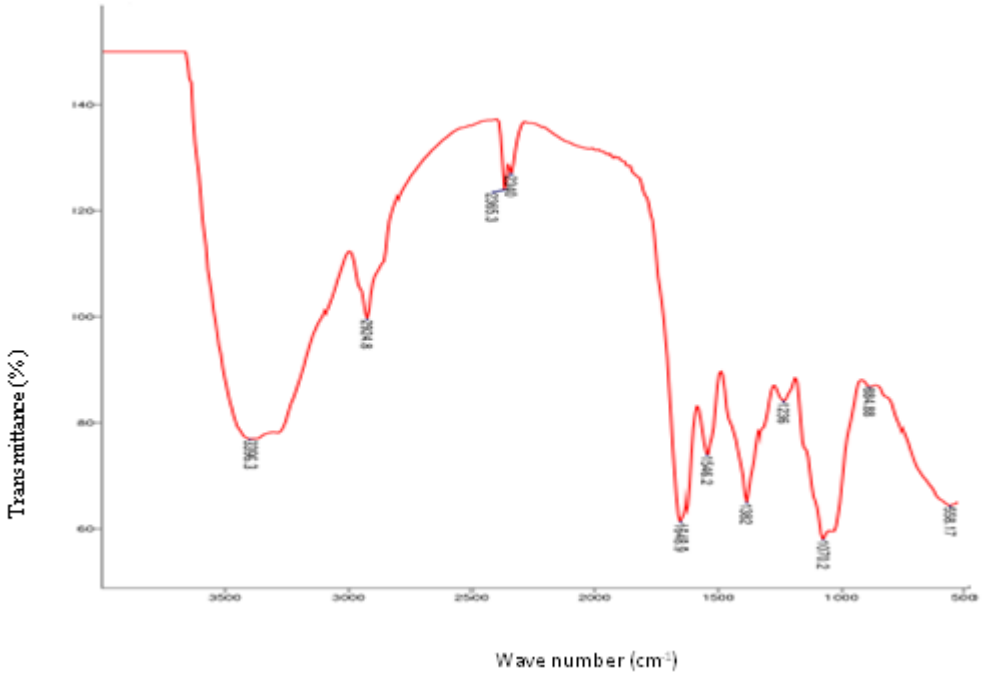

Fig 4: FTIR analysis recorded of Silver Nanoparticles synthesized from *Penicillium species*

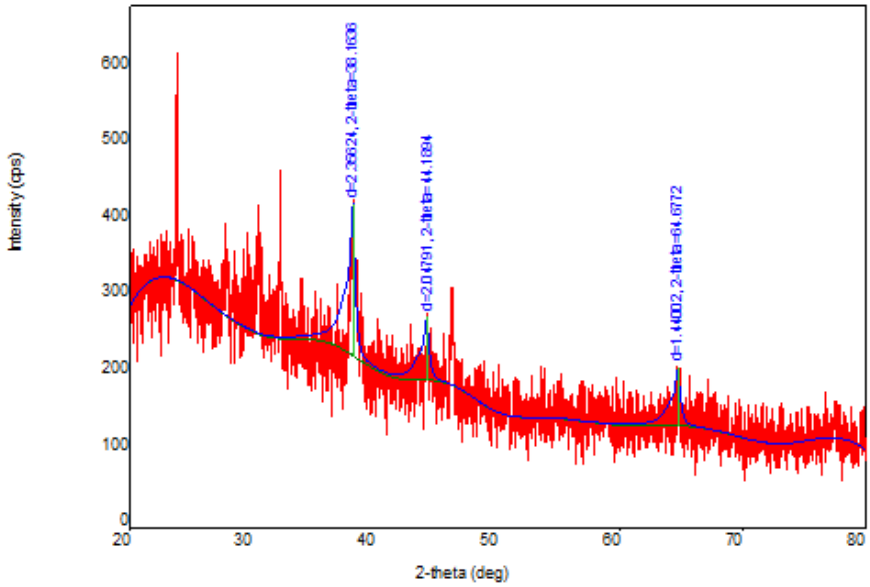

Fig 5: XRD pattern of silver Nanoparticles formed from *Penicillium species*

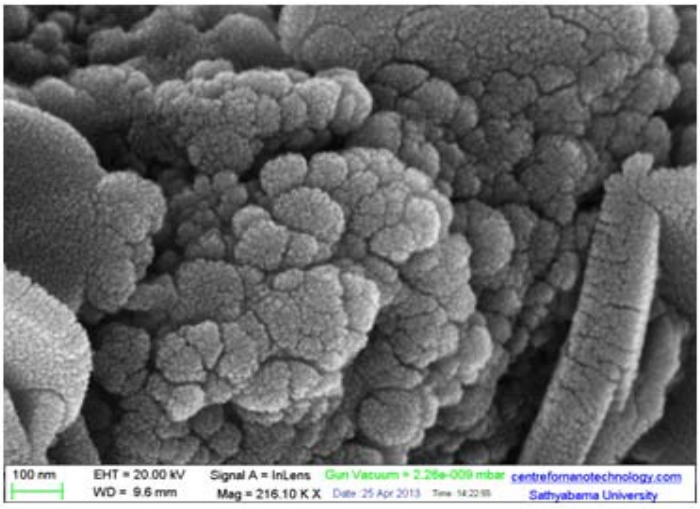

Fig 6 : Field emission Scanning electron microscope shows the particle size of silver Nanoparticles synthesized from Penicillium species (55to 65nm). Scale bar= 100nm

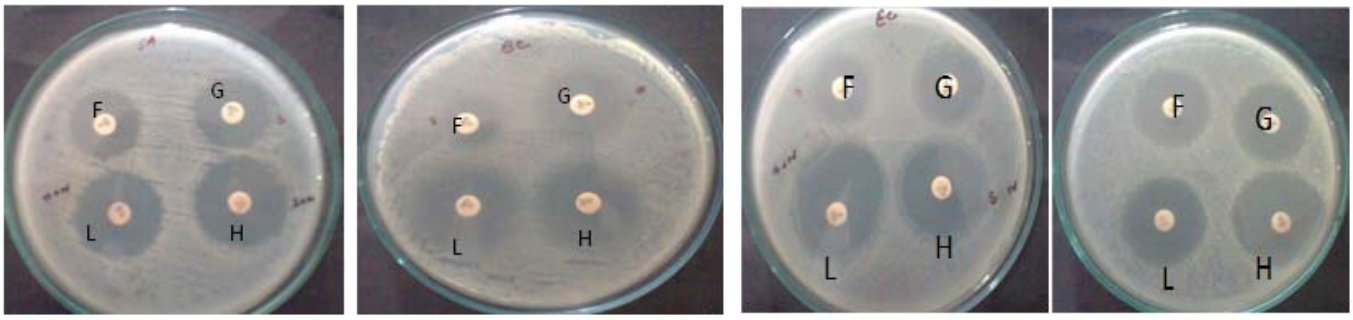

Fig 7: Synergistic antimicrobial activity of silver Nanoparticles and antibiotics on agar plates. F-Ofloxacin,G-Sparfloxacin, H-Sparfloxacin+AgNPs, L-Ofloxacin+AgNPs

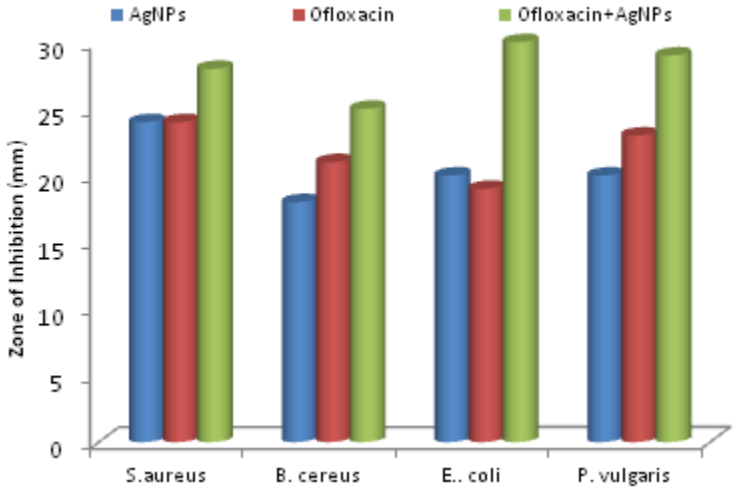

Fig 8: Graphical representation of the combination Ofloxacin with Ag-NPs against the selected pathogens

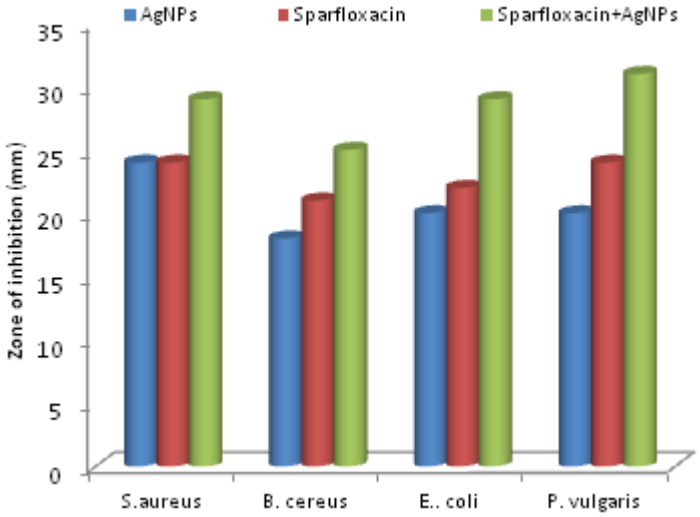

Fig 9: Graphical representation of the combination Sparfloxacin with Ag-NPs against the selected pathogens.

From the above results it suggest that the invitro biologically synthesized silver Nanoparticles from *Penicillium species* posses good antibacterial activity and also enhances the antibacterial activity of Ofloxacin and Sparfloxacin in combined form and these results were collaborated with the (Mudasir *et al*, 2013 and Monali *et al*, 2000) which demonstrates that the antibacterial activity of antibiotics enhances in presence of Silver Nan particles

CONCLUSION

This *penicillium species* is capable of producing the Silver nanoparticles extracellularly and these Nanoparticles are stable inside the solution due to the presence of proteins, molecules which stabilize the Nanoparticles and also as a capping agent, which are simple, efficient, ecofriendly, less toxic and easily amenable at large scale for the mass scale production. These biologically synthesized silver nanoparticles shows good antibacterial activity and also these Nanoparticles enhances the antibacterial activity of the Ofloxacin and Sparfloxacin were studied. Thus these Nanoparticles can be used as antibacterial agents alone or with the antibiotics and decrease the antibiotic dosage after animal trial experiment for its cytotoxicity.

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