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Research Article

Impact of Silver Nanoparticles on Pathogenic Bacteria

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ABSTRACT

Present study aimed was to synthesized AgNPs and investigate its antibacterial activity against different pathogenic bacteria. AgNPs was synthesized by polyol method. The characterizations of nanoparticles were done by XRD and FTIR method. Antibacterial activity was analyzed by disc diffusion and well diffusion method. It was found that prepared AgNPs were effective antibacterial activity. Silver nanoparticles (AgNPs) dissolved in three different solvent like Water, n-Hexane and 70% ethanol. All three different solvent with silver nanoparticles showed the zone of inhibition against bacteria like *E.coli, S.typhi, and S.aureus.* Maximum zone of inhibition was observed with *S.typhi* against in Silver Nanoparticles with 70% ethanol at 0.5 gm concentration. Minimum zone of inhibition showed by *S.aureus* against Silver Nanoparticles in water at 0.1 gm concentration. Zone of inhibition increased with increase in concentration of AgNPs. "Therefore the use of AgNPs in development of antibiotics for the treatment of different bacterial infection is novel approach in nanobiotechnology".

Keywords: AgNPs, Antibacterial activity,*E.coli, S.typhi and S.aureus.*

INTRODUCTION

Nanotechnology is a kind of technology deals with the design, synthesis and manipulation of structure of particles with the dimension smaller than 100nm. Now-a-days nanotechnology has dynamically developed as an important field, of modern research with potential effects in electronic and medicine[1]. Inorganic antibacterial factors have a very high bacterial resistance and thermal stability[2]. Nanobiotechnology represents an economic and eco friendly alternative for chemical and physical methods of nanoparticles synthesis. In the last two decades, a number of nanoparticles based therapeutic and diagnostic agents have been developed for the treatment of life threatening diseases such as cancer, diabetes, asthma, allergy and infectious diseases. The most important anticipated application in medicine includes drug delivery, both *In-vitro* and *In-vivo* diagnostics[1]. Silver in the form of nanoparticles may therefore be more reactive due to its catalytic properties and becomes more toxic to bacteria than silver ions[3].

Looking to importance of nanoparticles in various fields. The present investigation "Impact of Silver Nanoparticles on different microorganisms" was undertaken to find out the effect of nanoparticles against different pathogenic bacteria.

MATERIAL AND METHODOLOGY

Bacterial species –The following bacterial species were selected for present investigation:-

Escherichia Coli, *Staphylococcus aureus*, *Salmonella typhi.*

Synthesis of Silver Nanoparticles.
Silver nanoparticles were synthesized by polyol method[4].

Antibacterial test
Antibacterial tests were performed by disc diffusion and well diffusion method[5-6].

Media preparation:
The following media were used for microbiological study.

- Nutrient agar for *E.coli*and *S.typhi*
- Manittol salt agar for *S.aureus.*

RESULTS

XRD OF SILVER
The XRD pattern of the compacted AgNPs sample is presented in fig-1. The diffraction pattern mainly exhibited five peaks at 38.33°, 43.93°,64.39°,77.74° and 81.40° in a 2θ scale, which can be indexed to (111), (200) , (220),(311)and(222) reflections of fcc silver, indicating cubic phase of silver metal (JCPDS File: PDF# 040836). No other AgO or Ag_2O impurity peaks were observed in the spectra, suggesting that the synthesized particles were of high purity. It is known that silver nanoparticles rapidly oxidize on exposure to the atmosphere, which can result in particle aggregation[7] and could affect the antimicrobial properties of AgNPs.Scherrer equation was used to calculate crystallite size giving approximately 5.9891nm and lattice constant a was 3.611 Å.

FTIR of Silver Nanoparticles
FTIR analysis of silvernanoparticles showed different stretches of bonds visible at different peaks; 3437.13—N–H stretch, 2919.36—single aldehyde, 2855.61—C–H; O–H, 2337.84—C≡C, 1643.38—C=C, and 1110.05—C=O. The similar peak was observed by Markova which confirms the formation of silver nanoparticles[8].

Antibacterial activity of Silver nanoparticles.
The antibacterial activity of Silver nanoparticles were tested on the basis of disc diffusion and well diffusion methods. The results are summarized in tables 1-3 and presented in graphs 1-3.

Disc diffusion
In negative control water, N-Hexane and 70% ethanol was applied in disc of cultured petriplates. There were no inhibition zones against *E.coli*, *S.aureus*and *S. typhi* bacteria.

In positive control (experiment) the prepared silver nanoparticles were used with different solvent (such as water, 70% ethanol and N-Hexane) and with different concentration (0.1gm, 0.2gm, 0.3gm, 0.4gm, 0.5gm).They were applied over on bacterial culture plate. All three solvent with nanoparticles showed the zone of inhibition.
In different concentration of AgNps+ Water(0.1gm, 0.2gm, 0.3gm, 0.4gm, 0.5gm).The zone of inhibition were of 0.6 cm,0.8 cm,0.9 cm,1.0 cm,1.2 cm against *E.coli* , 0.4 cm,0.6 cm, 0.9 cm,1.2 cm,1.4 cm against *S.aureus* and0.7 cm,1.0 cm,1.3cm,1.6 cm,1.9 cm against *S.typhi* respectively
In different concentration of AgNps + n-Hexane(0.1gm, 0.2gm, 0.3gm, 0.4gm, 0.5gm).The zone of inhibition were of 0.5 cm,0.8 cm,1.2 cm, 1.4 cm,1.5 cm against *E.coli* ,0.6 cm,0.9 cm, 1.3 cm,1.5 cm,1.7 cm against *S.aureus* and 0.8 cm,1.3 cm,1.5cm, 1.8 cm, 2.0 cm against *S.typhi* respectively
In different concentration of AgNps+70% Ethanol (0.1gm, 0.2gm, 0.3gm, 0.4gm, 0.5gm). The zone of inhibition were of 0.9 cm,1.2 cm, 2.1 cm, 2.5 cm,2.8 cm against *E.coli* ,0.7 cm,1.0 cm, 1.3 cm,1.6 cm,1.8 cm. against *S.aureus* and 0.9 cm,1.4 cm, 1.9 cm, 2.4 cm, 2.9cm against *S.typhi* respectively.
The maximum zone of inhibition(2.9cm) was showed by *S.typhi* against Silver Nanoparticles with 70 % ethanol and minimum zone of inhibition (0.4 cm) was showed by *S.aureus* against Silver Nanoparticles with water.

Well diffusion method
In negative control water, N-Hexane and 70% ethanol was applied in well of cultured petriplates. There was no inhibition zone against *E.coli*, *S.aureus* and *S. typhi*.
In positive control (experiment) the silver nanoparticles used with different solvent (such as water, N-Hexane and 70% ethanol) and with different concentration (0.1gm, 0.2gm, 0.3gm, 0.4gm, 0.5gm) applied in well of bacterial culture plate. All three solvent with nanoparticles shows the zone of inhibition.
The inhibition zone were of 0.4 cm, 0.6 cm, 0.9 cm, 1.1 cm,1.3 cm against *E.coli*, 0.3 cm,0.7 cm, 1.0 cm, 1.4 cm, 1.9 cm against *S.aureus* and0.4 cm,0.8 cm,1.3 cm,1.8 cm,2.2 cm against *S.typhi* with different concentration of AgNps + Water (0.1gm, 0.2gm, 0.3gm, 0.4gm, 0.5gm) respectively.
The inhibition zones were of 0.6 cm, 0.9 cm, 1.2 cm, 1.4 cm, 1.8 cm against *E.coli.* 0.5 cm, 0.8 cm, 1.0 cm,1.3 cm,1.5 cm against *S.aureus* and 0.6 cm, 1.0 cm, 1.5cm, 1.9 cm, 2.1 cm against *S.typhi* with different concentration of AgNps + n-Hexane (0.1gm, 0.2gm, 0.3gm, 0.4gm, 0.5gm) respectively.

The zone of inhibition was of 0.9 cm, 1.3 cm, 1.7 cm, 2.2 cm, 2.7 cm against *E.coli*. 0.8 cm, 1.2 cm, 1.6 cm, 1.9 cm, 2.3 cm against *S.aureus*and0.5 cm, 1.0 cm,1.6 cm, 2.2 cm,2.5 cm against *S.typhi* with different concentration of AgNps+70% Ethanol (0.1gm,0.2 gm,0.3 gm, 0.4 gm, 0.5 gm) respectively
The maximum zone of inhibition 7 cm showed by *E.coli* against Silver Nanoprticles with 70 %ethanol and minimum zone of inhibition 0.3 cm showed by *S.aeureus* against Silver Nanoparticles with water.

DISCUSSION

In the present investigation Silver Nanoparticles were prepared by thermal decomposition method. These nanoparticles were characterized by XRD and FTIR method, and their antibacterial activities against different bacteria were investigated with the help of disk diffusion and well diffusion method. Silver nanoparticles mainly exhibited five peaks at 38.33^{o}, 43.93^{o}, 64.39^{o}, 77.74^{o}and 81.40^{o} in a 2θ scale. Present XRD and FITR data corroborate with the previous scientists finding[7,8].
In disc diffusion method Silver nanoparticles with 70% Ehtanol showed the maximum zone of inhibition 2.9 cm against *S.typhi* and minimum zone of inhibition 0.4 cm against *S.aureus with* AgNps+ Water. In well diffusion method Silver nanoparticles +70% Ethanol showed the maximum zone of inhibition 2.7 cm against *S.typhi* and minimum zone of inhibition 0.3 cm against *S.aureus* with AgNps+ Water. Ghosh *et al.*,Ramyadevi *et al.*, Tran *et al.*, Jamaran*et al.*, Jaiswal*et al.*, Velázquez-Velázquez *et al.*, and Ansari *et al.*, found that nanoparticles have effective antibacterial activity[9-15]. It was observed that nanoparticles have penetrated inside the bacteria and have caused damage by interacting with phosphorus and sulfur containing compounds such as DNA[16]. It is believed that DNA may have loose its replication power and cellular proteins and become inactive after treatment with nanoparticles. In addition, nanoparticles may prevent the growth and cell division. The nanoparticles have an additional contribution to the bactericidal efficacy. Heavy metals are toxic and react with proteins, therefore they bind protein molecules, as a result cellular metabolism is inhibited causing death of microorganism[17].

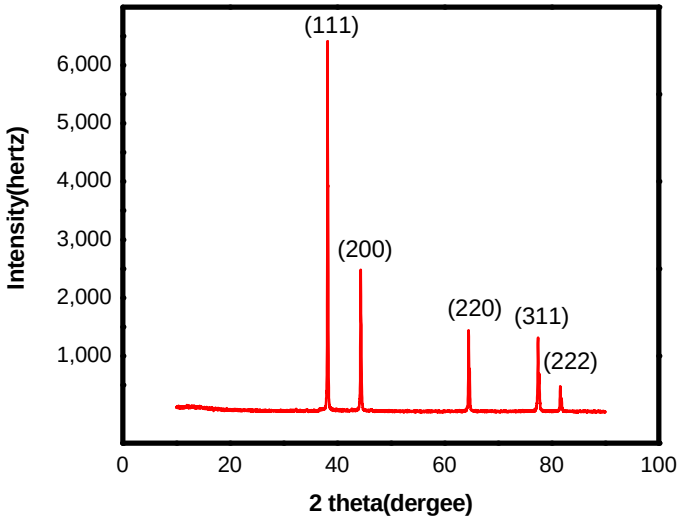

Fig 1
The XRD pattern of the Silver Nanoparticles.

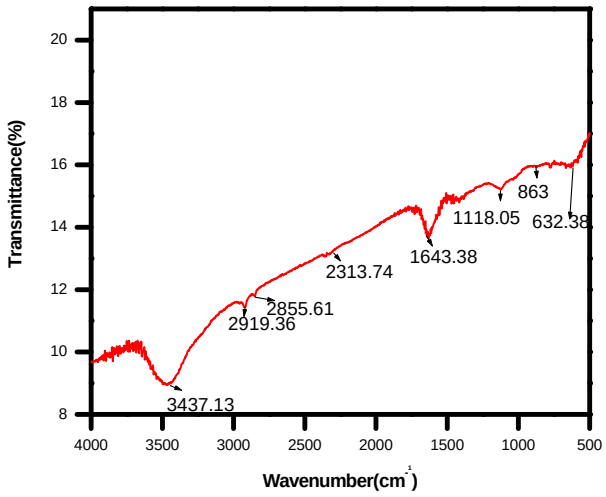

Fig 2
The FTIR analysis of the Silver Nanoparticles

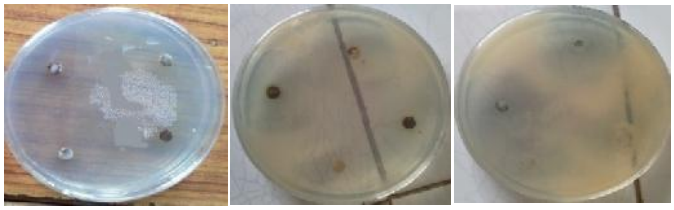

Fig-3 Fig-4 Fig-5

Fig 3-5
showing zone of inhibition against Silver nanoparticles with disc diffusion method

Fig-6 Fig-7 Fig-8

Fig 6-8
showing zone of inhibition against Silver nanoparticles with well diffusion method

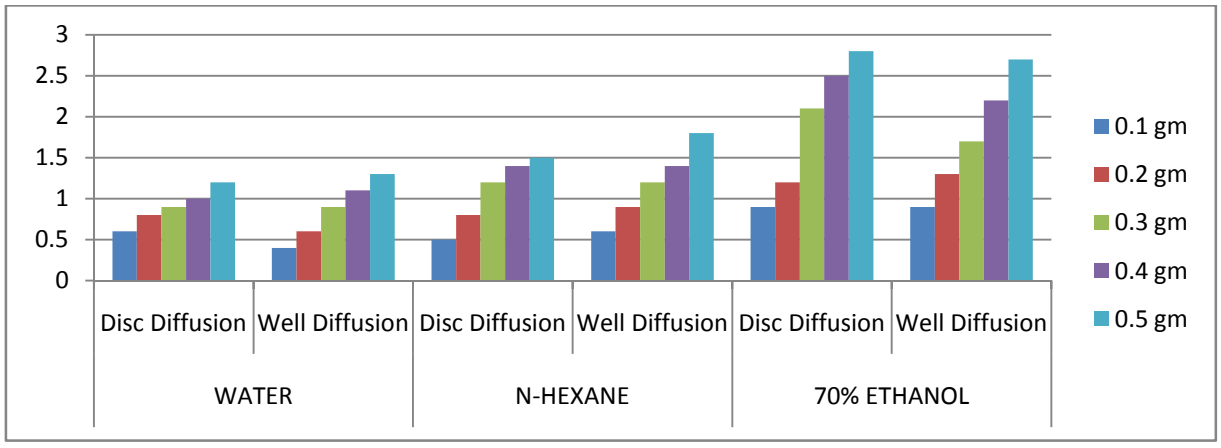

Figure 9
Antibacterial activity of water, n-Hexane and 70%ethanol + Silver nanoparticles against *E.coli* under disc diffusion and well diffusion method.

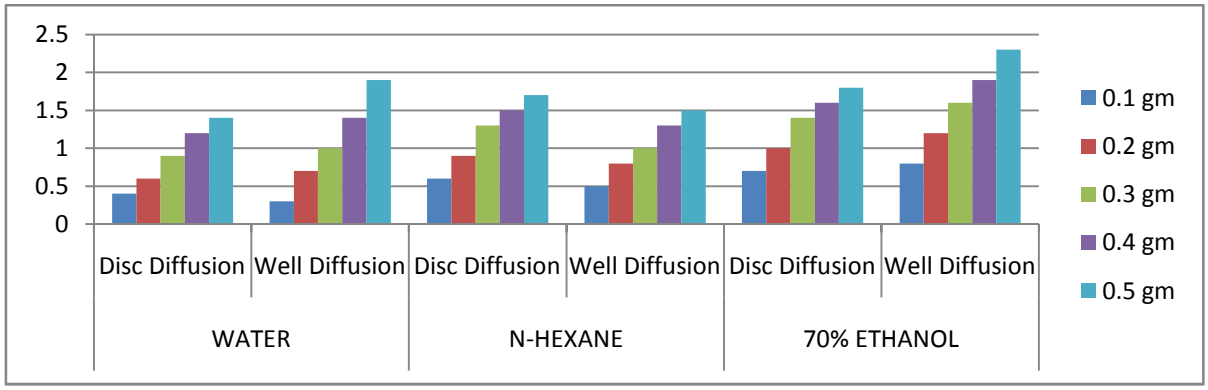

Figure 10
Antibacterial activity of water, n-Hexane and 70%ethanol + Silver nanoparticles against *S.aureus* under disc diffusion and well diffusion method

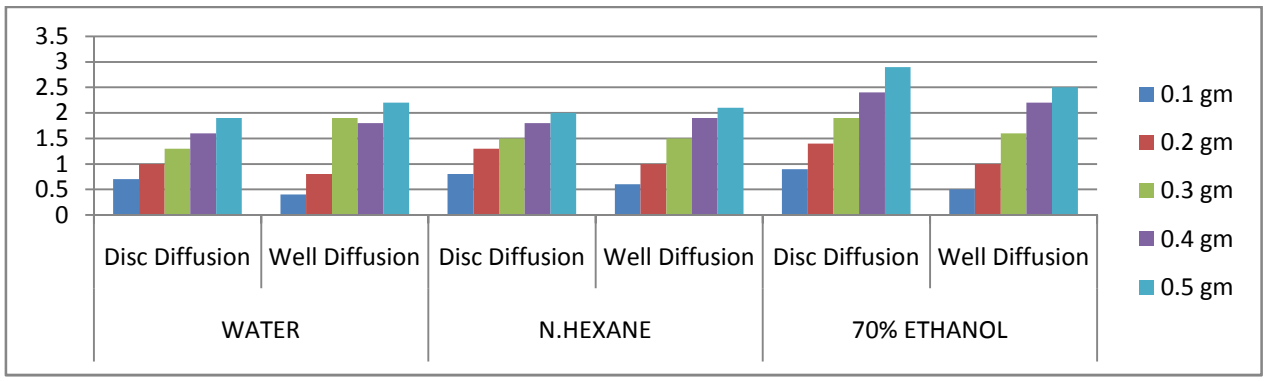

Figure 10
Antibacterial activity of water, n-Hexane and 70%ethanol + Silver nanoparticles against *S.typhi* under disc diffusion and well diffusion method.

Table 1
Antibacterial effect (zone of inhibition in radius) of Silver nanoparticles against *E.coli*

Conc of Nps	WATER		N-HEXANE		70% ETHANOL	
	Disc Diffusion	Well Diffusion	Disc Diffusion	Well Diffusion	Disc Diffusion	Well Diffusion
0.1 gm	0.6 cm	0.4 cm	0.5 cm	0.6 cm	0.9 cm	0.9 cm
0.2 gm	0.8 cm	0.6 cm	0.8 cm	0.9 cm	1.2 cm	1.3 cm
0.3 gm	0.9 cm	0.9 cm	1.2 cm	1.2 cm	2.1 cm	1.7 cm
0.4 gm	1.0 cm	1.1 cm	1.4 cm	1.4 cm	2.5 cm	2.2 cm
0.5 gm	1.2 cm	1.3 cm	1.5 cm	1.8 cm	2.8 cm	2.7 cm

Table 2
Antibacterial effect (zone of inhibition in radius) of Silver nanoparticles against *S.aureus* .

Conc of Nps	WATER		N-HEXANE		70% ETHANOL	
	Disc Diffusion	Well Diffusion	Disc Diffusion	Well Diffusion	Disc Diffusion	Well Diffusion
0.1gm	0.4 cm	0.3 cm	0.6 cm	0.5 cm	0.7 cm	0.8 cm
0.2gm	0.6 cm	0.7 cm	0.9 cm	0.8 cm	1.0 cm	1.2 cm
0.3gm	0.9 cm	1.0 cm	1.3 cm	1.0 cm	1.4 cm	1.6 cm
0.4gm	1.2 cm	1.4 cm	1.5 cm	1.3 cm	1.6 cm	1.9 cm
0.5gm	1.4 cm	1.9 cm	1.7 cm	1.5 cm	1.8 cm	2.3 cm

Table 3
Antibacterial effect (zone of inhibition in radius) of Silver nanoparticles against *S.typhi* .

Conc of Nps	WATER		N-HEXANE		70% ETHANOL	
	Disc Diffusion	Well Diffusion	Disc Diffusion	Well Diffusion	Disc Diffusion	Well Diffusion
0.1gm	0.7 cm	0.4 cm	0.8 cm	0.6 cm	0.9 cm	0.5 cm
0.2gm	1.0 cm	0.8 cm	1.3 cm	1.0 cm	1.4 cm	1.0 cm
0.3gm	1.3 cm	1.9 cm	1.5 cm	1.5 cm	1.9 cm	1.6 cm
0.4gm	1.6 cm	1.8 cm	1.8 cm	1.9 cm	2.4 cm	2.2 cm
0.5gm	1.9 cm	2.2 cm	2.0 cm	2.1 cm	2.9 cm	2.5 cm

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