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BIOSYNTHESIS, CHARACTERIZATION, COMPARATIVE AND SYNERGISTIC ACTIVITY OF SILVER NANO PARTICLES FROM BACILLUS SPECIES AGAINST HUMAN PATHOGENS

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ABSTRACT

Nanotechnology is useful in diagnostic techniques, drug delivery, sunscreens, antimicrobial bandage, and disinfectants. In the present investigate work synthesis of silver nanoparticles was prepared by biological method by reducing silver nitrate using the bacteria *Bacillus* species. The construction of nanoparticles was established by UV-visible spectroscopy with a maximum absorbance at 434nm, characteristic of silver nanoparticles. Further characterization of XRD and FESEM was used to determine the metallic character of AgNPs as well as morphological structure of AgNPs. The dimension of AgNPs is 41.13nm. The antimicrobial activity was done by disc diffusion method. Different concentrations of AgNPs was added to the plate and the concentration which gave maximum value is selected. Comparative analysis of synthesized AgNPs was done with water, AgNO3, extracted sample and AgNPs. Further synergetic activity was done to confirm the antimicrobial activity of AgNPs. AgNPs shows good antimicrobial activity against human pathogens so it can be used in drug delivery system.

KEYWORDS : ***Bacillus species, silver nanoparticles, $AgNO_3$, human pathogens, nanotechnology***

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INTRODUCTION

In recent trends, the enriched development in the field of nanotechnology has provided a wide range of application in fields of medicine, computer, bio-sciences and information technology. Formulation of nanomaterials based antimicrobial agents has received much attention due to its unique and physiochemical and biological properties due to its size effect and surface volume. A huge number of studies have been focused on the preparation of antibacterial activity using nanomaterials and nanocomposities [1-5.]—Among the nanomaterials, Silver nanoparticles (AgNPs) have shown very unusual biological activities. AgNPs possess both high electrical and thermal conductivity have a large surface area for Raman dispersion, catalytic activity, non-linear behaviour and prominent antibacterial properties, cause mainly by their large surfaces area to volume ratio. Moreover, AgNPs can be produced using different synthetic methodologies, thus providing specific morphologies and unique characteristics. Consequently AgNPs are arising as new bacteriostatic agents, because they are comparable in efficacy and even more potent antimicrobial compounds than conventional antibiotics. There are diverse compounds comprising Silver, such as materials containing ionic silver (Ag+) or metallic silver (Ag0), have been recently synthesized and demonstrated antibacterial activity against Gram negative bacteria such as *Escherichia coli* [6-10] .Microorganisms, such as bacteria in the living environment are often pathogenic and cause severe infections in human beings. There is a imperative necessitate to investigate for new antimicrobial agents from innate and inert substances. Among inert antimicrobial agents, silver has been in use most extensively since prehistoric times to struggle infections. The antibacterial activity of silver, silver ions, and Silver compounds has been thoroughly investigated[13-15.] Nanotechnology is the most active research arena in contemporary materials science. Nanoparticles reveal novel or enhanced properties based on specific characteristics such as size, distribution and morphology. There have been reproducible developments in the field of nanotechnology in the recent years, with various methodologies developed to synthesize nanoparticles of particular shape and size depending on specific requirements, applications of nanoparticles and nanomaterials. Nanotechnology can be termed as the synthesis, characterization, searching and application of Nano sized (1-100nm) materials for the development of science. It deals with the materials whose structures exhibit much novel and improved physical, chemical, and biological property, and functionally due to their Nano scaled size. Because of their size, nanoparticles have a larger surface area than macro-sized materials. Nanoparticles, because of their small size, have distinctive properties compared to the bulk form of the same material, thus offering many new developments in the field of biosensors, biomedicine and bio nanotechnology. Nanotechnology is also being utilized in medicine for diagnosis, therapeutic drug delivery and the development of treatments for abundant disorders. Nanotechnology is an enormously potent technology, which holds a huge pledge for the design and development of many types of novel products with its possible medical applications on early disease detection, treatment, and impediment. In the present revise silver nanoparticles was synthesized by using Bacillus spp. followed by characterization and antimicrobial activity of silver nanoparticles against test pathogens.

MATERIALS AND METHODS

Synthesis of silver nanoparticles

Media was prepared as per the procedure of manufacturing company (Hi-media). Sterilization for 15 mins at 121° Celsius. After sterilization, allow the broth to arrive at room temperature 35-37° Celsius. Inoculate broth by microorganism (Bacillus species) under sterile conditions (laminar flow). Inoculation was followed by Incubation for 24hrs at 37° C in rotary shaker at 150 rpm followed by Centrifuge at 6000 rpm to separate pellet and supernatant. The Pellet is washed thrice with double distilled water to remove the media solutes and dissolved in 100ml of Milli Q water and incubate further for 72hrs. After 72 hours of incubation the cell free filtrate was treated with $AgNO_3$, which consist of extra cellular metabolites. The treated biomass was incubated further for 24 to 72hrs. The Color change from white to yellow after 72 hours of incubation indicates the formation of silver nanoparticles.

Characterization of silver nano particles

The silver nanoparticles synthesis was characterized by various analytical techniques UV Spectrophotometer and FTIR. Further the size and morphology was confirmed by FESEM FIELD EMISSION SCANNING ELECTRON MICROSCOPE and X Ray diffraction (XRD) .

Anti-microbial activity of synthesized silver nanoparticles by disc diffusion method

The antimicrobial activity of synthesized silver nanoparticle was done by disc diffusion Muller hinton agar media as per the procedure of manufacturing company. Pathogens tested are:*Staphylococcus aureus, Escherichia coli, Vibrio cholera and Proteus vulgaris w*ere procured from PGIBMS, Chennai.Inoculate the pathogens into the peptone water and incubate it for 2-3 hrs at 37°C. After sterilization, allow the media to become cool and pour 15-20 ml of MHA in Petri plates under the sterile conditions (laminar flow). 4 discs are placed on each plate and marked as 1, 2, 3, 4 and 5. Different concentrations of AgNPs is added to the discs and marked as 1,2,3,4 respectively and 5th disc is +ve control (antibiotic). Equal concentrations of distilled water AgNO3, AgNPs, and bacterial extract without treated with AgNo3 were added to Disk 1, 2, 3, 4 respectively & 5th disk is +ve control(antibiotics). Synergetic activity of antibiotic and AgNPs was done to determine the efficiency of AgNPs. Zone of inhibition was measured to determine the efficiency of the synthesized silver nanoparticles and their comparative analysis after 24 hrs.

RESULTS AND DISCUSSION

Biosynthesis of silver nanoparticles

The *Bacillus* species isolated and was preferred in the current study for the potential biosynthesis of silver nanoparticles. The silver nanoparticles were synthesized by biological process where the solution is treated with the silver nitrate and the color is changed from white to yellowish color after 48 hrs of incubation. The changes into dark yellow color, indicates that the solution is reduced by silver nitrate into the silver nanoparticles (fig 1a, 1b). The produced silver nanoparticles from *Bacillus species* the color change to dark yellow indicated that the silver nitrate is reduced which was co-relating with the work [16].

Figure 1a
Before the addition of $AgNO_3$

Figure 1b
After the addition of $AgNO_3$

Characterization of silver nanoparticles

Uv-vis spectroscopy

The Biologically synthesized silver nanoparticles using *Bacillus* species was monitored by UV-Vis Spectroscopy. The bio diminution of pure AgNPs is monitored after 48hrs. 0.1 ml of sample is taken and diluted with Millipore water and the absorption peak was measured after 48hrs of incubation. The UV-Vis Spectrum of Silver nanoparticles produced by *Bacillus* species exhibits absorption band at 438nm, which is typical plasma band suggesting the formation of Silver nanoparticles (Fig. 2) which was accordance with the findings[16] in their study and the absorption range of silver nanoparticles was seen around 400-440 nm.

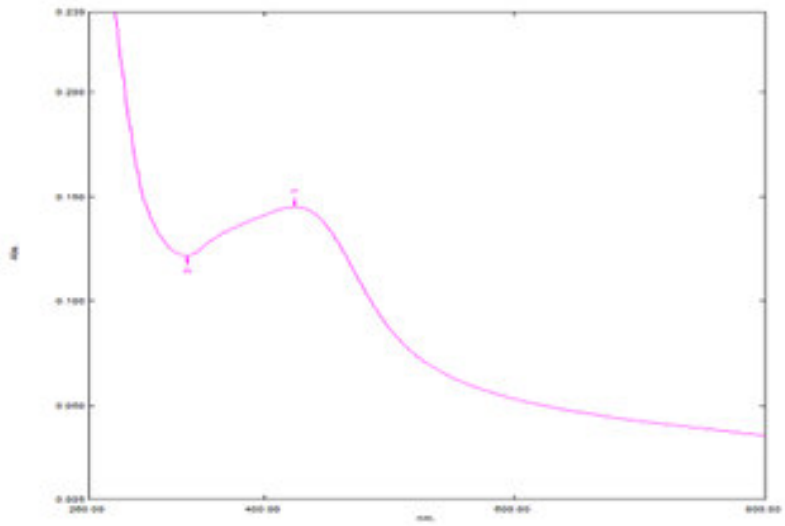

Figure 2
UV-visible absorption spectra of reduction of silver ions into silver nanoparticles using Bacillus species shows absorption peak at 438nm

X-ray diffraction

The graph (fig. 3) shows intelligent XRD peaks of Ag particles indicating its crystallinity. The peaks in XRD pattern (Table 1) can be indexed to a Face-centered cubic structure of silver nanoparticles which co-relates the research findings[16].

Table 1
XRD analysis of synthesized nanoparticles

No.	2 – theta(deg)	d(ang)	Height (cps)	FWHM(deg)	Int.I(cps eg)	Int.W(deg)	Asym. factor
1	27.86(2)	3.200(2)	236(24)	0.391(18)	117(4)	0.49(7)	1.0(2)
2	32.296(11)	2.7697(9)	593(39)	0.370(9)	290(4)	0.49(4)	1.37(18)
3	38.4(5)	2.34(3)	10(5)	2.8(5)	29(7)	3(2)	0.6(5)
4	46.24(2)	1.9619(8)	349(30)	0.459(16)	212(4)	0.61(6)	0.90(16)
5	54.80(4)	1.6738(11)	96(15)	0.47(3)	54(3)	0.56(12)	0.8(3)
6	57.46(4)	1.6026(10)	84(14)	0.53(3)	47(3)	0.57(13)	0.9(3)
7	76.63(3)	1.2424(5)	88(15)	0.38(7)	57(3)	0.65(14)	0.4(2)

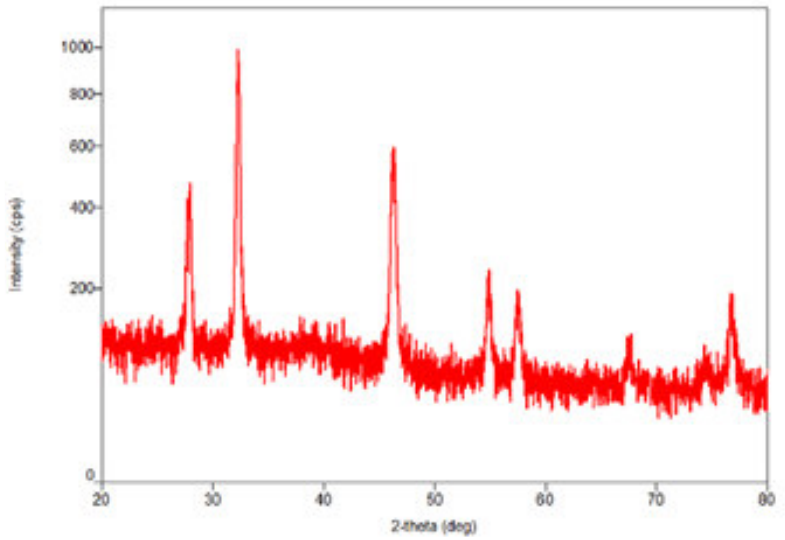

Figure 3
XRD analysis of synthesized silver nanoparticles

FESEM

FESEM usually reveals the relatively uniform roundish morphology. The sizes of particles (fig 4) are approximated to be in range of 41.13 nm to 200 nm and agglomeration of the Nano size crystallite can be seen quite clearly which was co-relating with[17].

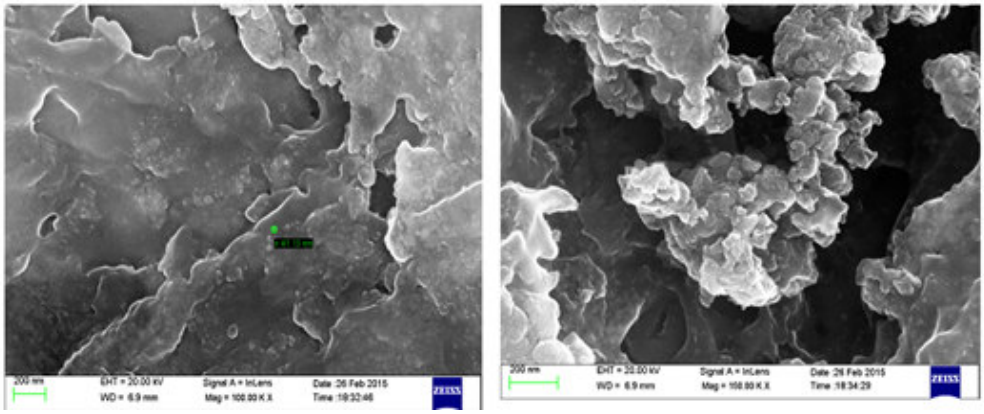

Figure 4
FESEM of biosynthesised AgNPs by Bacillus species (Scale bar 200nm)

EDAX

The formation of the biosynthesized AgNPs was examined by EDAX combined with FESEM. The appearance of signals (fig. 5) was liable due to X-ray emission from carbohydrates, proteins, enzymes nearby in the cell wall of the biomass. Through this graph it confirms the presence of biologically synthesized silver through EDAX which was correlated with the findings[18].

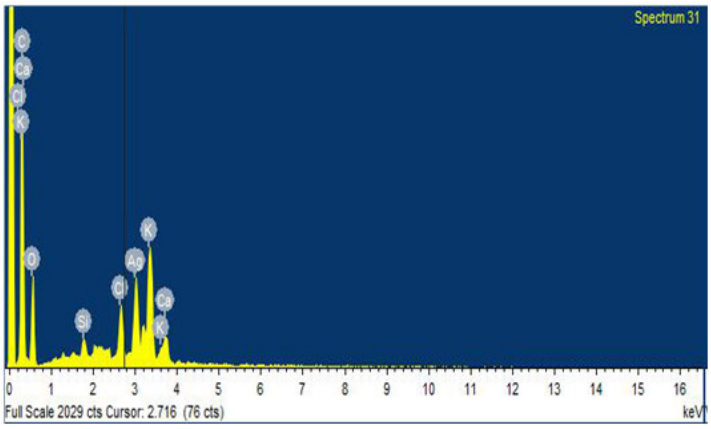

Figure 5
EDAX of biosynthesized AgNPs by Bacillus species

Anti-bacterial activity of silver nanoparticles against human pathogens

Silver nanoparticles synthesized from *Bacillus* species were assayed for the potential antibacterial activity against clinically isolated human pathogens. Different Human pathogens were grown on agar plates in order to check the antibacterial activity of silver nanoparticles at different concentrations (10µl, 15µl, 20µl, 25µl) in comparison with the positive control Amoxycillin 10mcg/disc**.** If the bacteria are susceptible to sample, an area of cleaning surrounds the disk where bacteria are not capable of growing (Zone of Inhibition). This is used to estimate the bacteria's sensitivity towards sample and compared with control. Good zone of inhibition is visible for different pathogens like *staphylococcus aureus, Escherichia coli, Bacillus cereus, Vibrio cholera and Proteus vulgaris.* Antibacterial activity of synthesized silver nanoparticles was made against human pathogens by diverse concentration such as 10µl, 15µl, 20µl and 25µl and Amoxycillin was used as a control by disc diffusion method that is Kirby Baver method (Table 2). The test pathogens are *S. aureus*, *V.cholerae, E.coli, P. vulgaris* and *B.cereus*. Among the five pathogens tested, the maximum antimicrobial activity was shown by *Vibrio cholera* (24mm) followed by *S. aureus*, (22mm),*Bacillus cereus (21 mm) and E coli(20mm) and P. vulgaris* (20mm) fig.6. These studies were correlating with the work[18] and 25µl have shown good results and were further processed for synergistic activity with commercial antibiotics.

Table 2
Antibacterial activity of silver nanoparticles against Human pathogens and its zone of inhibition

Human pathogens	10µl (AgNPs)	15µl (AgNPs)	20µl (AgNPs)	25µl (AgNPs)	Antibiotic (Amoxycillin)
Staphylococcusaureus	3±0.9	_	10±1	22±0.8	-
Vibrio cholerae	7±0.8	10±0.9	15±1	24±0.7	12±1
Escherichia coli	-	-	16±0.9	20±1	8±0.9
Bacillus cereus	7±0.7	12±0.9	11±0.8	21±1	12±.9
Proteus vulgaris	3±1	11±0.8	17±0.7	20±0.9	8±0.8

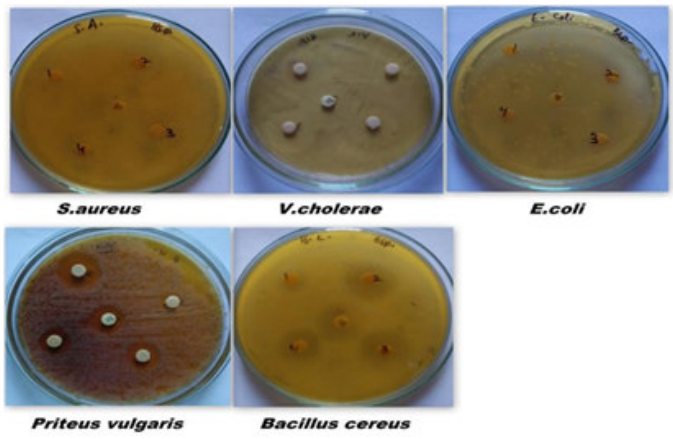

Figure 6
Antibacterial activity of silver nanoparticles from Bacillus species ranging in various concentrations Comparative activity

Antibacterial activity of AgNPs (Table 3) shows the good result against human pathogens while comparing with metabolic extract without the addition ofAgNO3. Then it was compared with antibiotic (Amoxycillin) but still AgNPs (fig. 7) showed the good result against human pathogens. [1. AgNPs 10µl 2. AgNPs 15 µl, 3. AgNPs 20µl, 4. AgNPs 25µl, 5. Amoxycillin 25µl].

Table 3
Zone of inhibition of AgNPs synthesized from Bacillus species and their comparative analysis withH2O, AgNO3, Metabolic Extract, AgNPs and antibiotic

Human pathogens	Distilled water	AgNO3	*Metabolic Extract*	AgNPs	Antibiotic (Amoxycillin)
Bacillus cereus	-	16±1	7±1	25±0.8	15±1
Escherichia coli	-	18±1	10±0.9	25±0.9	14±0.7
Staphylococcusaureus	-	16±0.9	6±0.8	23±1	12±0.8
Vibrio cholera	-	18±1	-	25±0.8	14±0.8
Proteusvulgaris	-	15±0.7	7±0.9	21±0.7	11±1

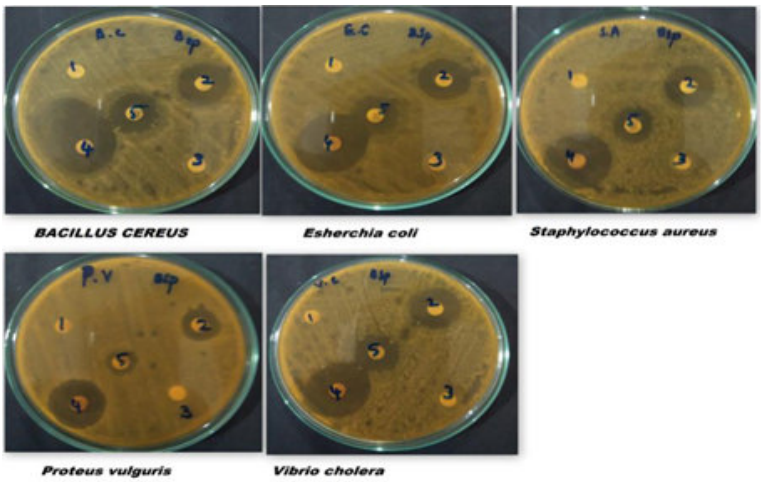

Figure 7
Zone of inhibition of AgNPs synthesized from Bacillus species and their comparative analysis withH2O, AgNO3, Metabolic Extract, AgNPs and antibiotic

Synergistic activity
Synergistic Activity of antibiotics and combination of antibiotics with AgNPs has been performed. We have used standard antibiotics such as Amoxicillin, Moxifloxacin, Streptomycin and Ampicillin. Later we added the AgNPs to the antibiotic (A1, B1, C1 and D1) in the concentration of 25µl, (Table 4) and then we found that the activity was efficient. The zone size ranged from 20 to 40 mm and exhibited efficient antimicrobial activity (fig. 8). The present study report was correlated with the study[18].

Table 4
Synergistic activity of AgNPs from Bacillus species

Human pathogens	Amox (10mcg)	Amox +AgNPs	Moxi (5mcg)	Moxi +AgNPs	Amp (10mcg)	Amp +AgNPs	Stp (10mcg)	Stp +AgNPs	AgNPs
Bacillus cereus	15±1	25±1	25±0.9	34±0.7	6±1	18±1	15±0.7	20±0.9	13
Staphylococcus aureus	11±0.9	20±1	25±0.7	39±1	24±0.8	27±0.9	21±1	25±1	17
Vibrio cholera	22±0.8	30±0.8	28±1	34±0.8	21±1	25±0.8	22±1	25±0.8	16
Escherichia coli	11±1	20±0.7	25±1	35±0.9	25±0.7	29±0.9	22±0.9	25±1	14
Proteus vulgaris	29±0.8	0±0.9	20±0.7	36±0.9	6±0.9	22±1	29±0.8	31±0.9	15

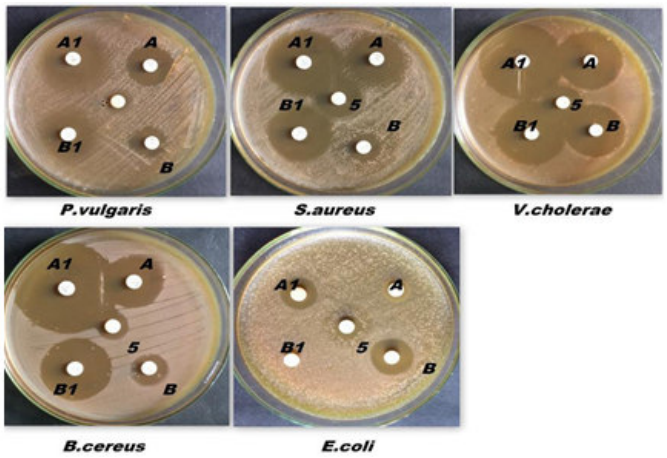

Figure 8
Synergistic activity of AgNPs synthesized from Bacillus species [A. Amoxycillin 10 mcg A1. Amoxycillin + AgNPs, B. Moxifloxacin 5 mcg, B1 . Moxifloxacin + AgNPs, 5. AgNPs 25 µl]

SUMMARY AND CONCLUSION

In present study AgNPs was produced from *Bacillus species* that is by biological method. The synthesized AgNPs was characterized to conform the produced materials consists of nanoparticles by various analytical methods such as UV-Visible spectroscopy, FTIR, XRD, FE-SEM and EDAX. In UV-Visible spectroscopy the absorption range was found to be around 432 nm. This indicated the presence of nanoparticles. Further it was confirmed by various parameters such as FTIR, XRD, FE-SEM and EDAX. In FESEM the absorption is found to be 41.13 nm. The Characterization confirmed the produced material is only AgNPs. These silver nanoparticles were subjected to antimicrobial activity to determine that the nanoparticles produced biologically from *Bacillus species* exhibits efficient antibacterial activity against human pathogens. The antimicrobial activity of AgNPs was determined by three different ways. One by adding AgNPs in various concentrations (10-25µl), second antimicrobial activity was determined with distilled Water, AgNO3, bacterial extract and AgNPs. Third by synergistic activity that is combining the AgNPs with standard antibiotics such as Amoxycillin, Moxifloxacin, Ampicillin and Streptomycin with the concentration of 25µl. Among all the 3 different antibacterial activity of AgNPs, synergistic activity of AgNPs showed efficient antimicrobial activity. In the antibacterial activity with AgNPs the zone of inhibition was around the range 10 nm but the synergistic activity the zone of inhibition around the range 15 nm. From these above results it was concluded that the AgNPs was synthesized by biological method was combined with standard antibiotics with the ratio of (10 mcg+25 µl and 5 mcg+25 µl) exhibited efficient antimicrobial activity when compared with AgNPs itself. So the synergistic activity of AgNPs method is applied in pharmaceutical industries after better toxicity testing and can be implemented for many multidrug resistance organisms in future studies.

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CONFLICT OF INTEREST

Conflict of interest declared none.

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