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Research Article

Comparative Study on the Synergistic Action of Garlic Synthesized and Citrate Capped Silver Nanoparticles with β-Penem Antibiotics

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Resistance to antimicrobial agents by pathogenic bacteria has emerged in recent years and is a major challenge for the health care industry. For developing a cheap broad-active agent that can be applicable against different pathogens, it is necessary to develop an alternative source for normal antibacterial agents. This paper mainly focuses on the combined action of green as well as citrate synthesized silver nanoparticles and β-penem antibiotics, which are β-lactam antibiotics with penem rings. These silver nanoparticles synergistic potential helps in the enhancement of antibacterial activity of broad spectrum antibiotics. The synergistic actions of citrate capped silver nanoparticles (Ag-NPs) were compared with that of garlic (*Allium sativum*) synthesized silver nanoparticles together with action of antibiotics, ampicillin and amoxyclav, and some of the pathogenic organisms showed an increase in the action of antibiotics.

1. Introduction

Over the last few years, the β-lactam classes of antibiotics were the most widely used and the most commonly prescribed drugs, which represent more than half of all the antibiotics used in medical field, especially for the treatment of infectious diseases. This is primarily because in general they work perfectly against bacteria, easy to deliver, and are of few side effects. But now most of the bacteria will show wide spread resistance against β-lactam antibiotics by means of production of β-lactam hydrolyzing enzyme (β-lactamases), the utilization of β-lactam-insensitive cell wall transpeptidases, and the active expulsion of β-lactam molecules from Gram-negative cells by way of efflux pumps [1].

Moreover bacteria are capable of producing extended spectrum β-lactamases (ESBLs). Therefore, a new choice of β-lactam antibiotics is necessary to overcome all these problems; thus, new β-lactam penems receive much attention. Recently, silver nanoparticles (Ag-NPs), which are well known for their antimicrobial activity, are also studied intensively due to the growing bacterial resistance to antibiotics. Silver nanoparticles (Ag-NPs) have been known to have inhibitory and bactericidal effects against Gram-negative and Gram positive bacteria especially multiresistant bacteria. 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 [2]. Therefore, silver nanoparticles can be exploited in medicine for wound healing, dental materials, catheters, coating stainless steel materials, textile fabrics, water treatment, sunscreen lotions, prevention of urinary tract infections, and so forth and possess low toxicity to human cells, high thermal stability, and low volatility. Moreover, silver-based antiseptics show broad-spectrum activity and far lower propensity to induce microbial resistance than antibiotics.

In our study, biogenic synthesis was used for the synthesis of silver nanoparticles which reduced the steps involved in downstream processing; thus, the process becomes more economical and ecofriendly. The synergistic action of new β-lactam penem antibiotics (ampicillin and amoxyclav) with silver nanoparticles can reduce the need for high dosages of antibiotics and minimize their side effects [3]. The present synergistic study clearly demonstrates that antibiotic and biogenically synthesized silver nanoparticles using garlic (*Allium sativum*) and chemically synthesized silver nanoparticles (CS-AgNPs) using sodium citrate exhibit improved bactericidal activities, and more interestingly, a synergistic activity becomes operational when both of the components act together.

2. Materials and Methods

2.1. Synthesis of Silver Nanoparticles. For biogenic synthesis of silver nanoparticles (Ag-NPs), garlic (*Allium sativum*) extract was used as the biological source for reduction and capping, which was purchased from local market, while in chemical synthesis sodium citrate was used as capping and reducing agent with silver nitrate ($AgNO_3$) as precursor, which was from Merck (Mumbai, India).

2.2. Biogenic Synthesis of Silver Nanoparticles. *Allium sativum* (garlic) weighing 5 gm was thoroughly washed in deionized water, cut into fine pieces crushed in 100 mL of deionized water, boiled for ten minutes, and filtered through Whatman no. 1 filter paper. Equal volume of pale white coloured garlic extracts was added to an equal quantity of 0.1 M aqueous silver nitrate ($AgNO_3$) solution by volume in the absence of ambient light to minimize the effects of photo activation. It was followed by the bioreduction of Ag^+ ions (Ag^+ to Ag^0), and the reaction was carried out at room temperature. The reactants were then characterized at intervals to detect the presence of silver nanoparticles. The percentage of silver nanoparticles in the reaction medium was enhanced by three cycles of centrifugation at 10,000 rpm for 3 min followed by redispersion in deionized water.

2.3. Lee and Meisel's Method of Synthesizing Silver Nanoparticles. The chemical synthesis of silver nanoparticle was done using Lee and Meisel's method [4]. 90 mg of silver nitrate was dissolved in 500 mL of deionized water, and solution was allowed to boil. 10 mL of 1% solution of sodium citrate was added dropwise to the boiling solution with vigorous stirring, and then the solution was kept boiling for a further period of 10 minutes, until a grey dispersion of silver resulted. The precipitate was separated by centrifugation, washed repeatedly using deionized water to remove any adsorbed impurities, and dried. The preparation and processing procedures were carried out in dark. The nanoparticles so formed were characterized using UV-visible spectrum analysis and SEM analysis.

2.4. Synergistic Interaction between Silver Nanoparticles and Antibiotics. Disc diffusion method was used to assay the synergistic effect of β-lactam penems with garlic synthesized and citrate capped silver nanoparticles for bactericidal activity against test strains on Muller Hinton agar plates. Here, the standard antibiotic discs were purchased from Himedia (Mumbai, India), and the inocula were prepared by diluting the overnight cultures with 0.9% NaCl to a 0.5 McFarland standard and were applied to the plates along with the standard and prepared discs containing differing amounts of Ag-NPs with antibiotic. After incubation at 35°C for 24–48 hours the zones of inhibition were measured [5].

3. Results and Discussion

In this work, silver nanoparticles were synthesized using garlic (*Allium sativum*) and sodium citrate, respectively. When the reducing agents were mixed with aqueous solution of the silver nitrate, they started to convert the color from colourless to yellowish brown due to reduction of silver ion, which indicated the formation of silver nanoparticles [6]. The reduction of silver ion was mainly due to the presence of phytochemicals such as flavonoids, phenols, allicin, and so forth in the *Allium sativum* extract. Then, the synthesis of silver nanoparticles by reduction of pure Ag^+ ions was monitored by measuring the UV-Vis spectrum. The UV-visible analysis showed that the absorption peaks at 406 and 425 nm were mainly due to the surface plasmon resonance (SPR) of silver nanoparticle, and the broadening of the peak indicated that the particles are polydispersed (Figures 1 and 2). The peaks at 406 and 425 nm are in good agreement with the theoretical simulation of SPR using Mie's theory [7] and different experimental results.

The SEM micrographs of these nanoparticles showed that nanoparticles formed are roughly spherical nanoparticles, and they are polydispersed (Figures 3 and 4). The combination of Ag-NPs (As-AgNPs, Cs-AgNPs) with two different β-lactam penem antibiotics (ampicillin and amoxyclav) was investigated against Gram-positive (*Bacillus subtilis, Staphylococcus aureus, Micrococcus luteus, Lactococcus lactis*) and Gram-negative (*Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, Klebsiella pneumoniae, Proteus vulgaris*) bacteria using the disc diffusion method. The diameters of zone (mm) of inhibition of different antibiotic discs with and without Ag-NPs against test strains were measured. Ampicillin, which is a β-lactam penem antibiotic, showed an enhanced antibacterial activity in combination with different concentration of Ag-NPs; it was found that AS-AgNPs (20 μg/mL) shows 36.3% fold increase against *Salmonella typhi* (Figure 5). Ampicillin also shows a slight enhancement of 3.5% fold increase against *Micrococcus luteus* at (20 μg/mL) of AS-AgNPs.

However, chemically synthesized silver nanoparticles (20 μg/mL) show 9% fold increase against *S. typhi*, 5% fold increase against *Lactococcus lactis,* and 5.2% fold increase against *Proteus mirabilis* (Table 1). Amoxyclav also showed enhanced synergistic activity in the presence of AS-AgNPs against different pathogens (Figures 6 and 7). It shows 30%, 15%, 11.1%, 10%, 9%, 8.3%, and 7.6% fold increase against *Pseudomonas aeruginosa, Proteus mirabilis, Escherichia coli, Staphylococcus aureus, Bacillus subtilis, Lactococcus lactis,* and *Micrococcus luteus*, respectively, at a concentration of 20 μg/mL (Table 2), while CS-AgNPs shows 22.2%, 16.6%, 15.3%, 10%, 9%, 7.6%, and 5% fold increase against *E. coli*, *P. aeruginosa, M. luteus, S. aureus, B. subtilis, Klebsiella pneumonia, and P. mirabilis*, respectively (Table 2), at 20 μg/mL. In the case of biogenic synthesized Ag-NPs, ampicillin showed the most pronounced antibiotic synergy against pathogens, while in chemically synthesized silver nanoparticles amoxyclav

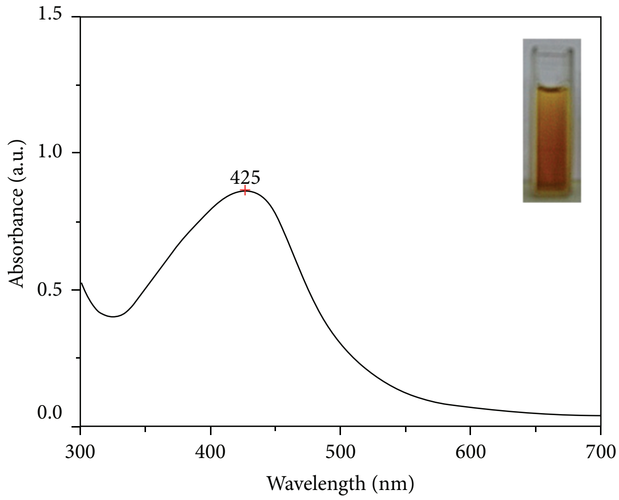


Figure 1: UV-visible spectrophotometer analysis of citrate capped silver nanoparticles.

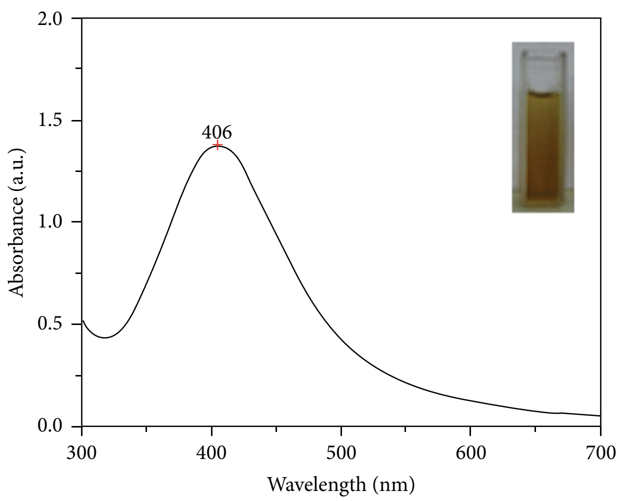


Figure 2: UV-visible spectrophotometer analysis of silver nanoparticles synthesized using extract of garlic.

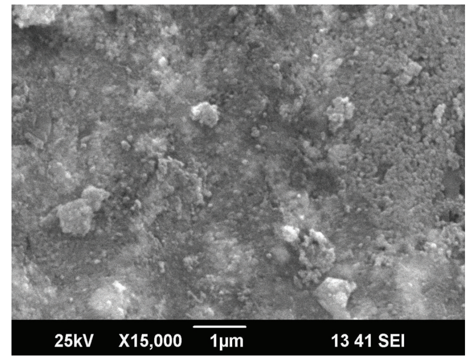


Figure 3: SEM image of AS-AgNPs.

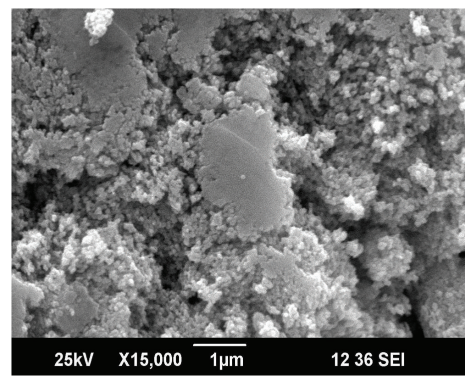
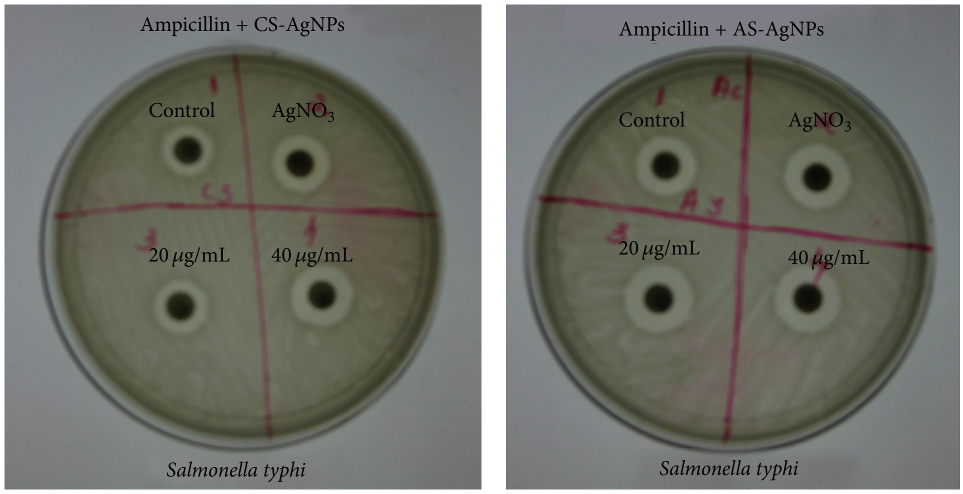


Figure 4: SEM image of CS-AgNPs.

showed effective synergistic activity. Moreover, the antibiotic activity of Ampicillin and amoxyclav increases with increases in concentration of silver nanoparticles. In some cases, antibiotics together with these AgNPs (As-AgNPs and Cs-AgNPs) result in an inhibition of bacterial growth due to some inhibitory mechanism. As with the case of enhancement of antibiotics, there has been a reverse mechanism of inhibiting the activity of antibiotics which has not been so far reported. Both of these silver nanoparticles (AS-AgNPs and CS-AgNPs) showed this type of inhibitory action together with some of the antibiotics. So these CS-AgNPs can be recommended together with antibiotics as well as without antibiotics in different areas of biology for controlling the pathogens. The antibacterial activity of ampicillin and amoxyclav increases in the presence of Ag-NPs against different test strains, and the synergistic effect of some of the Ag-NPs was found to be more prominent than the effect of antibiotics alone. High surface to volume ratio of silver nanoparticles allows them to interact with antibiotics. Moreover, the presence of active groups such as hydroxyl and amido groups in antibiotics may also enable them to interact with nanosilver by chelation which may result in enhanced synergistic effect. More recently, Batarseh's research showed that the bactericidal effect was caused by silver (I) chelating, which prevents DNA from unwinding [8]. The synergistic antibacterial effect with the combination of nanosilver and ampicillin has more potential, when compared with other antibiotics. Dhas et al. [9] reported the enhancement of antibacterial activities of gentamicin and chloramphenicol in combination with AgNPs against *Bacillus cereus, Staphylococcus aureus*, *Proteus mirabilis,* and *Escherichia coli*. In another report by Dar et al. [10], the AgNPs synthesized using *Cryphonectria* sp. also showed an increased antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi,* and *Candida albicans* in combination with streptomycin. Recently, Bhande et al. [11] have demonstrated that antibiotics showed enhanced synergism with ZnO nanoparticles against extended spectrum β-lactamases producers implicated in urinary tract infections. In their work, they proposed that the combination of ZnO nanoparticle and antibiotic increased the permeability of membrane which resulted in the leakage of protein from the membrane of bacteria. The results obtained in our study on combined effects of antibiotics with silver nanoparticles are similar to those of earlier studies reported.

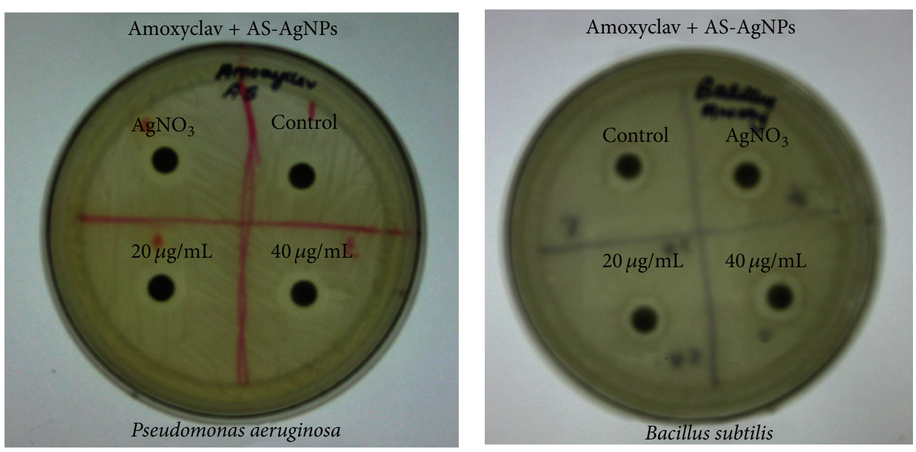
4. Conclusion

The study emphasis was on a possible combination of β-lactam penem antibiotics (ampicillin and amoxyclav) with



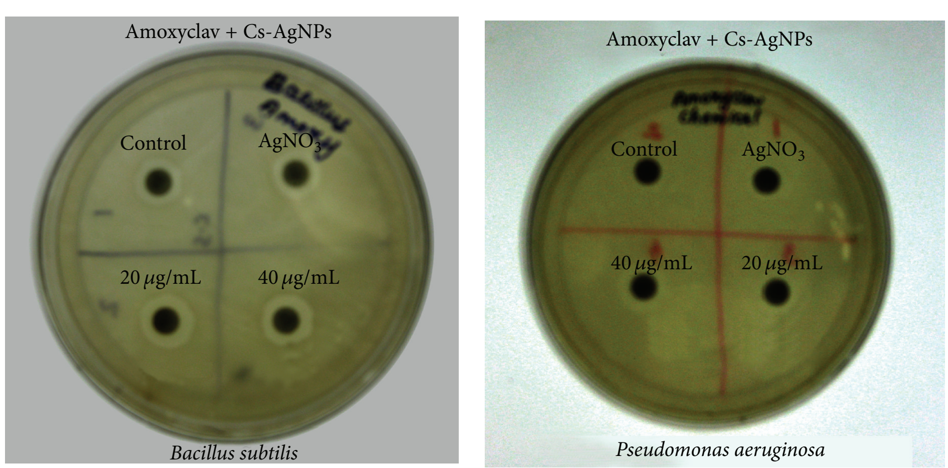
(a) (b)

Figure 5: Synergistic activity of ampicillin with CS-AgNPS and AS-AgNPS against *Salmonella typhi*.



(a) (b)

Figure 6: Synergistic activity of amoxyclav with AS-AgNPS against *P. aeruginosa* and *B. subtilis*.



(a) (b)

Figure 7: Synergistic activity of amoxyclav with CS-AgNPS against *Bacillus subtilis* and *Pseudomonas aeruginosa*.

Table 1: Synergistic activity of ampicillin (β-lactam penem antibiotics) against different pathogens.

Microorganisms	Ampicillin zone (mm) 30 μg/disc	Ag-NPs + ampicillin			
		Ag-NPs (AS) 20 μg/disc	Fold increase (%)	Ag-NPs (CS) 20 μg/disc	Fold increase (%)
Escherichia coli	26	0	−100	0	−100
Pseudomonas aeruginosa	45	39	−13.3	45	0
Staphylococcus aureus	11	11	0	11	0
Lactococcus lactis	20	20	0	21	5
Micrococcus luteus	28	29	3.5	28	0
Bacillus subtilis	24	14	41.6	24	0
Proteus vulgaris	36	36	0	36	0
Klebsiella pneumonia	7	0	−100	7	0
Salmonella typhi	11	15	36.3	12	9
Proteus mirabilis	19	18	−5.2	20	5.2

Table 2: Synergistic activity of amoxyclav (β-lactam penem antibiotics) against different pathogens.

Microorganisms	Amoxyclav zone (mm) 30 μg/disc	Ag-NPs + amoxyclav			
		Ag-NPs (AS) 20 μg/disc	Fold increase (%)	Ag-NPs (CS) 20 μg/disc	Fold increase (%)
Escherichia coli	18	20	11.1	22	22.2
Pseudomonas aeruginosa	10	13	30	12	16.6
Staphylococcus aureus	10	11	10	11	10
Lactococcus lactis	12	13	8.3	10	−16.6
Micrococcus luteus	13	14	7.6	15	15.3
Bacillus subtilis	11	34	9	12	9
Proteus vulgaris	35	0	−2.8	35	0
Klebsiella pneumonia	13	13	−100	12	7.6
Salmonella typhi	13	23	0	13	0
Proteus mirabilis	20	23	15	21	5

Allium sativum synthesized and citrate capped Ag-NPs, which showed enhanced antimicrobial effects and was concluded as synergism. Therefore, the combination of green and chemically synthesized Ag-NPs with ampicillin and amoxyclav in lower concentrations was found to be effective when compared to the individual antibacterial activity of silver nanoparticles as well as antibiotics and acts as the most promising strategy to combat the increasing spread of drug resistance.

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