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ANTIMICROBIAL ACTIVITY OF NANOPARTICLES AGAINST PATHOGENS OF OTITIS EXTERNA

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ABSTRACT

There is increase in use of nanoparticles in medicine like in ointments, coating medical devices, in diagnostics due to its efficacy. The study evaluated the antimicrobial activity of three nanoparticles Silver, Copper and Zinc against the bacteria and fungi of the common external infection of the ear. All the three nanoparticles having size 30-50nm were ordered from nanoresearch lab, Jamshedpur. The agar well diffusion technique was used to study their antimicrobial activity. Silver nanoparticles (AgNPs) evince good antimicrobial activity against all the bacteria and fungi with zone of inhibition ranging from 10 to 21 mm except *Staphylococcus* and *Pseudomonas.* Copper nanoparticles (CuNPs) displayed the greater antibacterial and antifungal activity than AgNPs and Zinc nanoparticles (ZnNPs). ZnNPs showed good antibacterial activity. As we talk about the antifungal activity of ZnNPs, it only inhibited the activity of *Penicillium polonicum* and *Candida Africana* with zone of inhibition 12mm and 14mm respectively.

KEYWORDS: Silver nanoparticles, Copper nanoparticles, Zinc nanoparticles, external ear infection

INTRODUCTION

The idea of Nanotechnology was first highlighted by Nobel laureate “Richard Feynman”, in his famous lecture at the California Institute of Technology, 29th December, 1959. In one of his articles published in 1960 titled, “There is Plenty of Room at the Bottom”, discussed the idea of nanomaterial. Nanotechnology has been provisionally defined as relating to materials, systems and processes which operate at a scale of 100 nanometers (nm) or less. A nanometer is one billionth of a meter. Overall nano refers to a size scale between 1 nanometer (nm) and 100 nm.[1] Nanotechnology and alongside nanostructured materials, play an ever-increasing rate in science, research and development as well as also in every day’s life. Nanotechnology has been used in various fields like medicine[2], Agriculture[3] etc. The use of nanotechnology in medicine offers some exciting possibilities. Some techniques are only imagined, while others are at various stages of testing, or actually being used today. Nanoparticles are used in drug delivery and in diagnostics like detection of cancer cells in blood stream.[4]

Silver nanoparticles have antimicrobial activity, anti-biofilm and also enhances the activity of antibiotics[5], it showed potent bactericidal effects against *Staphylococcus aureus*[6], *Staphylococcus epidermis.*[7] Copper nanoparticles also have good antimicrobial activity. The Copper nanoparticles inhibited the activity of bacteria and fungi also. The copper nanoparticles displayed antimicrobial activity towards *Micrococcus luteus*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*, fungus like *Aspergillus flavus*, *Aspergillus niger* and *Candida albicans.*[8] *Bacillus subtilis* depicted the highest sensitivity to copper nanoparticles compared to the other strains and was more adversely affected by the copper nanoparticles.[9] Zinc oxide nanoparticles also evinced good antimicrobial activity against *Bacillus cereus* (ATCC 11778), *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27853) and *Salmonella Typhimurium* (ATCC 1428).[10] Zinc oxide nanoparticles inhibited *C. albicans* growth, in a concentration-dependent manner.[11]

Present study investigated the antimicrobial activity of three nanoparticles AgNPs, CuNPs and ZnNPs.

MATERIAL AND METHODS

To determine the antibacterial and antifungal activity of nanoparticles, three nanoparticles Silver, Copper and zinc having an average particle size of 30-50nm were ordered from Nano Research Lab, Gopalpur, East Singhbhum, Jamshedpur-832102 (www.nanoresearchlab.in) with safety Clearance Certificate and Certificate of Analysis.

Preparation of Bacterial inoculum

The test organisms isolated and identified from otitis externa (*Klebsiella pneumoniae strain PPM1A* MT026979, *Staphylococcus simmie* MT027010, *Bacillus sp. Clone PPM2C* MT027034, *Acinetobacter sp. Clone PPM2D* MT02701, *Pseudomonas otitidis* OK035334, *Metabacillus malikii strain PPM* OK035442, *Exiguobacterium sp. Clone PPM9A* MT027057 and *Enterococcus sp. Clone PPM9B*MT027061) were inoculated into freshly prepared nutrient broth separately and incubated at 37^0C for 2 hours until the suspension turbidity reaches to 0.5 MacFarland standard (1.5×10^8CFU/ml); the OD of cell suspension was measured at 600nm.[12] The bacterial cells were diluted in normal saline to around 10^5 CFU/ml before use.[13]

Preparation of Fungal inoculum:

The fungal strains (*Aspergillus welwitschiae* MZ960141, *Aspergillus foetidus* MZ955454, *Aspergillus tamarii* MZ955985, *Aspergillus aflatoxiformans* OK035443, *Penicillium polonicum* ON811480, *Candida africana* ON811599) were inoculated separately in Sabouraud's dextrose broth and the suspensions were checked to provide approximately 1.5×10^8 CFU/ml.

Determination of antimicrobial activity of nanoparticles

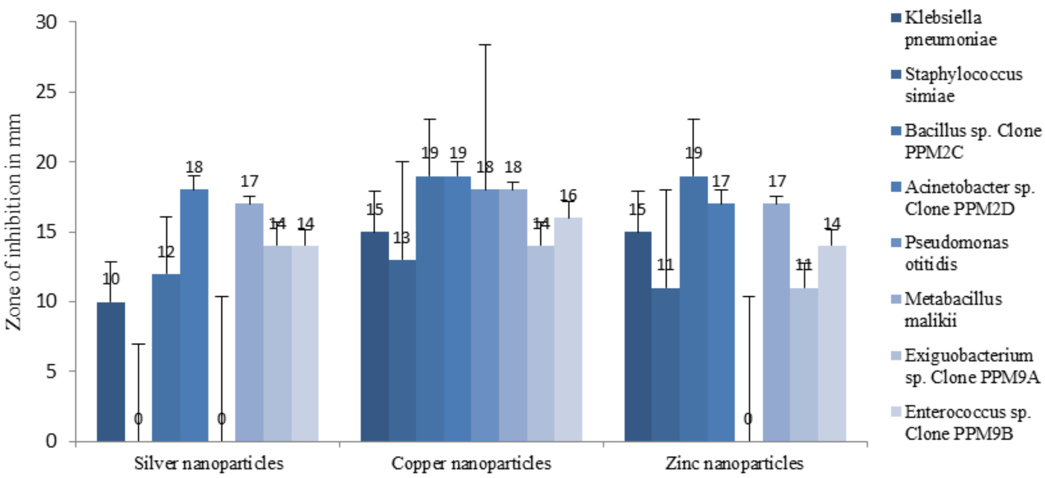
The lawn of bacterial culture was prepared on Sterilized Muller Hinton Agar Plates and the lawn of fungal culture was prepared on Antifungal Assay Agar plates with the help of a sterile swab and the holes in each plate were punched aseptically with 6mm sterile cork borer. 40µl of each nanoparticle (10mg/ml concentration) and solvent blanks (DMSO) were added separately in wells with the help of a micropipette. The antibiotics Imipenem and Nystatin were taken as positive control. Further, the plates were incubated at 37^0C for 24 hours for bacteria and 28^0C for 72hours for fungi to evaluate the clear zone formation surrounding the wells. The diameter of the zone was measured using a caliper. The whole experiment was done in triplicate.

RESULT AND DISCUSSION

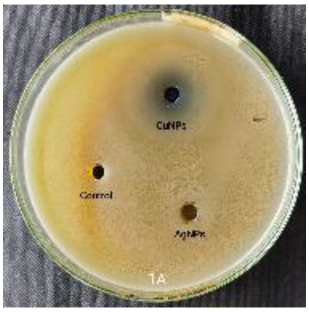
To determine the antibacterial and antifungal activity of nanoparticles, three nanoparticles Silver, Copper and zinc having an average particle size of 30-50nm were tested against the isolated pathogenic bacteria and fungi.

Antibacterial activity

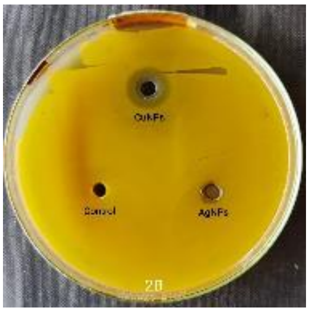
All the three nanoparticles evinced potent antibacterial activity against all bacteria except *Staphylococcus simiae* and *Pseudomonas otitidis* (Graph1). Silver nanoparticles showed zone of inhibition in the range of 10 to 18mm. 18mm zone is the maximum zone of inhibition shown against *Acinetobacter sp. Clone PPM2D.* Copper nanoparticles showed maximum zone of inhibition 19mm against *Bacillus sp. Clone PPM2C* and *Acinetobacter sp. Clone PPM2D, Pseudomonas otitidis* (18mm), *Metabacillus malikii* (18mm), *Klebsiella pneumonia* (15mm), *Enterococcus sp. Clone PPM9B* (16mm), *Exiguobacterium sp. Clone PPM9A* (14mm) and *Staphylococcus simiae* (13mm) (Graph1). Zinc nanoparticles exhibited maximum 19mm zone against *Bacillus sp. Clone PPM2C,* 17mm zone against *Acinetobacter sp. Clone PPM2D* and *Metabacillus malikii, Klebsiella pneumonia* (15mm), and *Enterococcus sp. Clone PPM9B* and 11mm against *Staphylococcus simiae* and *Exiguobacterium sp. Clone PPM9A* (Graph1). The error bars showing standard deviation indicated that the mean standard value of all the three nanoparticles deviates by large difference for all bacterial species except *Metabacillus malikii.*



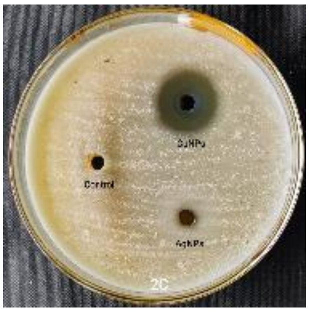
Graph 1: Zone of inhibition of nanoparticles against pathogenic bacteria. The error bars indicates the standard deviation.



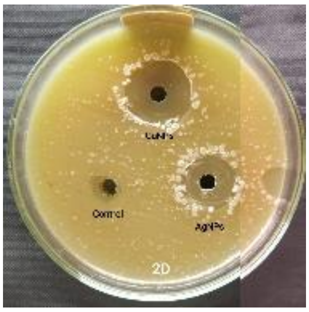
Klebsiella pneumoniae



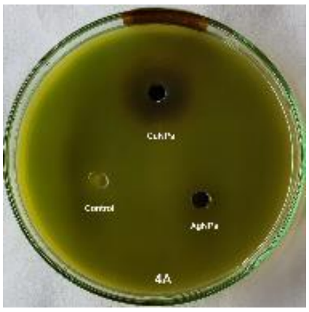
Staphylococcus sp. clone PPM2B



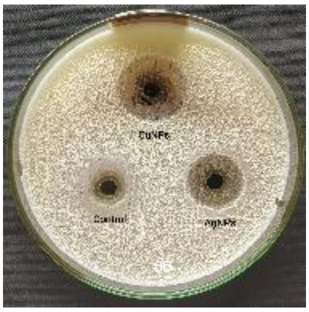
Bacillus sp. clone PPM2C



Acinetobacter sp. clone

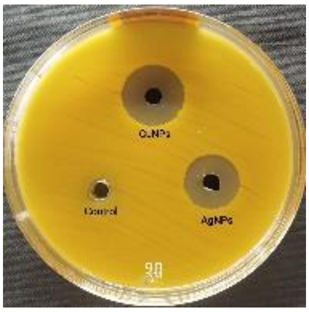


Pseudomonas otitidis



Metabacillus malikii

Exiguobacterium sp. clone PPM9A

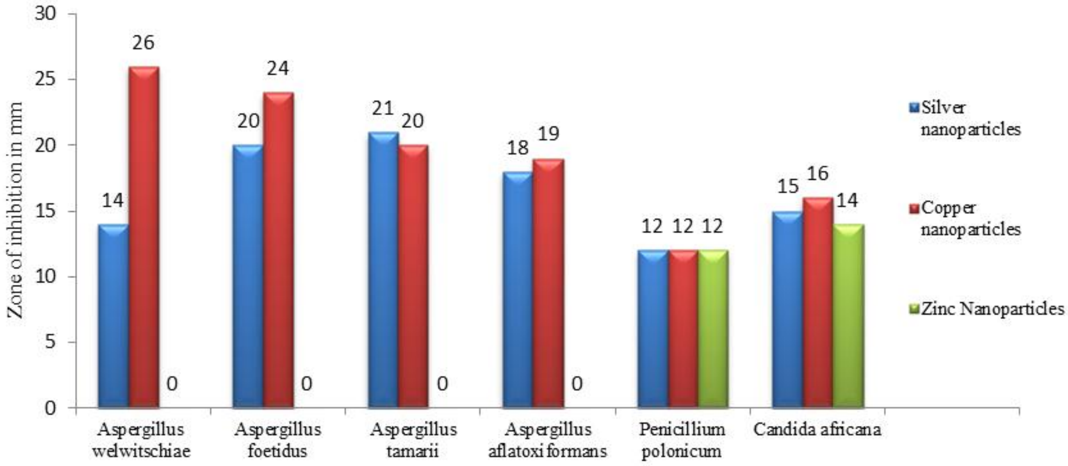


Enterococcus sp. clone PPM9B

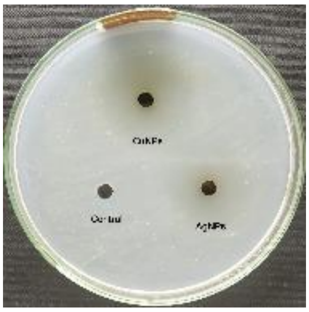
Figure 1: Antibacterial activity of Silver (AgNPs), Copper (CuNPs) and Zinc (ZnNPs) nanoparticles.

Antifungal activity

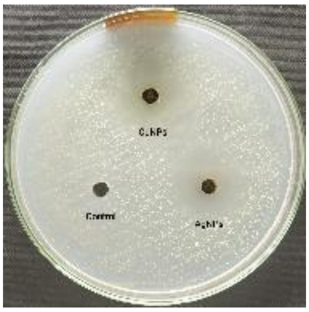
Silver and Copper nanoparticles evinced good antifungal results as compare to zinc nanoparticles. Copper nanoparticles showed maximum zone of inhibition against *Aspergillus welwitschiae* (26mm), *Aspergillus foetidus* (24mm) and *Aspergillus tmarii* (20mm) followed by *Aspergillus aflatoxiformans* (19mm), *Candida Africana* (16mm) and *Penicillium polonicum* (12mm). Silver nanoparticles showed 21mm and 20mm zone of inhibition against *Aspergillus tmarii* and *Aspergillus foetidus* respectively, *Aspergillus aflatoxiformans* (18mm), *Candida Africana* (15mm) and *Aspergillus welwitschiae* (14mm). Zinc nanoparticles only showed zone of inhibition against *Penicillium polonicum* (12mm) and *Candida africana* (14mm).



Graph 2: Zone of inhibition of Nanoparticles against fungal pathogens.



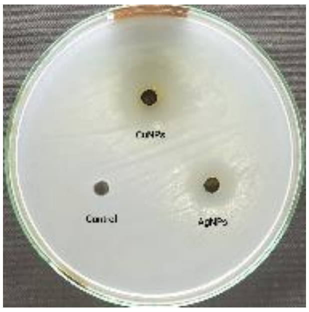
Aspergillus welwitschiae



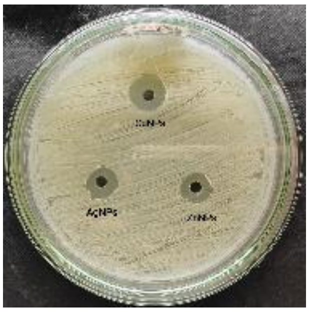
Aspergillus foetidus

Penicillium polonicum

Aspergillus tamarii



Aspergillus aflatoxiformans



Candida africana

Figure 2: Antifungal activity of Silver (AgNPs), Copper (CuNPs) and Zinc (ZnNPs) nanoparticles.

The antimicrobial activity of AgNPs was studied and found that at OD600, the growth of all pathogens, *Streptococcus sp.*, *Bacillus sp.*, *Staphylococcus sp.* and *Shigella sp.*, *EscheRichia coli*, *Pseudomonas aeruginosa* and *Klebsiella* sp.) as well as one fungus *Candida sp.* were entirely inhibited after treatments by all AgNPs gathered at the three different centrifugation speeds 4000, 8000 and 14,000 rpm.[14]

The antibacterial efficacy of CuNPs encapsulated with carbon was tested. The diameters of zone of inhibition for the Cu-NPs encapsulated with carbon were measured to be 14.4±0.5mm for *Escherichia coli*, 13.5±0.8mm for *Pseudomonas aeruginosa*, and 10.5±1.3mm for *Staphylococcus aureus* while those for n-Cu and Cu2O were 13.8±0.8 mm, 7.4±0.7 mm, 8.5±0.7 mm and 12.0±0.4 mm, 11.2±0.7 mm, 8.4±1.3 mm, respectively.[15]

The potential of Zinc oxide nanoparticles investigated to affect the growth curve of *Candida albicans.* It was observed that ZnO NPs inhibited *Candida albicans* growth, in a concentration-dependent manner. This was evaluated by a delay in entry to the logarithmic growth phase, and/or by a deceleration of the logarithmic growth.[11]

The antimicrobial activity of the biologically synthesized AgNPs having size 50nm was tested against microbiota of the acute ear infection.[16] The zone of inhibition increased with the increase in the concentration of silver nanoparticles and effectively kills them. AgNPs were found to have antibacterial activity against bacteria at 100µg/ml concentration.

The copper nanoparticles exhibited antibacterial activity toward the tested pathogenic strains of *M. luteus* (16±0.781), *S. aureus* (21±1.612), *E. coli* (26±0.985), *K. pneumoniae* (15±0.689), *P. aeruginosa* (5±1.136), and the antifungal activity was noticed toward *A. falvus* (13±0.577), *A. niger* (16± 0.354), *C. albicans* (23±1.258), respectively. *Bacillus subtilis* depicted the highest sensitivity to nanoparticles compared to the other strains.[8]

CONCLUSION

As the present study successfully presented the antimicrobial activity of nanoparticles against pathogens of otitis externa, Silver, Copper and Zinc nanoparticles can be used to target bacteria and fungi as an alternative to antibiotics.

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