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Synergistic Action of Silver Nanoparticles Synthesized from Silver Resistant Estuarine *Pseudomonas aeruginosa* Strain SN5 with Antibiotics against Antibiotic Resistant Bacterial Human Pathogens

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Abstract This study focuses on the extracellular synthesis of silver nanoparticles (AgNPs), carried out using the culture supernatant of silver resistant *Pseudomonas aeruginosa* strain SN5 isolated from Mandovi estuarine mangrove water sample. AgNPs were characterized using X-Ray diffraction (XRD) analysis which showed high intensity peaks at 28° and 32.5°, characteristic of silver oxide (Ag_2O) and confirmed its crystalline nature by referring Joint Committee on Powder Diffraction Standards (JCPDS), File No. 00–076-1393. Transmission electron microscopy (TEM) analysis revealed the nano sized AgNPS particles in the range of 35 nm – 60 nm. AgNPs showed antibacterial activity against both standard cultures of Gram positive and Gram negative bacterial human pathogens. Moreover, the AgNPs also showed antibacterial activity against ampicillin resistant *Staphylococccus aureus* strain VN3 and ciprofloxacin resistant *Vibrio cholera* strain VN1 isolated from Mandovi estuary, Goa India, polluted with human feces, domestic and hotel waste. These AgNPs exhibited better antibacterial activity as compared to AgNPs synthesized from plant extract of Honey suckle mistletoe and star anise. Interestringly, synergistic activity was observed when synthesized AgNPs were used in combination with antibiotics ampicillin and ciprofloxacin against ampicillin resistant *Staphylococccus aureus* strain VN3 and ciprofloxacin resistant *Vibrio cholera* strain VN1. Thus these AgNPs can be employed in cosmetics and wound dressings as a nanoweapon to control human bacterial pathogens.

Keywords Estuarine · *Pseudomonas aeruginosa* · Silver nanoparticles · Pathogens · Nanoweapon · Synergistic activity

Introduction

An increased and uncontrolled use of antibiotics to treat various bacterial diseases as well as due to long term exposure of microbes to antibiotics has led to the development of bacterial resistance to traditional antibiotics which over the years have evolved to become multi drug resistant bacterial pathogens due to the transfer of genes encoding resistant factors through horizontal gene transfer (Odonkor and Addo 2011; Kunkalekar et al. 2014). Diseases caused due to multidrug resistant strains of bacterial pathogens are very difficult to treat since the antibiotics used to treat are no longer effective on them (Nikaido 2009; Hwang et al. 2012). Hence there is pressing need to find an alternative and cost effective antimicrobial agent that will be effective against multidrug resistant bacterial pathogens.

Silver for years has been known for its natural antimicrobial properties (Kim et al. 2009). Moreover, silver nanoparticles (AgNPs) due to their small size and high surface-to-volume ratio, which are responsible for their high biological and chemical activity, enable them to act at various cellular levels (Ahmed et al. 2016). As a result AgNPs are gaining increased attention as an alternative to antibiotics. Green

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synthesis of AgNPs using bacteria, fungi, actinomycetes, algae and plant are gaining more attention due to cost effective and ecofriendly methods (Keat et al. 2015). Extracts from microorganisms as well as plants act as reducing as well as capping agents (Sintubin et al. 2009; Sukumaran and Eldho 2012). Biological synthesis of nanoparticles using bacteria is a more compatible method because over the years, bacteria have been exposed to different metals and hence have adapted to synthesize different metal nanoparticles (Kumar et al. 2010; Nikolaos and Louise 2014). Moreover, bacterial generation time is very less so they are economical to use (Li et al. 2011a; b).

Studies have shown that AgNPs influence many bacterial structures and metabolic processes simultaneously. AgNPs can inactivate bacterial enzymes affecting their metabolic processes, disrupt bacterial cell wall, accumulate in cell membrane and also increase its permeability, collapse bacterial plasma membrane, interact with DNA and generate reactive oxygen species which are known to damage biomolecules (Xu et al. 2011; Kunkalekar et al. 2014). Many studies have also revealed that use of antibiotic drugs in combination with metallic nanoparticles not only increase antimicrobial activity of both the components due to synergistic activity but also reduces their toxic effects. It can also restore the activity of some previously effective antibiotics (Panacek et al. 2006). Due to their multi-level mode of action on both, susceptible and antibiotic resistant bacteria, AgNPs are considered as a suitable candidate for its use in combination with antibiotics. Bacterial resistance to AgNPs or inactivation of its antibacterial action has not been proven yet (Franci et al. 2015).

In the present research, we have aimed to isolate Ag resistant estuarine bacteria and evaluate its potential to synthesize AgNPs that could be applied as nanoweapon against Gram positive and Gram negative bacterial pathogens and also explore prospect of these AgNPs in combination with antibiotics as synergistic effect on antibiotic resistant bacterial pathogens isolated from Mandovi estuary, Goa contaminated with human fecal, domestic and hotel waste.

Materials and Methods

Materials

Chemicals and microbiological media used in this study were purchased form Himedia Laboratories. All the chemicals used were of analytical grade. Glasswares were purchased form Merck Millipore.

Isolation of Silver Resistant Bacteria

The water sample was collected from the mangroves at the Mandovi estuary in a sterile glass bottle. In order to isolate silver resistant bacteria, the water sample was enriched in Minimal Salt Media (MSM) broth containing 0.2% glucose amended with 50 μM silver nitrate ($AgNO_3$). The flask was incubated at room temperature (28 ± 2 °C) for 24 h. The enriched broth was then serially diluted and spread plated (0.1 ml) on MSM agar plates containing 0.2% glucose amended with 50 μM $AgNO_3$. The plates were then incubated at room temperature for 24 h. Morphologically different bacterial colonies were selected and purified. Maximum tolerance concentration (MTC) of these isolates was checked by inoculating them in MSM media containing different concentrations of $AgNO_3$ i.e. 0.05 mM, 0.1 mM and 0.15 mM supplemented with 0.2% glucose as sole source of carbon. The bacterial isolate showing maximum tolerance to $AgNO_3$ was selected, identified biochemically and designated as strain SN5. The selected silver resistant estuarine bacterial isolate SN5 was used to carry out the synthesis of AgNPs.

Identification of Bacterial Strain SN5

Silver resistant bacterial strain SN5 was identified using biochemical tests with reference to Bergey's Manual of Systematic Bacteriology (Sneath et al. 1986). Pigment produced by strain SN5 in nutrient broth at 30 °C was analysed using UV-Vis spectrophotometry (Shimadzu, UV-2450, Japan) and spectrofluorometry (Shimadzu, RF-530 IPC) to further confirm the culture identity.

Synthesis of AgNPs

AgNPs were synthesized using $AgNO_3$. $AgNO_3$ (1 M) stock solution was prepared by dissolving 16.98 g in 100 ml distilled water, sterilized and stored in amber colored bottle.

Selected Silver (Ag) resistant bacterial strain SN5 was inoculated in 100 ml nutrient broth in an 250 ml Erlenmeyer flask and incubated for 24 h at 28 ± 2 °C. The overnight grown culture broth was centrifuged at 8000 rpm for 5 min and the culture supernatant was used for the synthesis of AgNPs. The resulting culture supernatant (100 ml) was taken in a 150 ml beaker and 1 ml of 1 M $AgNO_3$ stock solution was added slowly to make the final concentration 10 mM aqueous solution. The culture supernatant within a minute changed colour to greish brown from greenish yellow. The solution was centrifuged at 13000 rpm for 15 min. The supernatant was discarded and the pellet was washed with distilled water and then kept in the oven at 50 °C 12 h. The dried pellet obtained was then ground to fine powder using a mortar and pestle. Further, the powder was used for characterization.

Characterization of Nanoparticles

UV-vis Spectrophotometer

UV-visible spectrum of the culture supernatant with silver nitrate during the formation of AgNPs was monitored using

UV-Vis spectrophotometer-UV-2450, having operational range of wavelength between 190 nm and 800 nm.

X-ray Diffraction (XRD) Analysis

The crystalline nature of synthesized AgNPs was carried out by X-ray diffraction technology using a Rigaku Miniflex Diffractometer with CuK α radiation of wavelength 1.5418 Å and filtered through Ni absorber in the 2θ range of 5–80 (Prabhu et al. 2014).

Transmission Electron Microscopy (TEM)

A high-resolution TEM analysis of AgNPs was carried out to determine the particle size of the sample using a PHILIP CM200 transmission electron microscope at SAIF operating at IIT povoi Mumbai with a accelerating voltage of 200 kV and providing a resolution of 2.4 Å (Prabhu et al. 2014).

Antibacterial Activity of Synthesized AgNPs

Antibacterial activities of synthesized AgNPs was determined against standard cultures: *Staphylococcus aureus* ATCC 6538, *Klebsiella pneumoniae* ATCC1003, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 8739, *Salmonella abony* NCTC 6017 *and Staphylococcus epidermis* ATCC 12228 on Muller- Hinton Agar using well diffusion method (Prabhu et al. 2014). Experiment was done in triplicates.

a) *Determination of Minimum Inhibitory Concentration (MIC) of synthesized AgNPs*

The MIC is the lowest concentration of an antimicrobial agent that inhibits the visible growth of an organism. Different concentrations of synthesized AgNPs (0.1 mg/ml, 0.5 mg/ml, 1 mg/ml and 2 mg/ml) were prepared in sterile distilled water. MIC of AgNPs was determined by standard well diffusion method. Overnight grown pathogenic cultures (100 μl) were spread plated on different Muller- Hinton agar plates. Wells (6 mm diameter) were made using sterile cork borer under aseptic conditions. Different concentrations of AgNPs (0.1 mg/ml, 0.5 mg/ml, 1 mg/ml and 2 mg/ml) were loaded in the wells (40 μl) using a sterile micropipette. After incubation at 37 °C for 24 h, zones of inhibition in millimeter were measured using Hi-media Antibiotic Zonescale and tabulated. Sterile distilled water loaded in a well served as control. Experiment was done in triplicates and standard deviation was calculated.

b) *MIC of AgNPs in Muller Hinton broth*

MIC of AgNPs was tested on standard bacterial pathogens. Overnight grown bacterial cultures was inoculated in Muller Hinton broth containing different concentrations of AgNPs (0.1 mg/ml, 0.5 mg/ml, 1 mg/ml, 2 mg/ml, 2.5 mg/ml). Control tubes contained no AgNPs. After incubating the test tubes for 24 h at 30 °C, they were examined for possible growth and minimum inhibitory concentrations using spectrophotometer. MICs of AgNPs were determined as the lowest concentration of AgNPs that showed no growth of the bacteria in the test tube. Experiment was done in triplicate.

c) *Comparing the antibacterial activity of AgNPs with nanoparticles synthesized using plant materials*

Antibacterial activities of synthesized nanoparticles was compared with nanoparticles synthesized using plant extract of Honey suckle mistletoe leaves (*Loranthus falcutus*) (Enchimani and Naik 2015) and spice star anise (*Illicium vercum*) (Chari and Naik 2015). Antibacterial activity was determined using the well diffusion method (Prabhu et al. 2014). A stock of 2 mg/ml of nanoparticles (plant derived nanoparticles and synthesized AgNPs) was prepared in distilled water and 40 μl of each nanoparticles sample was tested against the standard pathogenic cultures namely *Staphylococcus aureus* ATCC 6538, *Klebsiella pneumoniae* ATCC 1003, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 8739, *Salmonella abony* NCTC 6017 and *Staphylococcus epidermis* ATCC12228 and zone of inhibition in millimeter was compared. Experiment was done in triplicates and standard deviation was calculated.

Synergistic Effect of Synthesized AgNPs with Antibiotics

A standard well diffusion method was used to assay the synergistic effect of synthesized AgNPs with antibiotics (ampicillin and ciprofloxacin) for antibacterial activity against the antibiotic resistant estuarine bacterial isolates from Mandovi estuary contaminated with human fecal, domestic and hotel waste i.e. ampicillin resistant (50 μg/ml) *S. aureus* strain VN3 and ciprofloxacin (50 μg/ml) resistant *V. cholera* strain VN1 (Vast and Naik 2016). Antibiotics (ampicillin and ciprofloxacin) were purchased from Himedia. Stock of each antibiotic (2 mg/ml) was prepared and filter sterilized and stored at 4 °C until use. Synergistic effect of antibiotics (ampicillin and ciprofloxacin – 50 μg/ml) with AgNPs (2 mg/ml) synthesized from silver resistant estuarine bacterial isolate SN5 was evaluated. Nanoparticle samples were mixed with individual antibiotic and 40 μl of this combined solution (final concentration of nanoparticles 2 mg/ml and antibiotics 50 μg/ml) was loaded in the wells and plates were then incubated at 37 °C for 24 h. The zone of inhibition was measured in millimeter to determine their antimicrobial potential.

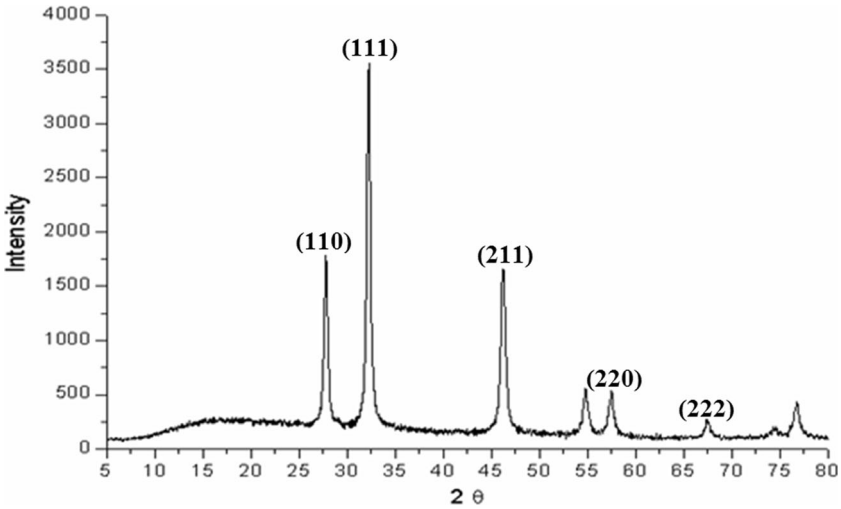

Fig. 1 XRD patterns of AgNPs synthesized from estuarine *Pseudomonas aeruginosa* strain SN5

Results and Discussion

Isolation and Identification of Silver Resistant Bacteria from the Mangrove Water Sample

Twenty silver resistant bacterial colonies were obtained after spread plating suitably diluted water sample of mangroves on MSM agar plates containing 0.2% glucose amended with 50 μM $AgNO_3$. Five bacterial colonies with different morphological characteristics were selected. The greenish bacterial colony found to grow on the plate having 0.1 mM $AgNO_3$ concentration was selected for further studies and designated as strain SN5. This bacterial isolate SN5 was found to be Gram negative short rod and motile. By comparing biochemical characteristics to Bergey's Manual of Bacteriology it was tentatively identified as *Pseudomonas aeruginosa*. UV–Vis spectrophotometric analysis of the greenish yellow pigment in nutrient broth clearly revealed presence of two types of siderophores such as pyochelin and pyoverdine (Fig. 1 in Online Resource 1). Pyochelin showed absorbance maxima at 247 (Peak 3) and 310 nm (peak 2) in UV range whereas pyoverdine showed strong absorbance at 370 nm (peak 1) (Naik and Dubey 2011). Spectrofluorimetric analysis of this yellow-green pigment produced by strain SN5 in nutrient broth clearly revealed presence of of siderophore pyochelin. Pyochelin emitted at 448 nm when excited at 350 nm (Fig. 2 in Online Resource 1), these results go hand in hand with *Pseudomonas aeruginosa* 4EA isolated by Naik and Dubey 2011, therefore we confirmed that strain SN4 is *Pseudomonas aeruginosa*.

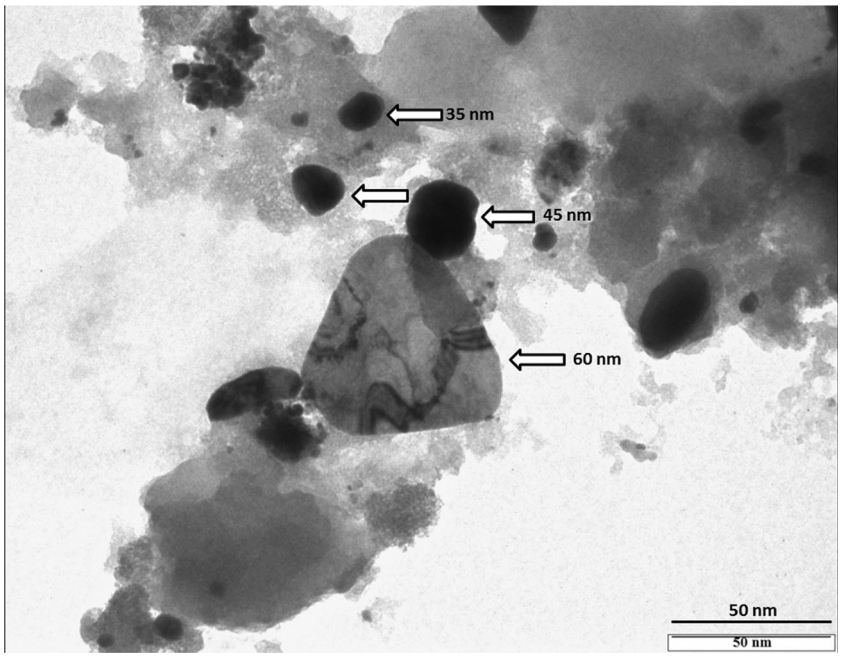

Fig. 2 TEM image of AgNPs synthesized from estuarine *Pseudomonas aeruginosa* strain SN5

Table 1 MIC results of AgNPs on standard cultures

Concentration of AgNPs	Zone of inhibition (mm)					
	S. aureus	*E. coli*	*B. subtilis*	*K. pneumoniae*	*S. abony*	*S. epidermis*
0.1 mg/ml	-	-	-	-	-	-
0.5 mg/ml	-	-	-	-	-	-
1 mg/ml	10 ± 0.5	9 ± 1	-	-	-	8 ± 0.5
2 mg/ml	16 ± 1	12 ± 1	9 ± 1.5	-	-	12 ± 0.5

Synthesis of AgNPs

The culture supernatant incubated with $AgNO_3$ showed a colour change in a minute to greish brown from greenish-yellow (Fig. 3 in Online Resource 1). The colour change was observed subsequently due to the reduction of silver ions and formation of AgNPs. The synthesized nanoparticles were designated as AgNPs-SN5.

Characterization of Nanoparticles

UV-vis Spectrophotometer

Surface plasmon resonance was recorded at 365 nm using UV-Vis spectrophotometer which is typical of AgNPs (Fig. 4 in Online Resource 1).

X-ray Diffraction (XRD)

XRD analysis was carried out to study the crystalline nature of the synthesized AgNPs. The XRD pattern (Fig. 1) showed peaks at 28°, 32.5°, 46.35°, 55.38°, 57.55°, 67.4°, and 77.5° in the spectrum of 2θ values ranging from 5 to 80. The two strong Bragg reflections were seen at 28° and 32.5° which corresponds to (110) and (111) of silver oxide (Ag_2O). Besides this, diffraction peaks at 46.35°, 55.38° and 67.4° can be indexed to (211), (220) and (222) planes of face centered cubic crystal structure of silver, respectively. These peaks corroborate with the standard Ag_2O pattern obtained from the Joint Committee on Powder Diffraction Standards (JCPDS), File No. 00–076-1393 (Janardhanan et al. 2009; Dhoondia and Chakraborty 2012). The appearance of two unknown peaks at 57.55° and 77.5° may correspond to the traces of bacterial pellet (Dhoondia and Chakraborty 2012). The XRD diffraction pattern obtained also matches with AgNPs synthesized using psychrotolerant *Pseudomonas mandelii* (Mageswari et al. 2015) indicating that nanoparticles synthesized by us were oxides of AgNPs and of crystalline nature.

Transmission Electron Microscopy (TEM)

The size of the AgNPs was measured by TEM analysis. It was observed that the particle sizes ranges from 35 to 60 nm (Fig. 2).

Antibacterial Activity of Synthesized Nanoparticles

a) *Determination of Minimum Inhibitory Concentration (MIC) of synthesized AgNPs*

The antibacterial activity of AgNPs was observed as a zone of inhibition around the wells (Table 1). The MIC observed for *S. aureus, E. coli* and *S. epidermis* was 1 mg/ml whereas MIC for *B. subtilis* was found to be 2 mg/ml. *S. abony* and *K. pneumoniae* did not show susceptibility to the synthesized AgNPs (showed resistance till 2 mg/ml) (Table 1).

Several studies have already revealed the mechanism of action of AgNPs on bacteria (Sukumaran and Eldho 2012; Prabhu et al. 2014). In case of *E. coli,* and *K. pneumoniae,* AgNPs are known to alter the membrane permeability and affect their respiration (Franci et al. 2015). AgNPs can cause irreversible damage to the bacterial cells and inhibit DNA replication in *S. aureus* (Kim et al. 2007; Li et al. 2011a; b; Franci et al. 2015). In addition to these two mechanisms,

Table 2 Comparison of antibacterial activity of synthesized AgNPs with AgNPs synthesized from plants

Test samples	Zone of inhibition (mm)					
	S. aureus	*E. coli*	*B. subtilis*	*K. pneumoniae*	*S. abony*	*S. epidermis*
Star anise	9 ± 0.5	9 ± 0.5	12 ± 1	-	8 ± 0.5	10 ± 0.5
Zanthophyllum	11 ± 0.5	10 ± 0.5	15 ± 1	12 ± 1	-	11 ± 1
Pseudomonas aeruginosa strain SN5	16 ± 1	12 ± 1	9 ± 1.5	-	-	12 ± 0.5

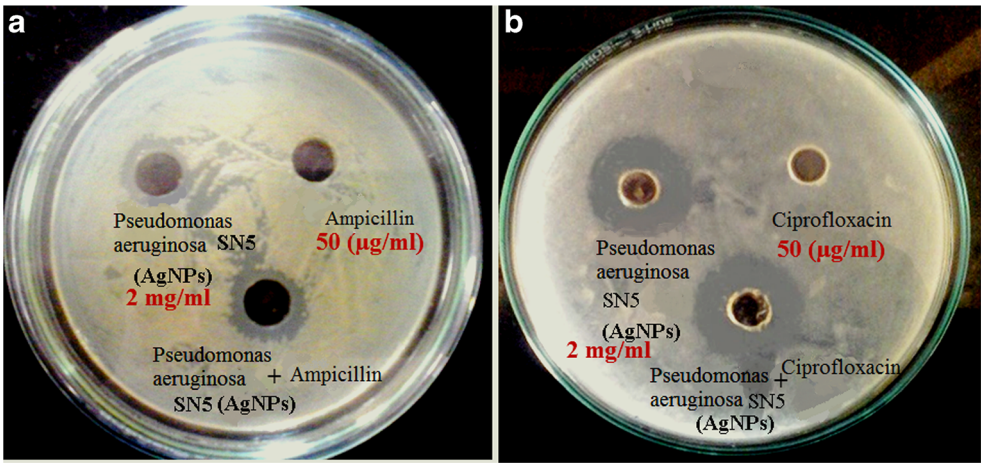

Fig. 3 Synergistic effect of antibiotic (ampicillin and ciprofloxacin) and AgNPs synthesised from *P. aeruginosa* strain SN5 on **a** ampicillin resistant *S. aureus* strain VN3 and **b** Ciprofloxacin resistant *V. cholera* strain VN1

AgNPs can also damage cytoplasmic membrane and modify intracellular ATP levels in *S. epidermis* (Franci et al. 2015).

b) *MIC of Silver nanoparticles in Muller Hinton broth*

The MIC observed for *S. aureus, E. coli* and *S. epidermis* was 1 mg/ml whereas MIC for *B. subtilis* was found to be 2 mg/ml. *S. abony* and *K. pneumoniae* showed resistance till 2 mg/ml and MIC is 2.5 mg/ml.

c) *Comparing the antibacterial activity of AgNPs with nanoparticles synthesized using plant extracts*

Antibacterial activity has been revealed as zone of inhibition around the wells as shown in Online Resource 1, Fig. 5. It was observed that AgNPs synthesized from *Pseudomonas aeruginosa* strain SN5 showed maximum antibacterial activity against *S. aureus* (16 ± 1 mm), *S. epidermis* (12 ± 0.5 mm) and *E. coli* (12 ± 1 mm) as compared to AgNPs synthesized from plant extract of Honey suckle mistletoe and a spice, star anise. AgNPs synthesized from plant extract of Honey suckle mistletoe (zanthophyllum) showed better antibacterial activity (in terms of zone of inhibition in millimeter) against *B. subtilis* (15 ± 1 mm) and *K. pneumoniae* (12 ± 1 mm) when compared to AgNPs synthesized from *Pseudomonas aeruginosa* strain SN5 and star anise. AgNPs synthesized from the extract of Honey suckle mistletoe and *Pseudomonas aeruginosa* strain SN5 did not show activity against *S. abony. S. abony* (8 mm) was found to be susceptible only to AgNPs synthesized from star anise whereas AgNPs synthesized from Honey suckle mistletoe and *Pseudomonas aeruginosa* strain SN5 could not inhibit the growth of *S. abony.* AgNPs synthesized from star anise did not show activity against *K. pneumoniae.* These results substantiate the fact that AgNPs synthesized from bacteria have better antibacterial activity than AgNPs syntesized from plant materials (Table 2).

Silver is considered as a safe and effective antibactericidal metal because it is non-toxic to animal cells. By varying some experimental conditions AgNPs of different size can be synthesized. The possible antibacterial activity of AgNPs may be due to the electrostatic attraction between negatively charged bacterial cell membrane and positively charged nanoparticles in the cell wall (Dibrov et al. 2002; Hamouda et al. 2000). These AgNPs react with bacterial cell wall proteins and destroy its structural rigidity and increasing its permeability. This allows AgNPs to penetrate inside the bacterial cell and affect its DNA and generate reactive oxygen species (Woo et al. 2008). It can also interrupt the proton motive force across the cytoplasmic membrane (Martınez-Castanon et al. 2009; Pal et al. 2007; Panacek et al. 2006). Recently is has been shown that AgNPs can simultaneously induce apoptosis and inhibit new DNA synthesis in the cells in a positive concentration dependent manner (Huijing et al. 2015).

Table 3 Zone of inhibition (mm) of antibiotic (ampicillin and ciprofloxacin) with and without AgNPs against ampicillin resistant *S. aureus* strain VN3 and Ciprofloxacin resistant *V. cholera* strain VN1

Test culture	Zone of inhibition (mm)				
	Ampicillin (50 µg/ml)	Ciprofloxacin (50 µg/ml)	AgNPs (2 mg/ml)	Ampicillin + AgNPs	Ciprofloxacin + AgNPs
Ampicillin resistant *S. aureus* VN3	Resistant	Not done	14 ± 1	16 ± 1	Not done
Ciprofloxacin resistant *V. cholera* VN1	Resistant	Resistant	17 ± 0.5	Not done	20 ± 0.5

Synergistic Effect of Synthesized AgNPs with Antibiotics on Antibiotic Resistant Cultures

The antibiotic resistant cultures *S. aureus* strain VN3 and *V. cholera* strain VN1 used were found to be resistant to 50 μg/ml of the antibiotic ampicillin and ciprofloxacin respectively. The synthesized AgNPs (2 mg/ml) were tested against these antibiotic resistant cultures and it was observed that both the cultures were susceptible to the synthesized AgNPs as shown in Fig. 3. But when AgNPs were used in combination with the antibiotics (ampicillin and ciprofloxacin) an increase in the zone of inhibition was observed as compared to the zone of inhibition showed when AgNPs used individually (Fig. 3). These results support synergistic effect of AgNPs and antibiotic.

Results proved that efficacy of the tested antibiotics was increased when used in combination with AgNPs. The zone of inhibition increased from 14 ± 1 mm to 16 ± 1 mm in case of ampicillin (against *S. aureus* strain VN3) and from17 ± 0.5 mm to 20 ± 0.5 mm in case of ciprofloxacin (against *V. cholera* strain VN1) when combined with AgNPs (Table 3). This indicates that the combination of antibiotics and AgNPs could increase the antibiotic efficacy against antibiotic resistant pathogens. Ashley et al. 2012 reported that ampicillin interact with the AgNPs through Van der Waals forces and other weak bonds and surrounds AgNPs. Ampicillin is a class of antibiotic that inhibits the cell wall synthesis in bacteria. The AgNPs and anitibiotic complex acts on the cell wall of bacteria leading to cell lysis leading to easy penetration of AgNPs into the cell (Fayaz et al. 2010). A more serious damage to the bacterial cell is done due to the action of AgNPs-ampicillin complex on DNA, preventing DNA unwinding (Fayaz et al. 2010). In present study AgNPs and ampicillin complex acts on the cell wall of ampicillin resistant *S. aureus* strain VN3 leading to easy penetration of AgNPs into the cell and AgNPs can act on bacterial DNA. Simillarly ciprofloxacin-AgNPs complex may have better action on DNA gyrase as compared to ciprofloxacin or AgNPs alone.

Antibiotics contain some active functional groups on their surface such as hydroxyl groups which can easily react with AgNPs by chelation (Fayaz et al. 2010). Previous studies reported that nanoparticles due to their smaller size have large surface area which allows them to closely interact with the antibiotics and can thus either inhibit peptidoglycan synthesis or AgNPs-antibiotics complex can interact with DNA leading to the damage of the bacterial cells. AgNPs are reported to render bacteria susceptible to antibiotic treatment (Arunkumar et al. 2013). The antibacterial activity and the synergistic effect of antibiotics with the biosynthesized AgNPs is summarized in Table 3.

Conclusion

AgNPs were synthesized extracellularly using the culture supernatant of silver resistant *Pseudomonas aeruginosa* strain SN5 isolated from the mangrove ecosystem (Mandovi estuary). The synthesized AgNPs were characterized by XRD and TEM analysis. The AgNPs were of crystalline nature and 35–60 nm size. The AgNPs showed potent broad spectrum activity against standard cultures *Staphylococcus aureus* ATCC 6538, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 8739, and *Staphylococcus epidermis* ATCC 12228 and antibiotic resistant organisms such as ampicillin resistant *S. aureus* strain VN3 and ciprofloxacin resistant *Vibrio cholera* strain VN1. AgNPs synthesized using estuarine bacteria showed better antibacterial activity against the test cultures as compared to the AgNPs synthesized from extract of Honey suckle mistletoe leaves and star anise due to unknown reason and we are working on this aspect. The antibacterial activity of ampicillin and ciprofloxacin increased greatly when combined with AgNPs on ampicillin resistant *S. aureus* VN3 and ciprofloxacin resistant *Vibrio cholera* VN1. This enhanced effect could probably be due to the synergistic action of nanoparticles and antibiotics. This is a first report of AgNPs synthesized from silver resistant estuarine *P. aeruginosa* strain SN5 isolated from mangrove ecosystem of Goa, which show synergistic activity with antibiotics and they can be used as a nanoweapon to control infectious pathogens.

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Compliance with Ethical Standards

Conflict of Interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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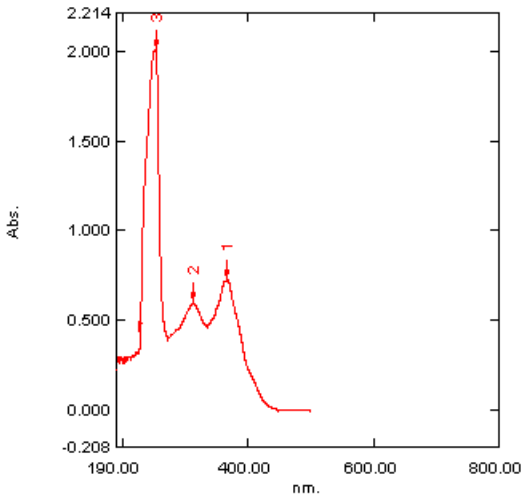

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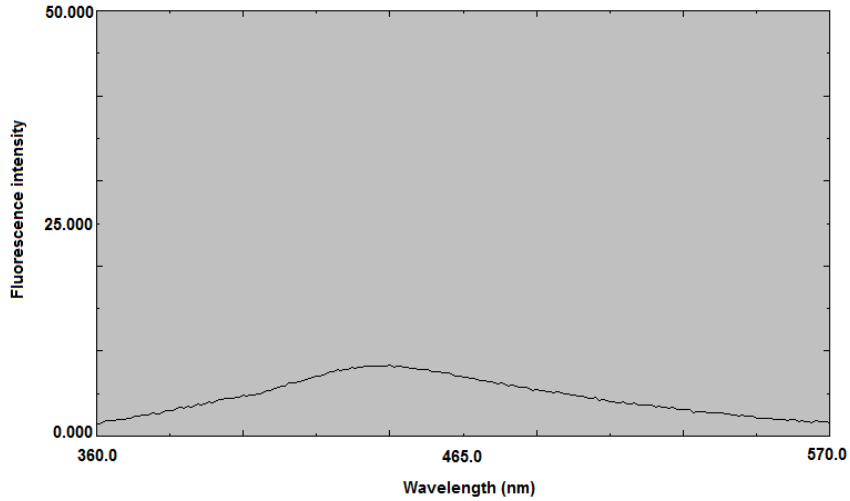

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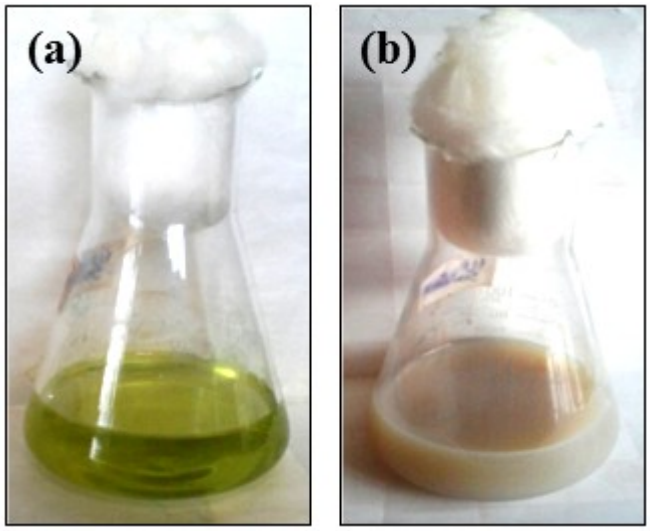

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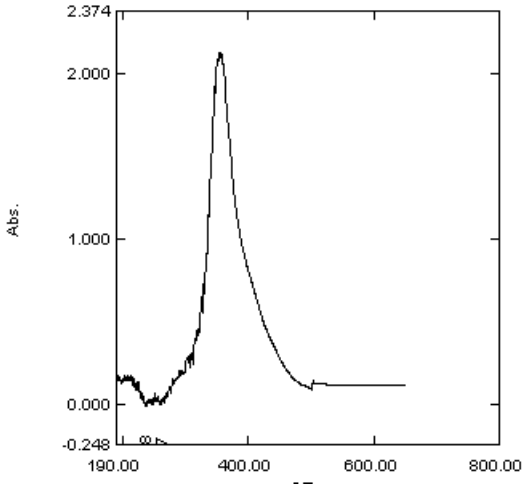

Fig. 4 AgNPs formation was monitored using UV-visible spectrophotometer by recording surface plasmon resonance at 365 nm.

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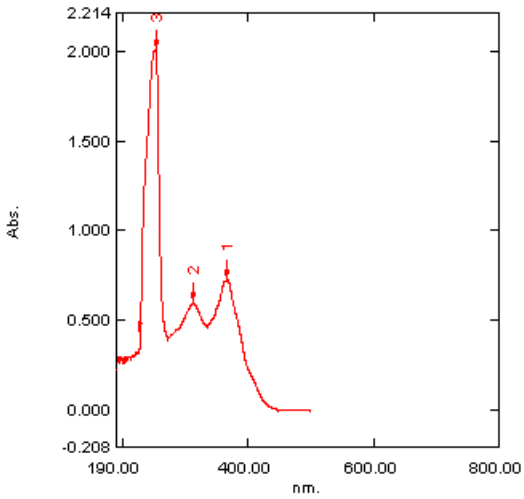

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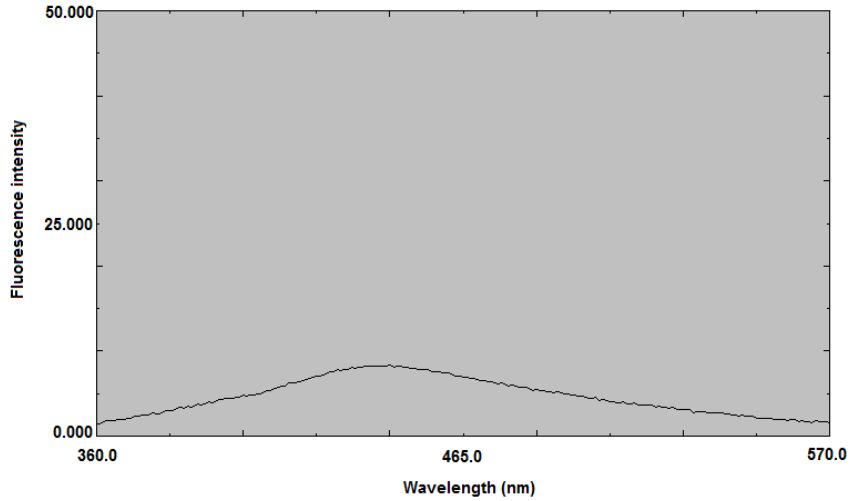

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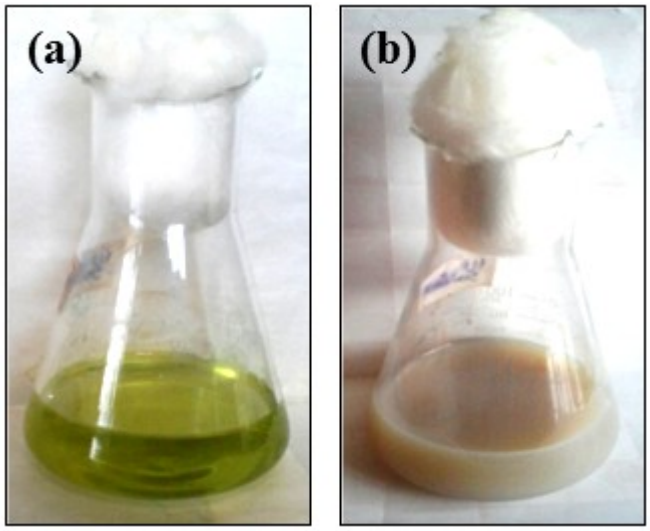

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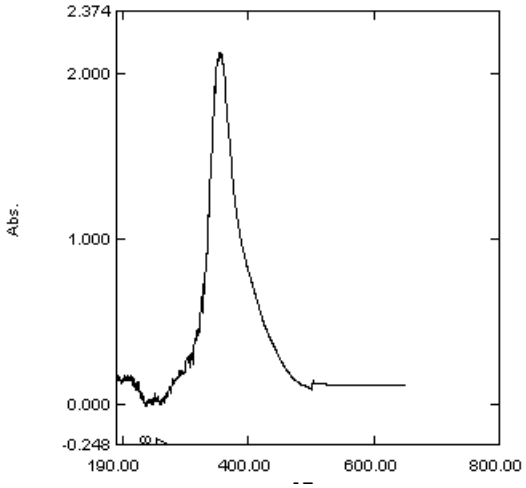

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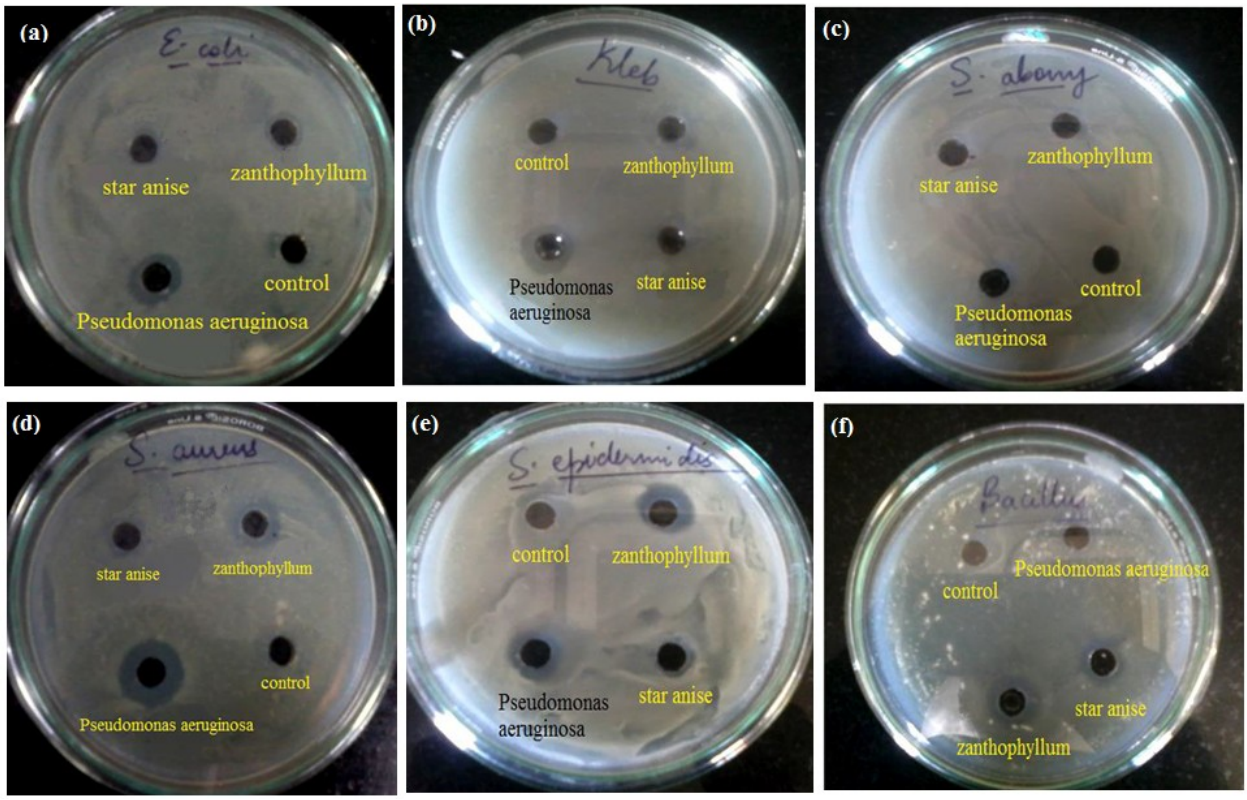

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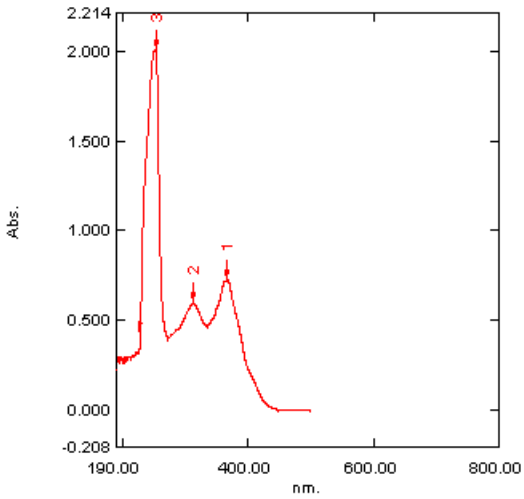

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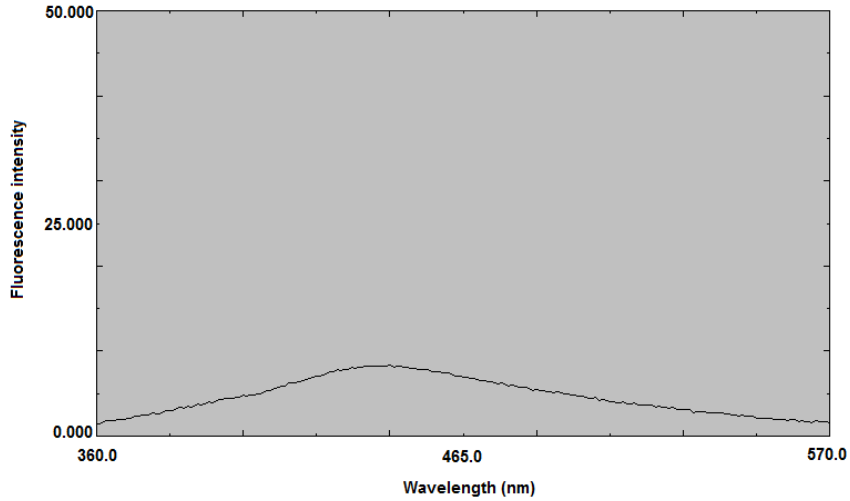

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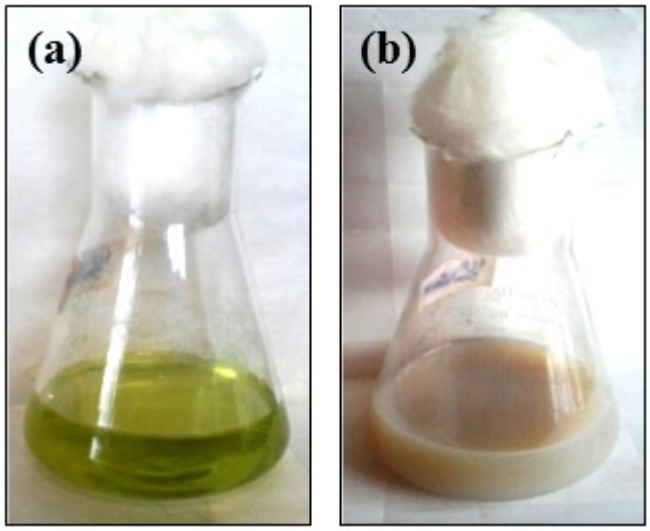

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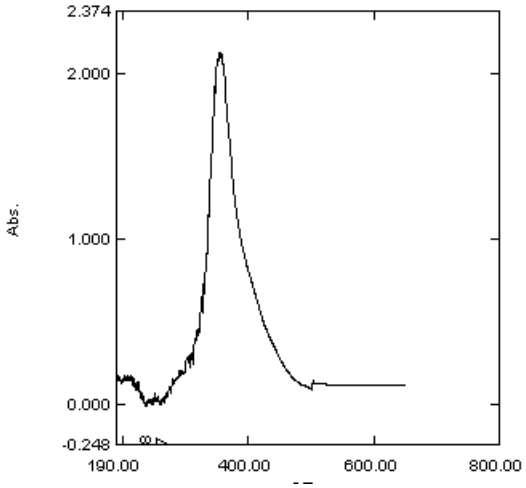

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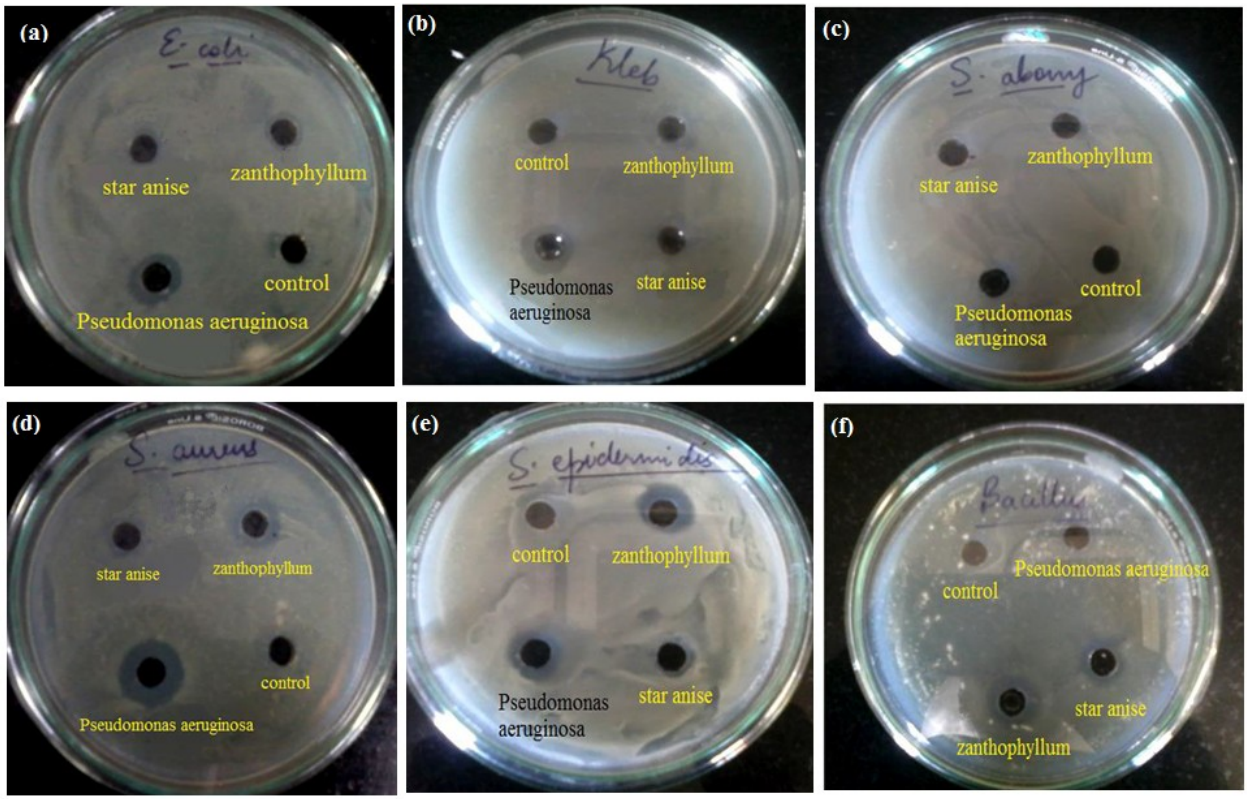

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