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### RESEARCH ARTICLE

#### EVALUATION OF ANTIBACTERIAL BEHAVIOUR OF SILVER NANOPARTICLES.

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#### Manuscript Info

##### Manuscript History

Received: 17 January 2018  
Final Accepted: 19 February 2018  
Published: March 2018

##### Keywords:-

AgNps, Nutrient agar, XRD, *Staphylococcus aureus*.

#### Abstract

It is well known that Silver nanoparticles has great affinity against many pathogenic microbes i.e silver nanoparticles possess good antimicrobial properties against many bacteria and fungi. *Staphylococcus aureus* is one of the most pathogenic bacteria causing infections on skin, respiratory tract, not only this it also cause life threatening diseases such as pneumonia, meningitis etc. Inhibitory effect of silver nanoparticles against staphylococcus aureus gram positive bacteria was evaluated by *in-vitro* disc diffusion method. The bacterial strains were treated with silver nanoparticle discs at varying concentrations. The antibacterial activity was evaluated by measuring zone of inhibition around antimicrobial disc.

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#### Introduction:-

Since from ancient ages living beings are affected by infinite number and types of microbes, which include fungi, nematodes, bacteria, virus etc.<sup>1</sup> Bacteria are ubiquitous organisms present in everywhere in living environment. These bacteria causes serious health issues including infections on skin, respiratory tract, malfunctioning of body organ systems and other chronic and fatal diseases.<sup>2-5</sup> One of the pathogenic bacteria is *Staphylococcus aureus* causing infection in human body, such as boils, folliculitis, impetigo, and cellulitis.<sup>6-9</sup> Due to the increase in antibiotic resistance it is very difficult to treat such bacteria and this can lead to hazardous health issues. *Staphylococcus aureus* is one of them which shows makeable resistance towards methicillin.<sup>10</sup> Many researchers are focusing on other alternative methods to overcome this problem. Metal nanoparticles as antimicrobial agents have proved to be the best alternative solution for the above problem.<sup>11-15</sup> As silver as bulk is well known antimicrobial agent since time immemorial.<sup>16</sup> Silver is a non-toxic and hypo allergic naturally occurring element, it does not accumulate in human system and never cause any harm.<sup>17</sup> Silver ions expose and remove K<sup>+</sup> ions from bacterial and cytoplasmic membrane of the bacterial cell ruptures which causes halt of DNA replication and finally leading to cell death.<sup>18</sup> In the present scenario due to the advancement of research in the field of metal nanoparticles, silver nanoparticles attained much more attention than any other metal nanoparticles because of its antimicrobial affinity and therefore are being exploited by many pharmaceutical companies for making antibiotics, used in biomedical applications.<sup>19</sup> Due to antimicrobial affinity of silver nanoparticles, it has so many commercial applications too like manufacturing of washing machines, air conditioners and refrigerators which use linings of silver nanoparticles.<sup>20-24</sup> The medical field also is using products with silver nanoparticles, such as heart valves & other implants, medical face masks, wound dressings and bandages.<sup>25-28</sup>

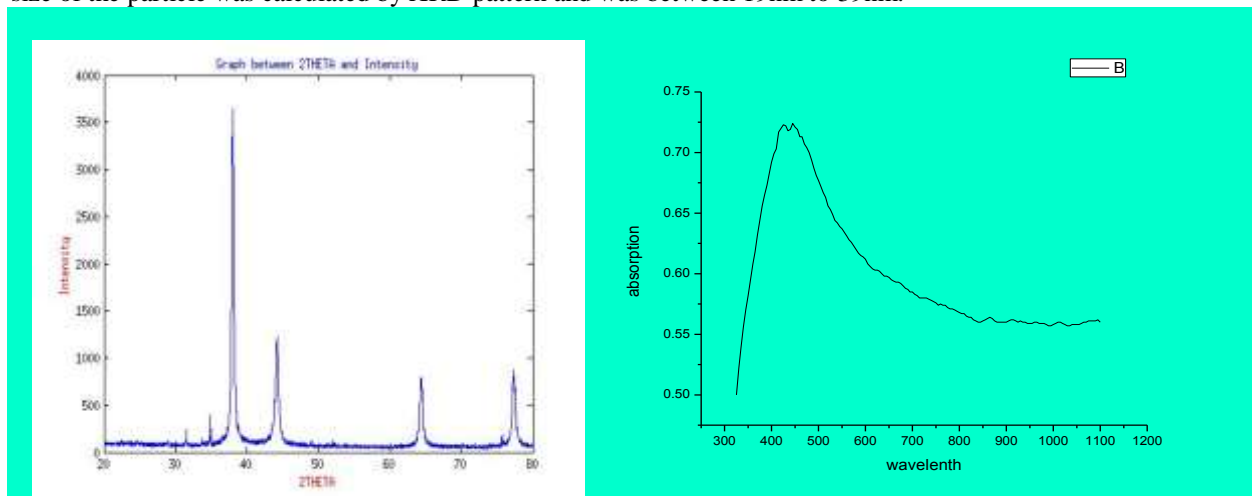
In this study we have tested the synthesized silver nanoparticles for the antibacterial activity against *S.aureus* by disc diffusion method calculating diameter of zone of inhibition of bacterial strains.

### Material and Method:-

Selected bacterial culture *Staphylococcus aureus* (MTCC 11949) for antibacterial assay was procured from Microbial Type Culture Collection Chandigarh, India, in freeze dried condition. Nutrient agar powder was purchased from Science Corporation for the preparation of culture media. Ciprofloxacin of with 98.0% purity was as positive control was procured Sigma Aldrich. Plant sample of *Azadirachta indica* was collected from women campus, University of Allahabad.

### Synthesis of silver nanoparticle:-

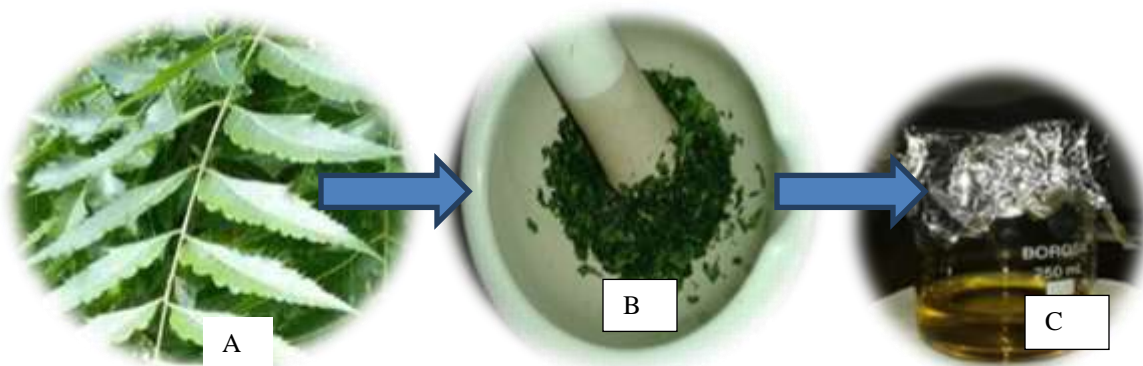
Silver nanoparticles were already synthesized at low temperature using sodium borohydride as reducing agent. The prepared sample was characterized by Uv-visible measurement which showed absorption at 425nm. The crystallite size of the particle was calculated by XRD pattern and was between 19nm to 39nm.<sup>29</sup>



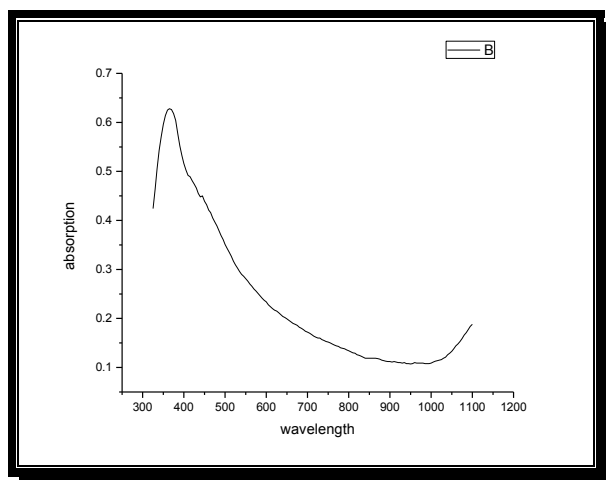
**Figure: 1:-**X-ray diffraction pattern of silver nanoparticles[29]      **Figure:2** UV-vis spectra of silver nanoparticle[29]

### Preparation of plant extract of *Azadirachta indica*

For herbal product as antimicrobial agent we have selected *Azadirachta indica* i.e. neem as it possess excellent antibacterial property.<sup>30</sup> Selected plant was washed thoroughly thrice in running water to remove dirt and dust and other contaminants. And shade dried at room temperature. Extract was prepared in distilled water in the ratio 5: 100 (5gm of crushed leaves in 100 ml of solvent). The Uv-visible absorption spectra of extract of plant extract exhibited at 365 nm as in figure 4.



**Figure: 3:-** (A) plant sample of *Azadirachta indica*, (B) extraction by pestle and mortar, (C) crude extract



**Figure: 4:-UV-vis spectra of Plant extract.**

#### **In-vitro assay of AgNp against *Staphylococcus aureus***

In-vitro evaluation of synthesized silver nanoparticles and plant extract was performed against gram positive bacteria *Staphylococcus aureus* by disc diffusion method.

#### **Disc diffusion method**

The bacterial strains in freeze dried condition were first of all revived in culture media and incubated for 24hrs at 37°C on Nutrient agar plate followed by refrigeration storage at 4°C. AgNp powder was diluted at different concentrations (10, 30, 50, 70, and 90, in µg/ml) in double distilled water. The agar plates were inoculated with the bacterial strains along with the wattman filter paper disc impregnated with AgNp at different concentration. Ciprofloxacin was used Positive control. The zone of inhibition around the disc was measured after 24 hours of incubation at 37°C.

#### **Result and Discussion:-**

Silver nanoparticles and neem extract both shows broad spectrum antibacterial activity against pathogenic bacteria *Staphylococcus aureus*. The quantitative analysis both the antibacterial agents can be observed by the diameter (in mm) of the zone of growth inhibition of bacteria around the diffused discs. The comparative study of antibacterial activity of silver nanoparticles and plant extract reflect that silver nanoparticles were more effective against *Staphylococcus aureus* than that of plant extract, although plant extract also exhibit good inhibitory effect as compared to the positive control. From the table 1, it is clearly proved that at higher concentration of silver nanoparticles, the zone of inhibition is large.

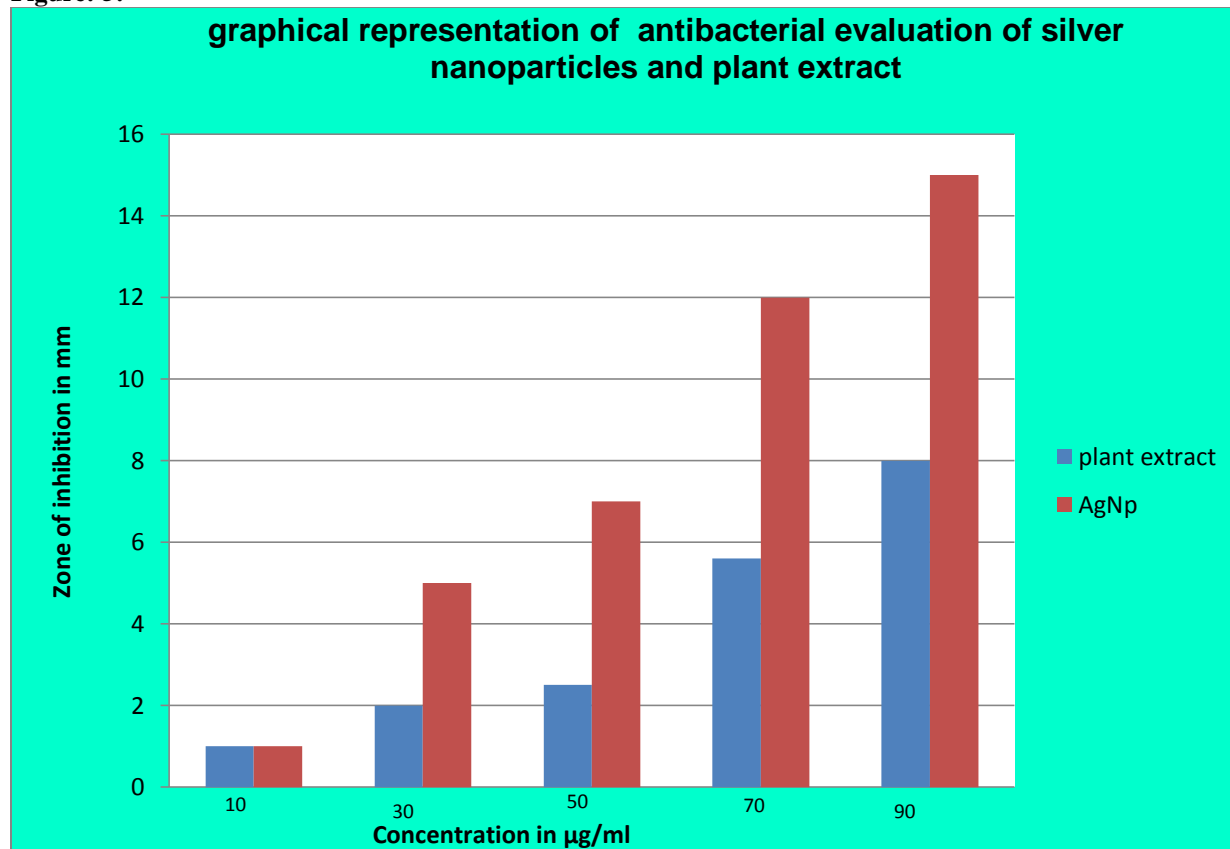
**Table: 1:-**

<b>Comparative study of antibacterial activity of silver nanoparticles and plant extract (<i>Azadirachta indica</i>)</b>		
<b>Concentration in(µg/ml)</b>	<b>Diameter of zone of inhibition In mm</b>	
	<b>plant extract</b>	<b>AgNp</b>
<b>10</b>	<b>1</b>	<b>1</b>
<b>30</b>	<b>2</b>	<b>5</b>
<b>50</b>	<b>2.5</b>	<b>7</b>
<b>70</b>	<b>5.6</b>	<b>12</b>
<b>90</b>	<b>8</b>	<b>15</b>
<b>Ciprofloxacin (positive control)</b>	<b>4 mm</b>	

The graphical presentation of zone of inhibition clearly indicates that as the concentration of the silver nanoparticle increases there is dynamic increase in the diameter of the zone around which the bacterial growth was restricted.

Ciprofloxacin has been in use to treat the tested bacterial infections since years but with time the microbe become highly resistant to this antibiotic and now there is utmost need to treat such infections with some alternative and for this silver nanoparticles can efficiently be formulated as medicine to treat severe infections caused by *Staphylococcus aureus*.

Figure: 5:-



### Conclusion:-

From the above research work we can conclude that silver nanoparticles (AgNp) shows much greater inhibitory effect against bacterial growth than that of neem (*Azadirachta indica*) extract. Action of silver nanoparticles was also compared with antibiotic ciprofloxacin which shows negligible effect against bacteria as it is highly resistant towards such antibiotics. Hence silver nanoparticles can be formulated as antibiotic against *Staphylococcus aureus* which used in treatment of various skin infections caused by such pathogens.

### Acknowledgement:-

The authors are thankful to the Department of Physics, University of Allahabad for providing UV characterization facility. The authors are also thankful to the Department of Botany for providing all the necessary facilities for this research work. Last but not the least, great vote of thanks to all our labmates for their valuable support. Financial support was provided by the UGC research fellowship and contingency.

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