Mycobased biosynthesis of Silver nanoparticles and Studies of its Synergistic antibacterial activity combined with Cefazolin antibiotic against selected organisms

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Abstract: Silver nanoparticles were synthesized biologically from non pathogenic fungus and its synergistic antibacterial activity with the antibiotic cefazolin was studied in detail. The nanoparticles were characterized using UV–Vis spectroscopy and Transmission Electron Microscopy (TEM). In the UV – Vis spectrum the peak at 420nm confirms the synthesis of silver nanoparticles and from TEM studies silver nanoparticles with different shapes and sizes were observed. The size of the silver nanoparticles was observed in the range of 30-50 nm with spherical shape. The synthesis of nanoparticles was rapid, stable for several months and involves purely ‘green synthesis’ which does not involve any toxic chemicals and by products. The antibacterial activity for silver nanoparticles, antibiotic and antibiotic conjugated with silver nanoparticles was assessed using the standard broth dilution method and pour plate technique for determining the minimum inhibitory concentration (MIC). Organisms used for this study are Escherichia coli (Gram negative) and Staphylococcus aureus (Gram positive). The antibacterial effect of the antibiotic and the silver nanoparticles was found separately and compared with that of combined effect of antibiotic and silver nanoparticles and the combination of antibiotic with silver nanoparticles was found to be very effective compared to its individual effect. The current study opens light to a novel drug with broad spectrum antibacterial activity.

Key words: Antibacterial activity, Green synthesis, MIC, Cefazolin, E. Coli, S. aureus

INTRODUCTION

With the emergence and increase of microbial organisms resistant to multiple antibiotics, and the continuing emphasis on health-care costs, many researchers have tried to develop new, effective antimicrobial reagents free of resistance and cost. Such problems and needs have led to the resurgence in the use of Ag-based antiseptics that may be linked to broad-spectrum activity and far lower propensity to induce microbial resistance than antibiotics (Jun Sung Kim). Research in antibacterial material containing various natural and inorganic substances (Cho K, Kim TN) has been intensive. Among metal nanoparticles (Me-NPs), silver nanoparticles (Ag-NPs) have been known to have inhibitory and bactericidal effects (Cho K). It can be expected that the high specific surface area and high fraction of surface atoms of Ag-NPs will lead to high antimicrobial activity as compared with bulk silver metal (Cho K). The biomedical application of silver nanoparticles also attracted increasing interest (Sun R.W), such as antimicrobial activity of silver nanoparticles for wound healing (Wright J.B), and silver nano-coated medical devices (Furno F), etc. Duran and co workers investigated that use of silver ion or metallic silver as well as silver nanoparticles can be exploited in medicine for burn treatment, dental materials, coating stainless steel materials, textile fabrics, water treatment, sunscreen lotions, etc. and posses low toxicity to human cells, high thermal stability and low volatility.

Nature of Silver Nanoparticles:

Silver is non toxic, safe inorganic antibacterial agent being used for many centuries and is capable of killing of 650 microorganism that causing diseases (H.Jeong). The antibacterial activity of silver species has been well known since ancient times (Holt , Siddhartha Shrivastava) and it has been demonstrated that, in low concentrations, silver is non toxic to human cells [Raimondi F, Zhang Y). Among the inorganic antibacterial agents, silver (Ag) has been known most extensively since ancient times to fight infections and control spoilage. The antibacterial and antiviral actions of Ag, Ag+ and Ag, Au compounds have been thoroughly investigated (Oka, M. T - Oloffs, A). Silver ion and silver-based compounds are highly toxic to microorganisms (Slawson, R. M), showing strong biocide effect against as many as 16 species of bacteria, including Escherichia coli, S.aureus (Oka, M. T).
Synthesis Of Silver Nanoparticles:

An array of physical, chemical and biological methods have been used to synthesis nanoparticles (Narayanan Kannan Badri). The chemical method involves the use of reducing agents such as sodium tetrahydridoborate, citrate, or ascorbate is most commonly used (Chen, M, Kuo, P. L, Lou, X. W). Considering that such reducing agents may be associated with environmental toxicity or biological hazards, the development of a green synthesis for silver nanoparticles is desired (Dongwei Wei). Eco-friendly bio-organisms in plant extracts contain proteins, which act as both reducing and capping agents forming stable and shape-controlled AgNPs (Virender K). Microbes produce inorganic materials either intra- or extracellular often in nanoscale dimensions with exquisite morphology. Bacterial and fungal species are generally used to synthesize AgNP (silver nanoparticles). Klaus and co-workers (2001) have shown that the bacterium Pseudomonas stutzeri AG259, isolated from a silver mine, when placed in a concentrated aqueous solution of silver nitrate, played a major role in the reduction of the Ag+ ions and the formation of silver nanoparticles (AgNPs) of well-defined size and distinct topography within the periplasmic space of the bacteria (Kreibig U). Shahverdi et al. reported the rapid synthesis of metallic nanoparticles of silver using the reduction of aqueous Ag+ ion using the culture supernatants of Klebsiella pneumonia, Escherichia coli, and Enterobacter cloacae (Enterobacteriaceae). A novel biological method for the synthesis of silver nanoparticles using the fungus Verticillium is reported (Klaus, T et al, 1999). Biosynthesis of Ag-NPs using a common fungus, Alternaria alternate was reported by [Monali Gajbhiye]. Trichoderma viridae has also been used in the biosynthesis of AgNP (Mohammed Fayaz) which is the source of AgNP in this study.

Stabilization Of Silver Nanoparticles:

Proteins can bind to nanoparticles either through free amine groups or cysteine residues in the proteins (A. Gole, C. Dash, Mandal, S) and via the electrostatic attraction of negatively charged carboxylate groups in enzymes present in the cell wall of mycelia (M. Sastry) and therefore, stabilization of the silver nanoparticles by protein is a possibility. Reported the formation of triangular, hexagonal and spheroidal Ag-containing nanoparticles at different cellular binding sites of the bacterium, Pseudomonas stutzeri AG259 (Klaus T). Though the mechanism of silver resistance offered by bacteria using silver binding protein. Hence, it is advantageous to work with fungus for the large-scale production and purification of silver nanomaterials. A model Gram-negative bacterium E. coli, when treated with silver nanoparticles, showed the formation of “pits” in its cell wall with the accumulation of nanoparticles in the cell membrane, leading to increased permeability and cell death [Sondi I]. Hence, the synthesized nanoparticles could be of immense use as antimicrobial agent.

Effect of Antibacterial Activity Using Silver Nanoparticles:

Recently, due to the emergence of antibiotic-resistant bacteria and limitations of the use of antibiotics the clinicians have returned to silver wound dressings containing varying level of silver (Chopra I, Gemmell CG). There lies a strong challenge in preparing nanoparticles of silver stable enough to significantly restrict bacterial growth. The major mechanism through which silver nanoparticles manifested antibacterial properties was by anchoring to and penetrating the bacterial cell wall and modulating cellular signalling be dephosphorylating putative key peptide substrates on tyrosine residues [Siddhartha Shrivastava]. Silver was first registered as a pesticide in the United States in 1954. Several studies propose that Ag NPs may attach to the surface of the cell membrane disturbing permeability and respiration functions of the cell. Smaller Ag NPs having the large surface area available for interaction would give more bactericidal effect than the larger Ag NPs (Kvitek L). It is also possible that Ag NPs not only interact with the surface of membrane, but can also penetrate inside the bacteria (Morones JR). The mechanism of action of silver is linked with its interaction with thiol group compounds found in the respiratory enzymes of bacterial cells. Silver binds to the bacterial cell wall and cell membrane and inhibits the respiration process (Klasen HJ). In case of E. coli, silver acts by inhibiting the uptake of phosphate and releasing phosphate, mannitol, succinate, proline and glutamine from E. coli cells (Bragg P. D, Haeftili C, Rosenkranz HS - Schreurs WJA, Yamanaka M).

Effect of Nanoparticles Size and Shape on the Antimicrobial Activity:

The surface plasmon resonance plays a major role in the determination of optical absorption spectra of metal nanoparticles, which shifts to a longer wavelength with increase in particle size. The size of the nanoparticle implies that it has a large surface area to come in contact with the bacterial cells and hence, it will have a higher percentage of interaction than bigger particles (Kreibig U, Morones JR, Pal S). The nanoparticles smaller than 10 nm interact with bacteria and produce electronic effects, which enhance the reactivity of nanoparticles. Thus, it is corroborated that the bactericidal effect of silver nanoparticles is size dependent (Pal S - Raimondi F). When silver nanoparticles enter the bacterial cell it forms a low molecular weight region in the center of the bacteria to which the bacteria conglomerates thus, protecting the DNA from the silver ions. The nanoparticles preferably attack the respiratory chain, cell division finally leading to cell death. The
nanoparticles release silver ions in the bacterial cells, which enhance their bactericidal activity (Feng QL, Morones JR, Sondi I). According to (Pal S) truncated triangular nanoparticles show bacterial inhibition with silver content of 1 µg. While, in case of spherical nanoparticles total silver content of 12.5 µg is needed. The rod shaped particles need a total of 50 to 100 µg of silver content. Thus, the silver nanoparticles with different shapes have different effects on bacterial cell.

**Synergistic Effect:**

The minimum inhibitory concentrations (MIC) of extracellular biosynthesized AgNPs on gram-positive and gram-negative bacteria were determined by broth dilution method [Siddhartha Shrivastava] and pour plate technique. The combination of these AgNPs with different antibiotics was investigated against gram-positive and gram-negative bacteria using the disk diffusion method. The diameter of the inhibition zone (mm) around the different antibiotic disks with and without AgNPs against test strains was found. The highest percentage of fold increase was found for ampicillin followed by kanamycin, erythromycin, and chloramphenicol (Mohammed Fayaz). Also in the article cited by [Shahverdi AR] nanoparticles are evaluated for their part in increasing the antimicrobial activities of various antibiotics against *Staphylococcus aureus* and *Escherichia coli*. The antibacterial activities of penicillin G, amoxicillin, erythromycin, clindamycin, and vancomycin were increased in the presence of Ag-NPs against both test strains. The highest enhancing effects were observed for vancomycin, amoxicillin, and penicillin G against *S.aureus*.

**MATERIALS AND METHODS**

**Materials Source Of Microorganisms:**

The four fungal species was obtained from the Culture Collection Center (CAS in Botany, University of Madras, India) and maintained in potato dextrose agar, nutrient agar and selective media (HiMedia, Mumbai, India) slant at 27°C. Two bacterial strains were obtained from the ATCC.

**Fungus Species:**

*Alterneria alternata,*
*Fusarium oxysporum,*
*Phoma capitulum ,*
*Trichoderma harzianum,*

Above fungus species were cultured and maintained in PDA medium.

**Bacterial Organism Under Study:**

*Staphylococcus aureus* (ATCC 29737)  – Gram positive cocci
*Escherichia coli* (ATCC10536) -- Gram negative rod

**Antibiotic:**

Cefazolin (APEX lab, Chennai & Stribog lab HYD)

**Silver Nanoparticles:**

Concentration of synthesized silver nanoparticles is found to be 1 mg/ml of the silver nanosolution and the size of the silver nanoparticle is 30 – 50 nm. Appropriately synthesized silver nanoparticles were taken based on the requirement of usage.

**Cfazolin Antibiotic:**

Cefazolin - 1mg
Distilled water - 1ml
25mg of cefazolin antibiotic weighed and dissolved in 25ml of sterilized distilled water.

**Methods Maintenance Of Cultures:**

Culture was maintained by repeated sub culturing on appropriate medium.

Hours bacterial culture was prepared for each experimental procedure.

1. **Fungus:**

Fungi cultures were inoculated into the PDA plates. The plates were maintained at room temperature for week.
2. **Bacteria:**

Bacterial strains were confirmed using Mannitol Salt Agar medium and Eosin Methylene Blue medium, respectively for *Staphylococcus aureus* and *Escherichia coli*. Bacterial cultures were inoculated in respective medium and incubate at room temperature for 24 hours.

**Synthesis Of Silver Nanoparticle:**

**Production of Biomass:**

To prepare the biomass for biosynthesis, the fungus obtained were grown aerobically in liquid broth containing malt extract powder, glucose, yeast extract, peptone. The culture flasks were incubated on room temperature at 27°C. The biomass was harvested after 120 hours of growth by sieving through a plastic sieve followed by extensive washing with sterile double-distilled water to remove any medium components from the biomass.

**Synthesis of Silver Nanoparticles:**

Typically 15 g of biomass (wet weight) were brought into contact with 100 mL sterile double-distilled water for 48 hours at 27°C in an Erlenmeyer flask and agitated 150rpm. After incubation the cell filtrate was filtered by Whatman filter paper No.1 (Oakland, California, USA). After filtration the observed pH of cell filtrate was 7.2. Into these 100 mL of cell filtrate, a carefully weighed quantity of silver nitrate was added to the Erlenmeyer flask to yield an overall Ag+ ion concentration of 10⁻³ M, and the reaction was carried out under dark conditions.

**Characterization Of Silver Nanoparticles:**

Further included time-dependent formation of AgNPs using ultraviolet-visible (UV-visible spectrophotometer (Beckman DU-20 spectrophotometer), samples for Transmission Electron Microscopy(TEM) were prepared by drop-coating the AgNPs solution into the carbon-coated copper grid, and their size and morphology were characterized by TEM (JEOL 2000 FX MARK II, Tokyo). Also changing of solution colour from pale yellow to dark brown was confirmed, thus silver nanoparticles was used further to continuing the experiment.

**Conjugating Cefazolin With Silver Nanoparticles:**

Silver nanoparticles were mixed with Cefazolin of different concentration such as 50 µg, 100 µg, 150 µg, 200 µg, 250 µg was added and incubated overnight . This concentration of silver nanoparticles is used to treat the *Staphylococcus aureus*. Silver nanoparticles were mixed with Cefazolin different concentration such as 50 µg, 100 µg, 150 µg, 200 µg, 250 µg and incubated overnight . This concentration of silver nanoparticles is used to treat *E.coli*.

**Enhancement Of Cefazolin Activity:**

**Broth dilution method for finding MIC for S.aureus:**

**Cefazolin Activity:**

To the 50ml of Nutrient broth prepared in side arm conical flasks 100µg, 200µg, 300µg, 400µg and 500µg per ml concentration of Cefazolin was added. To measure the bacterial growth rate and to determine the growth curve 0.15 ml (1 × 10⁸ CFU) over night culture of *S.aureus* was added to the broth containing Cefazolin. The OD was read at 600 nm at 2 hours intervals for 30 hours.

MIC was confirmed by pour plate technique.

**Silver Nanoparticles Activity:**

To the 50ml of Nutrient broth prepared in side arm flasks 500µg, 600µg, 700µg, 800µg and 900µg per ml concentration of Silver nanoparticles were added. To this 0.15 ml (1 × 10⁸ CFU) over night culture of *S.aureus* was added. The OD was read at 600 nm at 2 hours intervals for 30 hours.

MIC was confirmed by pour plate technique.

**Cefazolin with Silver Nanoparticles Activity:**

To the 50 ml of Nutrient broth prepared in side arm flasks 50µg, 100µg , 150µg, 200µg and 250µg per ml concentration of Cefazolin with 1µg/ml Silver nanoparticles were added .To this 0.15 ml (1 × 10⁸ CFU) over night culture of *S.aureus* was added . The OD was read at 600 nm at 2 hours intervals for 30 hours.

MIC was confirmed by pour plate technique.

**Broth Dilution Method For Finding Mic Of Escherichia Coli:**

**Cefazolin Activity:**
To the 50 ml of Nutrient broth prepared and sterilized in side arm conical flasks 50µg, 100µg, 150µg, and 200µg per ml concentration of Cefazolin was added. To measure the bacterial growth rate and to determine the growth curve 0.15 ml (1 × 10^8 CFU) over night culture of *E.coli* was added to the broth containing Cefazolin. The OD was read at 600 nm at 24 hours intervals for 24 hours.

MIC was confirmed by pour plate technique.

**Silver Nanoparticles Activity:**

To the 50 ml of Nutrient broth prepared in side arm flasks 50µg, 100µg, 150µg, 200µg, 250 µg per ml concentration of Silver nanoparticles were added. To this 0.15 ml (1 × 10^8 CFU) over night culture of *E.coli* was added. The OD was read at 600 nm at 2 hours intervals for 24 hours.

MIC was confirmed by pour plate technique.

**Cefazolin With Silver Nanoparticles Activity:**

To the 50 ml of Nutrient broth prepared in side arm flasks 50µg, 100µg, 150µg, 200µg, 250µg per ml concentration of Cefazolin containing 1µg/ml Silver nanoparticles were added. To this 0.15 ml (1 × 10^8 CFU) over night culture of *E.coli* was added. The OD was read at 600 nm at 2 hours intervals for 24 hours.

MIC was confirmed by pour plate technique.

**MIC (Minimum inhibitory Concentration) (Qi L, Xu Z, Jiang X 2004) in S.aureus & *E.coli*:**

The minimum inhibitory concentration (MIC) the lowest concentration of material that inhibits the growth of an organism. MIC of silver nanoparticles was also determined by Pour plate method according NCCLS (CLSI). MIC method was determined based on cultures containing varying concentration of silver nanoparticles in suspension (1mg per ml). Subsequently, the flasks were inoculated with 150 µl of the freshly prepared 24 hours of bacterial suspension in order to maintain initial bacterial concentration .2 Cells per ml, and then incubated in an orbital shaker for 120 rpm at room temperature. Bacterial growth was measured as increase in absorbance at 600 nm determined using a spectrophotometer. The experiments also included a positive control (flask containing inoculums and nutrient media, devoid of silver nanoparticles) and a negative control (flask containing silver nanoparticles and nutrient media, devoid of inoculums). The positive controls indicated the microbial growth profile in the absence of nanoparticles. The absorbance values for positive controls were subtracted from the experimental values (flasks containing nutrient media, inoculums and nanoparticles).

**Pour Plate Method:**

**Antibiotic:**

*Escherichia coli* and *Staphylococcus aureus* cultures were inoculated in 100ml nutrient broth and incubate in shaker for 24 hour. Prepare nutrient broth for each culture and inoculate 150µl the appropriate culture flasks. Then add the antibiotic ranges from 50-500ml in each broth and incubate in shaker about 18-22 hours. Incubated broth cultures (1ml) were taken and serially diluted using sterile distilled water up to 10^-7. 1ml of serially diluted culture was taken and transfer culture to the sterile plates. Then add the prepared nutrient agar medium to the Petri plates. Allowed to solidify the medium and then look for inhibition of colonies.

**Silver Nanoparticles:**

*Escherichia coli* and *Staphylococcus aureus* cultures were inoculated in 100ml nutrient broth and incubate in shaker for 24 hour. Prepare nutrient broth for each culture and inoculate 150µl the appropriate culture flasks. Then add the silver nanoparticles ranges from 50-500ml in each broth and incubate in shaker about 18-22 hours. Incubated broth cultures (1ml) were taken and serially diluted using sterile distilled water up to 10^-8.1ml of serially diluted culture (10^-5 and 10^-8) were taken, transfer culture to the sterile plates. Then add the prepared nutrient agar medium to the Petri plates. Allowed to solidify the medium and then look for inhibition of colonies.

**Antibiotic Combined With Silver Nanoparticles:**

*Escherichia coli* and *Staphylococcus aureus* cultures were inoculated in 100ml nutrient broth and incubate in shaker for 24 hour. Prepare nutrient broth for each culture and inoculate 150µl the appropriate culture flasks. Then add the antibiotic combining silver nanoparticles ranges from (100-300 µl for *E.coli* and 50-250µl for *S.aureus*) in each broth and incubate in shaker about 18-22 hours. Incubated broth cultures (1ml) were taken and serially diluted using sterile distilled water up to 10^-8. 1ml of serially diluted culture (10^-5 and 10^-8) were taken, which transfer to the sterile plates. Then add the prepared nutrient agar medium to the Petri plates. Allowed to solidify the medium and then look for inhibition of colonies.

**RESULTS AND DISCUSSION**
A detailed study on the extracellular biological synthesis of silver nanoparticles using the fungal species was studied and its antibacterial effect with commercially available antibiotic cefazolin against Gram positive and Gram negative bacteria by taking *Escherichia coli* and *Staphylococcus aureus* as model organisms are reported in this work.

**Synthesis of Silver Nanoparticles:**

Four different fungal species was screened for biological synthesis of silver nanoparticles and among them *Trichoderma harzianum* was found to be capable of synthesizing silver nanoparticles with high stability. For the screened organism culture filtrate colour has changed from pale yellow to dark brown after reduction for 24 hours in dark condition which is shown in Figure 1. The formation of silver nanoparticles was preliminary confirmed by the colour changes from pale yellow to dark brown. Formation of dark brown is due to the Surface Plasmon Resonance property of silver nanoparticles.

**UV –VISIBLE Spectroscopy:**

The formation of silver nanoparticles was confirmed by the colour changes from pale yellow to dark brown. The nanoparticles show maximum absorbance at 420 nm on UV - Vis spectra which is shown in Figure 2.

**Transmission Electron Microscope (TEM):**

A TEM study reveals the silver nanoparticles size and shape nature. The transmission electron micrograph revealed the formation of polydisperse nanoparticles with the size range of 30–50 nm and the TEM micrograph which is shown in Figure 3.

The silver nanoparticles were found to be stable for two months. The nanoparticles were also evaluated for their increased antibacterial activities with cefazolin antibiotics against selected Gram positive and Gram negative bacteria. The results showed that the combinations of antibiotics with silver nanoparticles have better antibacterial effect.

Fig. 1:
(a) Control – Pale Yellow Culture Solution with Culture Filterate.
(b) Silver Nanoparticle – Dark Brown Colour Solution after 24 Hours of Incubation.

Fig. 2: TEM Image of Silver Nanoparticles
Size 30-50 nm
Antibacterial Activity of Silver Nanoparticles:

Antibacterial activity of silver nanoparticles has been checked in Nutrient broth were 0.15mL \( (1 \times 10^{-8} \text{ CFU}) \) of \( E. coli \) cells and \( S. aureus \) is supplemented with different concentration nanoparticles and the OD value is checked for every 2hrs. For \( E. coli \) the minimum inhibitory concentration was found to be 300μg/ml and the range is between 300-400μg/ml and for \( Staphylococcus aureus \) the minimum inhibitory concentration was found to be 800μg/ml and the range is between 800-900μg/ml. This shows that silver nanoparticles has better activity on \( E. coli \) than \( Staphylococcus aureus \).

Minimum inhibitory concentration was confirmed by pour plate technique were no colonies were observed for \( E. coli \) from the plates starting from the silver nanoparticles concentration from 300μg/ml and 800μg/ml for \( S. aureus \) which confirms the MIC of silver nanoparticles for both organisms.

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Table 2: \( E. coli \) Vs Silver nanoparticle
Antibacterial Activity Of Silver Nanoparticles:

Graph. 1: *S.aureus* Vs silver nanoparticles

Graph.2: *E.coli* Vs silver nanoparticles

**Antibacterial Activity of Cefazolin:**

Antibacterial activity of Cefazolin has been checked in Nutrient broth were 0.15mL (1 × 10⁸ CFU) of *E. coli* and *S.aureus* is supplemented with different concentration of Cefazolin and the OD value is checked at 2 hours interval for 24 hours. For *E.coli* the minimum inhibitory concentration was found to be 400μg/ml and the range is between 400-450μg/ml and for *Staphylococcus aureus* the minimum inhibitory concentration was found to be 150μg/ml and the range is between 150-200μg/ml.

Minimum inhibitory concentration was confirmed by pour plate technique were no colonies were observed for *E.coli* from the plates starting from the antibiotic concentration from 400 μg/ml and 150μg/ml for *S.aureus* which confirms the MIC of antibiotic cefazolin for both organisms.

**Enhancement of Antibacterial Activity (cefazolin + nanoparticles):**

Cefazolin is conjugated with silver nanoparticles by overnight incubation. Enhancement of antibacterial activity of Cefazolin conjugated nanoparticles has been checked in Nutrient broth were 0.15ml (150μl) of *E. coli* cells and *S.aureus* was added with different concentration of Cefazolin conjugated nanoparticle and the OD value is checked for every 2hrs. For *E.coli* the minimum inhibitory concentration was found to be 200μg/ml and the range is between 200-250μg/ml for silver, where as for *Staphylococcus aureus* the minimum inhibitory concentration was found to be 100μg/ml and the range is between 100-150μg/ml for silver. This shows an enhancement in antibacterial activity on *E.coli* and *Staphylococcus aureus.*

**Table 3:** *S.aureus* Vs Cefazolin

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Table 4: *E. coli* Vs Cefazolin

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<td>0.2</td>
<td>0.219</td>
<td>0.245</td>
<td>0.169</td>
<td>0.046</td>
<td>0.024</td>
<td>0.044</td>
<td>0.046</td>
</tr>
<tr>
<td>500</td>
<td>0.2</td>
<td>0.217</td>
<td>0.201</td>
<td>0.107</td>
<td>0.01</td>
<td>0.02</td>
<td>0.033</td>
<td>0.012</td>
</tr>
</tbody>
</table>

**Antibacterial activity of cefazolin:**

Graph 3: *S. aureus* Vs Cefazolin

Graph 4: *E. coli* Vs Cefazolin

Minimum inhibitory concentration was confirmed by pour plate technique were no colonies were observed for *E. coli* from the plates starting from the antibiotic concentration from 200 μg/ml and 100μg/ml for *S. aureus* which confirms the MIC of antibiotic cefazolin for both organisms.

Table 5: *S. aureus* Vs silver nanoparticles + Cefazolin

<table>
<thead>
<tr>
<th></th>
<th>0</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>10</th>
<th>12</th>
<th>14</th>
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</thead>
<tbody>
<tr>
<td>Positive</td>
<td>0.2</td>
<td>0.255</td>
<td>0.31</td>
<td>0.434</td>
<td>0.726</td>
<td>0.92</td>
<td>1.297</td>
<td>1.418</td>
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<tr>
<td>50</td>
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<td>0.209</td>
<td>0.298</td>
<td>0.389</td>
<td>0.6</td>
<td>0.692</td>
<td>0.787</td>
<td>0.796</td>
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<tr>
<td>100</td>
<td>0.2</td>
<td>0.232</td>
<td>0.266</td>
<td>0.315</td>
<td>0.415</td>
<td>0.4</td>
<td>0.372</td>
<td>0.333</td>
</tr>
<tr>
<td>150</td>
<td>0.2</td>
<td>0.228</td>
<td>0.236</td>
<td>0.254</td>
<td>0.289</td>
<td>0.268</td>
<td>0.241</td>
<td>0.211</td>
</tr>
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</table>
Table 6: E.coli Vs Silver nanoparticles + Cefazolin

<table>
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<tr>
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<th>4</th>
<th>6</th>
<th>8</th>
<th>10</th>
<th>12</th>
<th>14</th>
</tr>
</thead>
<tbody>
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<td>0.22</td>
<td>0.224</td>
<td>0.223</td>
<td>0.222</td>
<td>0.218</td>
<td>0.212</td>
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</tr>
<tr>
<td>250</td>
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<td>0.226</td>
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<td>0.207</td>
<td>0.214</td>
<td>0.212</td>
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</tbody>
</table>

Enhancement of antibacterial activity (Cefazolin and Nanoparticles):

Graph.5: S.aureus Vs cefazolin and nanoparticles

Graph.6: E.coli Vs cefazolin and nanoparticles
Fig. 8: Nanoparticles with E.Coli:

Fig. 9: Antimiotics with E.Coli.
Fig. 10: Nanoparticles with Antibiotics Against E.Coli.

Fig. 11: Nanoparticles with S. Aureus
Fig. 12: Antibiotics with S. Auresus

Fig. 13: Nanoparticles with Antibiotics
Table 7:

<table>
<thead>
<tr>
<th>Organism</th>
<th>Antibacterial agent</th>
<th>MIC(µg/ml)</th>
<th>Range</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Cefazolin</td>
<td>150</td>
<td>150-200</td>
</tr>
<tr>
<td></td>
<td>Silver nanoparticles</td>
<td>800</td>
<td>800-900</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>Cefazolin conjugated Silver</td>
<td>100</td>
<td>100-150</td>
</tr>
<tr>
<td></td>
<td>nanoparticles</td>
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<td></td>
</tr>
</tbody>
</table>

Table 8:

<table>
<thead>
<tr>
<th>Organism</th>
<th>Antibacterial agent</th>
<th>MIC(µg/ml)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>Cefazolin</td>
<td>400</td>
<td>400-450</td>
</tr>
<tr>
<td></td>
<td>Silver nanoparticles</td>
<td>300</td>
<td>300-400</td>
</tr>
<tr>
<td></td>
<td>Cefazolin conjugated Silver</td>
<td>200</td>
<td>200-250</td>
</tr>
<tr>
<td></td>
<td>nanoparticles</td>
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</tbody>
</table>

Conclusion:

In this current work nanoparticles were synthesized biologically using the fungus *Trichoderma harzianum*, which is a pure green chemistry and completely toxic free compared to chemical synthesis methods. Minimum inhibition concentration of silver nanoparticles, Cefazolin antibiotics and antibiotics conjugated with silver nanoparticles was found out by broth dilution method and was compared. Enhancement study of Cefazolin antibiotics along with silver nanoparticles against *E.coli* and *S.aureus* was studied. For *E.coli* the minimum inhibitory concentration was found to be 200µg/ml and the range is between 200-250µg/ml for silver. For *Staphylococcus aureus*, the minimum inhibitory concentration was found to be 100µg/ml and the range is between 100-150µg/ml for silver. From the above results obtained, we can conclude that a silver nanoparticle plays a vital role in enhancing the antibacterial activity of Cefazolin. When nanoparticles conjugated with Cefazolin, in lower concentrations also it was found to be effective when compared to the individual antibacterial activity of nanoparticles as well as antibiotic.

Cefazolin antibiotic have proved to have many side effects but used as a life saving antibiotic when other antibiotics fails. At the same time the cost of the Cefazolin is more compared to other commercially available antibiotics. By our findings, since the concentration of Cefazolin is reduced, the side effects caused due to the antibiotics can be minimized up to an extend and at the same time cost effective also.

REFERENCES


