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Biomimetic Synthesis and Optimization of *Aloe vera* Conjugated Silver Nanoparticle for Use against Multidrug Resistant Microorganisms

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ABSTRACT

The rise of multidrug-resistant (MDR) microbes is a significant and formidable public health challenge, garnering substantial attention from the scientific community. The current study attempted to manufacture silver nanoparticles utilizing the leaves of *Aloe vera*, a widely used medicinal herb. The bioreduced silver nanoparticles were characterized by UV-Vis spectrophotometer, dynamic light scattering (DLS), fourier transform infra-red (FTIR) spectroscopy, transmission electron microscopy (TEM) and energy-dispersive X-ray spectroscopy (EDX). The mean particle size of the produced NPs was around 80 nm and spherical in shape. A qualitative examination of the reducing potential of leaf extract was also performed, indicating the presence of a large amount of reducing entities. FTIR studies demonstrated that the AgNPs were stabilized by the extract's eugenols, terpenes and other aromatic components. The described nanoparticles exhibited growth inhibitory effects on many strains, including gram-positive and gram-negative bacteria such as *Bacillus subtilis, Pseudomonas aeruginosa, Staphylococcus aureus* and *Escherichia coli*. when analyzed using the Agar Well-diffusion method. A significant decrease in the zone of inhibition was seen upon the application of the drug-conjugated silver nanoparticle. The green synthesis strategy opened up the possibility of creating extremely stable Ag nanoparticles with strict particle shape/size distribution from various leaf extracts to develop improved anti-bacterial medicines.

Key words: Antibacterial activity, silver nanoparticles, characterization, optimization

INTRODUCTION

Nanotechnology, a branch of technology that operates at a sub-micron scale, has gained significant attention in recent years. It involves manipulating individual atoms and molecules. In recent decades, nanotechnology has been widely used in various fields. It is a convergence of several sciences that allows for atomic-level work and the creation of novel structures. Nanotechnology involves developing and controlling nanoscale materials and technologies to utilize their exceptional properties (Sharma *et al.,* 2022). Plant mediated synthesis of biological nanoparticles is a sustainable and eco-friendly method for nanoparticle production. This approach utilizes plant extracts containing various phytochemicals such as alkaloids, phenolic, terpenoids and flavonoids to reduce metal ions into nanoparticles. The plant extracts serve as both reducing and capping agents, facilitating nanoparticle formation without the use of harsh chemicals or extreme conditions (Berta *et al.,* 2021). This green synthesis approach not only minimizes environmental impact but also improves the biocompatibility of the nanoparticles, making them suitable for biomedical applications such as drug delivery, imaging and antimicrobial treatments (Hernandez Diaz *et al*., 2021). Furthermore, plant-mediated synthesis is cost-effective and scalable, offering a promising alternative for producing nanoparticles with controlled size and shape, tailored for specific applications (Adeyemi *et al*., 2022). Recent research has concentrated on physico-chemical optimization of silver nanoparticles using aqueous extract of *Aloe vera* plant. The amount of plant extract utilized in green synthesis significantly influences nanoparticle production. The plant extract functions as an electron donor, enabling the conversion of metal ions into nanoparticles. The use of nanomedicine for drug distribution may improve antibiotic effectiveness for therapy. Nanosystems for

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prescribing antibiotics and infection site targeting have several benefits over traditional formulations. The *in vitro* antibacterial activity of nanoformulated medicines chloramphenicol and gentamycin coupled with silver nanoparticles was further investigated.

MATERIALS AND METHODS

Twenty g of fresh *Aloe vera* leaves were used to prepare the aqueous extracellular extract of leaves. The leaves were thoroughly rinsed with double-distilled water and then cut into small pieces. Then 100 ml of double-distilled water was added to the finely chopped pieces, and the mixture was allowed to boil for 5 min. It was passed through Whatman Filter Paper No. 1 and stored at freezing temperature. The remaining extract was stored in the refrigerator at 4°C, sealed with aluminium foil for future use.

To synthesize AgNPs, 2 ml of aqueous leaf extract from *Aloe vera* and 10 ml of $AgNO_3$ solution at ration 1:5 were combined in a conical flask. The initial synthesis was monitored under various incuabation times (1-7 h). The solution was stirred on a hot magnetic plate, under different salt concentrations (0.5, 1, 1.5, 2, 2.5 and 3 mM), temperature 30 to 70°C and pH (3, 5, 7, 9 and 11) for optimization and stable formation of silver nanoparticles at 7 h of incubation. The reduction of Ag^+ ions to metallic Ag^0 occurred during the process of incuabtion. The change in colour from pale yellow to reddish brown, indicated the initial synthesis of AgNPs. The little aliquot of coloured suspension was centrifuged at 10,000 rpm for 15 min thricely to get rid of biological debris and impurities presnt in the nanoparticles. Fig. 1 illustrates the complete process of synthesizing silver nanoparticles (AgNPs) mediated by *Aloe vera* leaf extract. The techniques of ultraviolet visible spectroscopy (UV–Vis) over the spactrakl range from 200-700 nm, dynamic light scattering (DLS) and zeta potential were used for monitoring the synthesis of silver nanoparticles. Fourier transformed infrared spectroscopy (FTIR) and transmission electron microscopy (TEM) were used for the characterization of the AgNPs.

The *in vitro* antibacterial efficacy of chloramphenicol and gentamycin coated silver nanoparticles was examined utilizing the well diffusion method on Mueller-Hinton agar

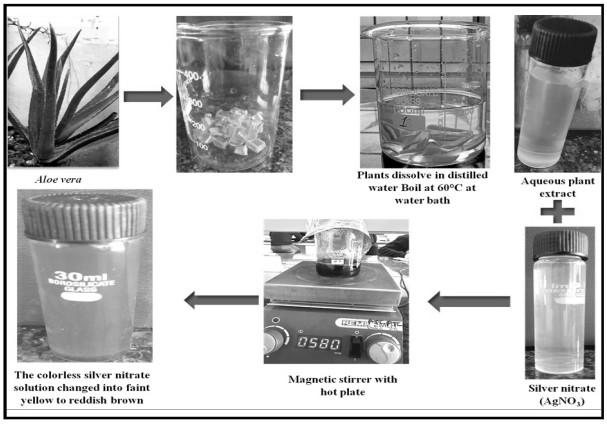

Fig. 1. Graphical representation for the synthesis of *Aloe vera* leaf extract mediated AgNPs.

plates. Initially, Mueller-Hinton agar plates were inoculated with spore suspensions of the microorganisms. The antibiotic-coated AgNPs were subsequently placed on agar plates and incubated for a minimum of one hour at 25°C to facilitate pre-incubation diffusion, hence mitigating the impact of timing discrepancies when different solutions were employed. The plates were assessed for antibacterial activity after 12-24 h of incubation at 37°C by measuring the diameter of the inhibition zones for each bacterial culture (Sharma *et al.,* 2024).

RESULTS AND DISCUSSION

Silver nanoparticles possess distinctive optical characteristics that enable them to interact effectively with particular wavelengths of light (Pal *et al.,* 2023). The creation of silver nanoparticles was primarily assessed by observing the change in colour of the solution within the initial 60 min, but the completion on the reduction of solution was observed at 1-7 h of post-incubation showing change in colour from light yellow to a dark brown indicating the existence of AgNPs due to surface plasmon resonance (SPR). Fig. 2 illustrates the colour change observed in AgNPs solutions. To further validate the creation of nanoparticles under various physico-chemical parameters, UV-Vis spectroscopic investigation was conducted.

UV-Vis spectroscopy is an effective, reliable, sensitive and selective technique employed for the first identification of various types of nanoparticles (Liu *et al.,* 2021; Raj *et al.,* 2024). Fig. 3(A) illustrates the UV-Vis absorption spectrum of silver nanoparticles (AgNPs) at 380

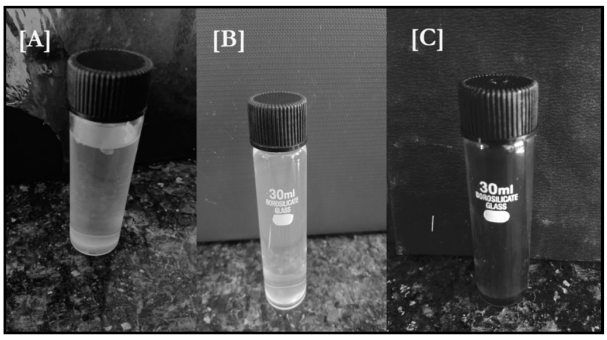

Fig. 2. Preliminary synthesis: (A) Yellow colour from aqueous leaf extract of *Aloe vera,* (B) White colour of $AgNO_3$ and (C) Dark brown colour change indicating the formation of silver nanoparticles.

nm for 1.5 mM salt concentration. Similar intensities in this region were also seen for different concentrations thus indicating the presence of AgNPs in the reaction mixture. The absorption peaks were identified at various time intervals as shown in Fig. 3(B). The highest absorption peak was seen at 7 h of incubation at 410 nm. Figure 3(C) exhibits the effects of temperature on synthesis of silver nanoparticles. The absorption in the visible spectrum progressively increases from room temperature to 70°C at 420 nm. This illustrates that the absorption intensity showing red-shift as a result of the increase in particle size over time with change in temperature of the solution. The free electron oscillates between the conduction band and the valence band, generating a spectrum of absorption peaks as a result of the mass oscillation of electrons in silver nanoparticles resonating with the optical wave at surface plasmon resonance (SPR). Fig. 3(D) indicates the stable synthesis of silver nanoparticles at variable pH. At pH 11, the maximum absorbance was seen at 420 nm as it promoted the reduction of silver ions (Ag^+) to form nanoparticles. This alkaline pH favoured the deprotonation of certain secondary metabolites present in *Aloe vera* aqueous leaf extract, which have then acted as reducing agents to convert Ag^+ to Ag^0 (metallic silver). Additionally, the alkaline pH influenced the size, shape and stability of the resulting silver nanoparticles (Singh *et al.,* 2022).

The biofunctionalized silver nanoparticles synthesized at 1.5 mM salt concentration at

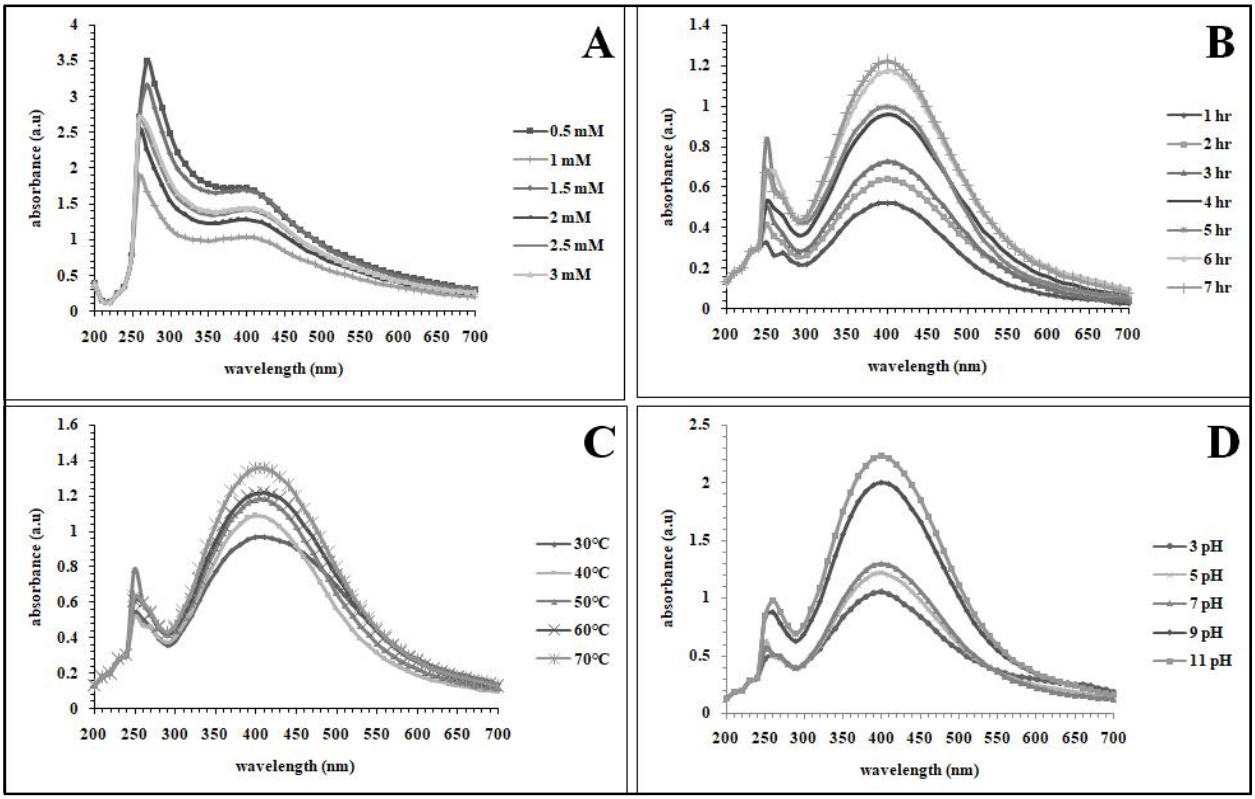

Fig. 3. Absorption spectra of AgNPs: (A) At different concentrations of $AgNO_3$ solution, (B) At different time intervals for $AgNO_3$ solution, (C) At different temperature for $AgNO_3$ solution and (D) At different pH $AgNO_3$ solution.

70°C and pH 11 were characterized for particle size distribution. The maximum quantity and maximum amplitude recorded illuminated the dimensions and size distribution of silver nanoparticles. The determined particle size distribution by intensity was seen within the 10-150 nm range (Fig. 4A). The average particle size was approximately 49.63 nm, with some particles exhibiting sizes ranging from 10 to 150 nm. The polydispersity index (PDI) was 20.7. Fig. 4B depicts the zeta potential graphs of the synthesized AgNPs. The zeta potential of the nanoparticles is -21.6 mV. This value signifies that the particles possessed a negative surface charge. The existence of an anionic surface charge indicates that this chemical is capable of interacting with other molecules.

The shape of AgNPs was examined using HR-TEM. Fig. 5 depicts a combination of plates (spherical and hexagonal) and spheres. Representative TEM pictures indicated that the size distribution of AgNPs ranged from 40 to 100 nm. The high-resolution TEM revealed distinct lattice fringes on the surfaces of the particles.

The FTIR spectra were utilized to determine the photochemical constituents in *Aloe vera* leaf extract and leaf extract-mediated synthesized AgNPs as shown in Fig. 6A and 6B. The maximum transmittance at 3362/cm is displayed to the presence of –OH stretching responsible for capping and reducing agent. Highly conjugated carboxylic functional groups were identified at the absorption band of 1975/cm. The peak at 568/cm confirmed the production of AgNPs, corresponding to the –OH phenolic bond. Consequently, the fingerprint indicates that the bonds associated with aromatic compounds, ethers and polyphenols serve as bioactive constituents that function as reducing and stabilizing agents for AgNPs mediated by *Aloe vera* leaf extract. The absorption peak exhibited a little shift from *Aloe vera* leaf extract to AgNPs due to nanoparticle production. Table 1 illustrates the many bioactive functional groups that validate the conversion of Ag^{+} ions to AgNPs (Singh *et al.,* 2021; Raj *et al.,* 2024).

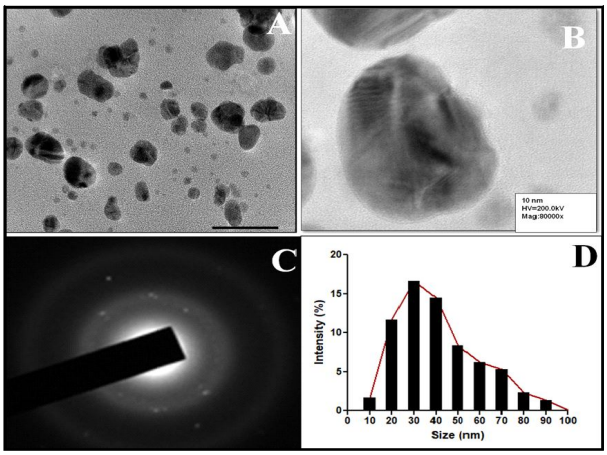

Fig. 5. (A and B) Transmission electron microscopy (TEM) images, (C) SAED images and (D) Histogram distribution plot of silver nanoparticles.

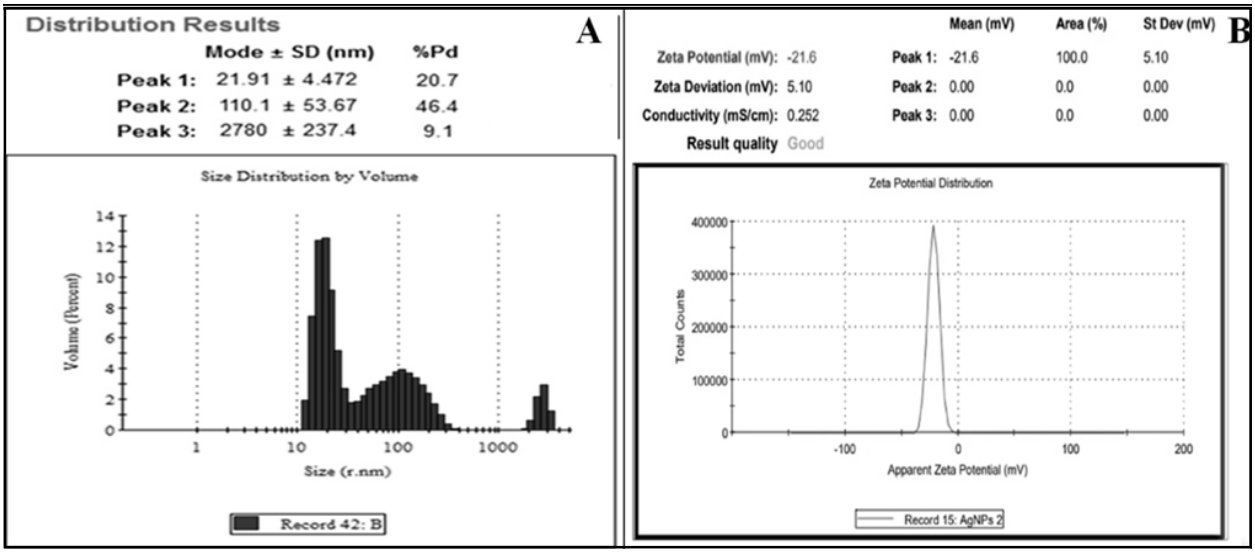

Fig. 4. (A) Size distribution pattern of AgNPs using DLS technique and (B) Indicates the measured zeta potential value of AgNPs in the dispersed medium (-21.6 mV).

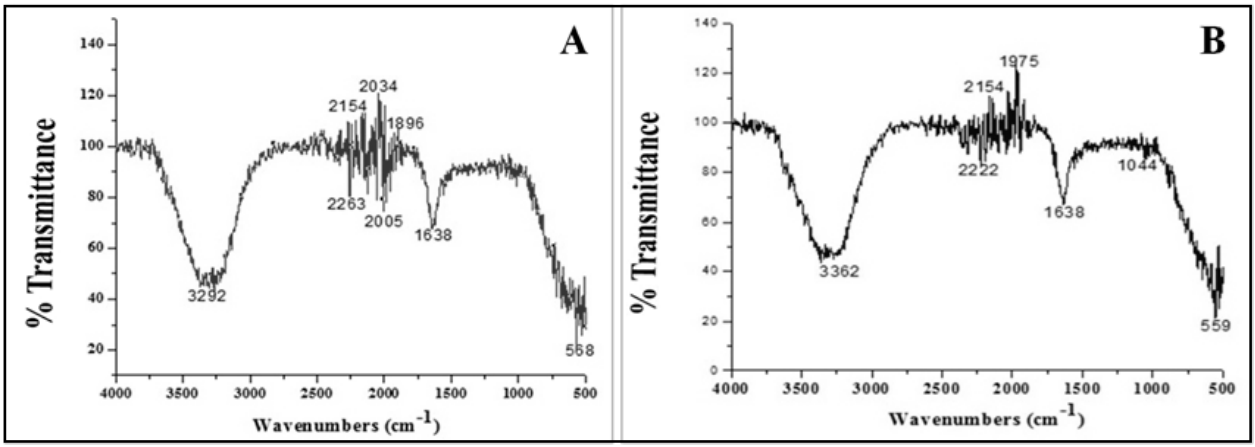

Fig. 6. FTIR spectra (A) *Aloe vera* extract and (B) Aqueous leaf extract of AgNPs.

Table 1. Functional group at different characteristics absorptions of FT-IR data cm.

Sample name	O–H (stretching)	K-H (stretching)	C=C (stretching)	–C–O (stretching)	O–H (stretching)
Leaf extract	3292	2263	2034	-	568
AgNPs	3362	2222	1975	1044	559

Energy dispersive X-ray spectroscopy (EDX) profiling of the sample was investigated for confirmed synthesis. It showed the presence of strong silver signal along with weak oxygen, carbon and copper peaks, which may have originated from the biomolecules bound to the surface of the silver nanoparticles (Fig. 7). However, the signal of copper peak was also strong, most likely due to background from supporting copper grids. The presences of these signals are the fingerprints in the elements of the sample (Table 2).

Table 2. Energy dispersive X-ray (EDX) qualification results of Av-AgNPs

Element	Line type	Weight (%)	Atomic (%)
C	K series	53.88	52.19
O	K series	1.66	4.60
Cu	K series	14.16	21.10
Ag	K series	30.30	22.11
Total		100.00	100.00

The antibacterial evaluation of both the nanoparticles and silver nanoparticles immobilized with drugs (Chloramphenicol and Gentamycin) were tested against the MDR strains of Gram positive (*S. aureus* and *Bacillus subtilis*) and Gram negative (*E. coli* and *Pseudomonas aeruginosa*) bacteria. The results are summarized in Figs. 8 and 9. Nanoparticles (NPs) can be regarded as efficient antibacterial

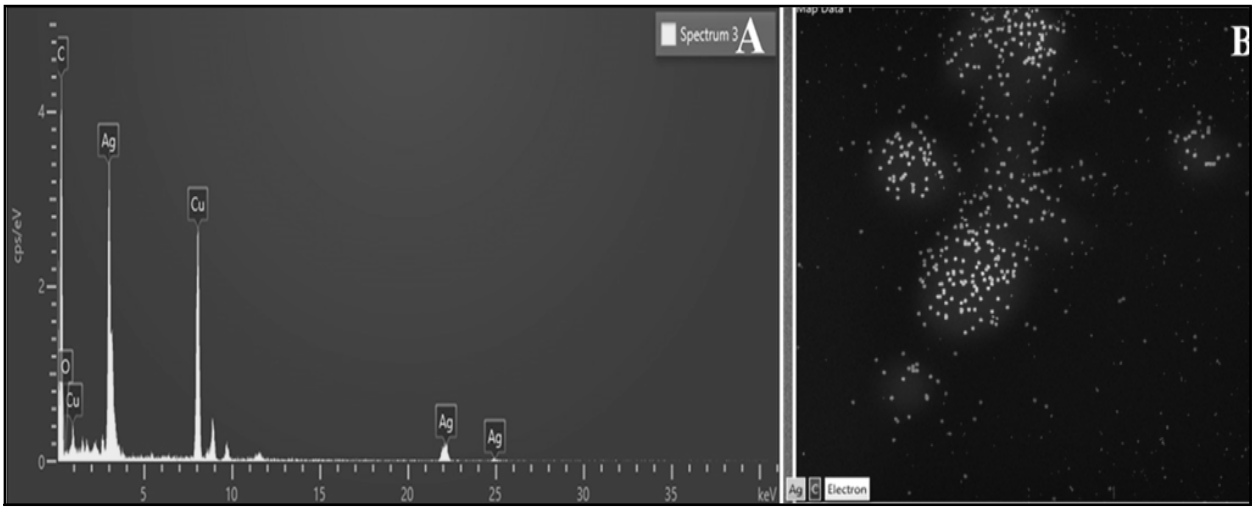

Fig. 7. Energy dispersive X-ray (EDX) spectrum of Av-AgNPs.

agents when they demonstrate a substantial zone of inhibition (ZOI) against bacteria (Gangwar and Joseph, 2024). The well diffusion approach was utilized to assess the zone of inhibition of *Aloe vera* assisted AgNPs (Yaseen *et al.,* 2024). A UV-visible spectrophotometer (JASCO V-600, Japan) was employed to adjust the optical density (OD 0.1) of the bacterial culture. The reduction in the microbial growth was measured and all the pathogens were found sensitive to silver nanoparticle and drug conjugated silver nanoparticles. Interestingly, the biofabricated silver nanoparticle with gentamycin showed significant reduction of pathogens (Fig. 8) and silver nanoparticle with chloramphenicol showed highly significant reduction (Fig. 9) despite the fact that the concentration of AgNPs with drug was very less (10 µg/ml) in 1:1 ratio. The NPs, immobilized in drug suspension, were found to be potent against *S. aureus*, as evident from the zone of inhibition values. The surface chemistry and size distribution of nanometer-scale metallic ions facilitated our examination of the efficacy of silver nanoparticles as drug carriers.

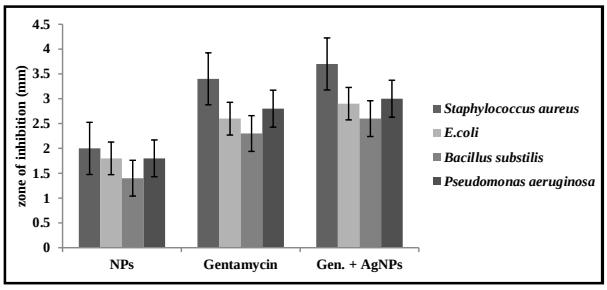

Fig. 8. Histogram of zone of inhibition for gentamycin drug mixed with AgNPs against the test bacteria.

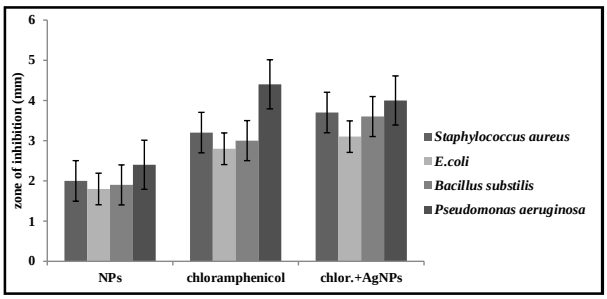

Fig. 9. Histogram of zone of inhibition for chloramphenicol drug mixed with AgNPs against the test bacteria.

CONCLUSION

The biosynthesis of AgNPs represented a cost-efficient and eco-friendly method. The AgNPs were optimized under various physico-chemical parameters (molar concentration, incubation time temperature and pH). The UV-Vis spectra of the synthesized products exhibited maximal absorption at 420 nm, confirming the generation of the desired AgNPs. The other characterization method such as DLS, FTIR, TEM and EDX confirmed the formation and presence of spherical shaped silver nanoparticle with average size range of about 40-100 nm. The biofabricated silver nanoparticle with gentamycin and chloramphenicol showed significant reduction in bacterial growth in comparison to drug and silver nanoparticle alone when tested against MDR strains of Gram positive and Gram negative bacteria. This method of immobilization could act as an efficient drug carrier that could act as a potent probe for developing drug delivery system in the near future.

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