

STUDY ON ANTIMICROBIAL ACTIVITY OF ZINC AND COPPER-NANOPARTICLES

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Article Received on
06 April 2018,

Revised on 27 April 2018,
Accepted on 17 May 2018,

DOI: 10.20959/wjpr201811-12399

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ABSTRACT

The aim of this study was to investigate the antimicrobial activity of ZnO and CuO nanoparticles on bacteria Gram-negative (*Escherichia coli*) Gram-positive bacteria (*Bacillus*) and fungi *Aspergillus* and *Penicillium*. Test method Well diffusion agar is used. Results, showed antimicrobial properties by inhibiting growth of bacterial strains by Zinc nanoparticles on *Bacillus* (0.7mm), *E-coli* (1.2mm) and fungal strains on *Aspergillus*(1.3mm), *Penicillium*(1.1mm). Inhibiting growth of bacterial strains by Copper nanoparticles on *Bacillus* (0.5mm), *E-coli* (0.8mm) and fungal strains on *Aspergillus*(0.8mm), *Penicillium*(0.7mm). It was observed that Zinc nanoparticles have more inhibition on *E.Coli* and *Aspergillus* than Copper nanoparticles

so Zinc nanoparticles have more antimicrobial activity than copper nanoparticles.

KEYWORDS: Antimicrobial, *Bacillus E-coli*, *Aspergillus*, *Penicillium*, ZnO nanoparticles, CuO nanoparticles, Inhibition.

INTRODUCTION

Metals have been used for centuries as antimicrobial agents. Among those used is silver, copper, zinc, gold and nickel each with different properties and spectrum of activity. The advent of nanotechnology, it was attempted to replace the biocides from antimicrobial paints with various nano-sized substances such as: zinc oxide, titanium dioxide and silver (Niegisch *et al.*, 2002). The development of antimicrobial nanocoatings through the green chemistry

methods could be a promising way for potential environmental applications (Yamauchi *et al.*, 2005).

In recent year, received a nanoparticle material because of a growing interest in physical and chemical properties unique that much different from their conventional counter parts (Stoimenov *et.al*, 2005). The application of copper oxide (CuO) NPs for antimicrobial applications was introduced by (Ren *et al.*, 2009). Recent studies have demonstrated activities of antimicrobial from various material nanoparticles. Including silver (Kim *et al.*, 2008) and copper (Cotti *et al.*, 2005) and titanium dioxide (Kwak *et al.*, 2010) and oxide zinc (Lilite *et a.*, 2010).

The considerable antimicrobial activities of inorganic metal oxide nanoparticles such as ZnO, MgO, TiO₂, SiO₂ and their selective toxicity to biological systems suggest their potential application as therapeutics, diagnostics, surgical devices and nanomedicine based antimicrobial agents (Mohsen and Zahra, 2008; Sobha *et al.*, 2010; Laura *et al.*, 2006; Sawai and Yoshikawa, 2003; Reddy *et al.*, 2007).

MATERIALS AND METHODS

Preparation of test culture media

- **Bacterial culture:** Bacterial culture was prepared by serial dilution method. The culture was then inoculated into Nutrient broth. Bacillus and E-coli are the two bacterial test cultures.
- **Fungal culture:** Fungal culture was prepared by inoculating in Potato Dextrose broth. Aspergillus and Penicillium are the two fungal test cultures.

Preparation of ZnO nanoparticles

Zinc sulfate heptahydrate (ZnSO₄(H₂O)₇) and Ammonium hydroxide (NH₄OH) were the two starting materials for the synthesis of ZnO nanoparticles. 0.1M of ZnSO₄ solution is prepared by taking 1.61 grams of ZnSO₄ in 100ml of double distilled water. This solution of irradiated to electromagnetic waves which enhances the ZnSO₄ particles. 25ml of 25% Ammonia solution per 100ml is added drop wise by continuous stirring at 60°C. This results in the formation of ZnO nanoparticles. The obtained nanoparticles are washed twice with distilled water. These particles are filtered by Whatmann Filter paper or by centrifugation techniques. Now these particles are dried at 60°C. These particles are then grinded to obtain the fine particles (figure-1).



Figure 1: Zinc nanoparticles.

Preparation of CuO nanoparticles

Copper sulphate was used as a basic precursor, tea decoction as a stabilizer, L- ascorbic acid as an anti oxidizing agent, Sodium hydroxide was used as a catalyst and also to adjust the pH of the solution to 12. Tea decoction was desperately prepared. The copper sulphate solution (0.04M) and L- ascorbic acid (0.001M) were prepared separately. The solutions of tea decoction and L- ascorbic acid were added to copper sulphate under rapid stirring. The sodium hydroxide was also added to the mixed copper salt solution under constant stirring. The initial blue colour of the solution eventually turns to green colour. Stirring was continued for another 1hour to complete the reaction. The precipitate was filtered and washed twice with methanol and dried to obtain copper nanoparticles(figure-2).



Figure 2: Copper nanoparticles.

RESULTS AND DISCUSSION

Antimicrobial activity of Zinc nanoparticles

In this study, the prepared Zinc nanoparticles exhibited high antimicrobial activity against *Bacillus* (0.7mm), *E-coli* (1.2mm) and fungal strains on *Aspergillus*(1.3mm), *Penicillium*(1.1mm). Figure 4(a,b,c,d) shows the photographic image of an inhibition zone

produced by Zinc nanoparticles on *Bacillus*, *E-coli* and fungal strains on *Aspergillus*, *Penicillium*. In the study done by Reddy *et al.* (2007) and by Selahattin *et al.* (1998), emphasizing on the higher susceptibility of Gram-positive bacteria in comparison with Gram-negative bacteria, it has been proposed that the higher susceptibility of Gram-positive bacteria could be related to differences in cell wall structure, cell physiology, metabolism or degree of contact. Lingling *et al.*, (2006) work shows the particle concentration seems to be more effective on the inhibition of bacterial growth than particle size.

The enhanced bioactivity of smaller particle probably is attributed to the higher surface area to volume ratio (Nagarajan and Rajagopalan (2008). According to the results, it can be concluded that ZnO nanoparticles are effective antimicrobial agents both bacteria on Gram-positive, Gram-negative bacteria and fungal strain *Aspergillus* and *Penicillium*(Table-1). The same results were confirmed in the study of Zhongbing *et al.* (2008).

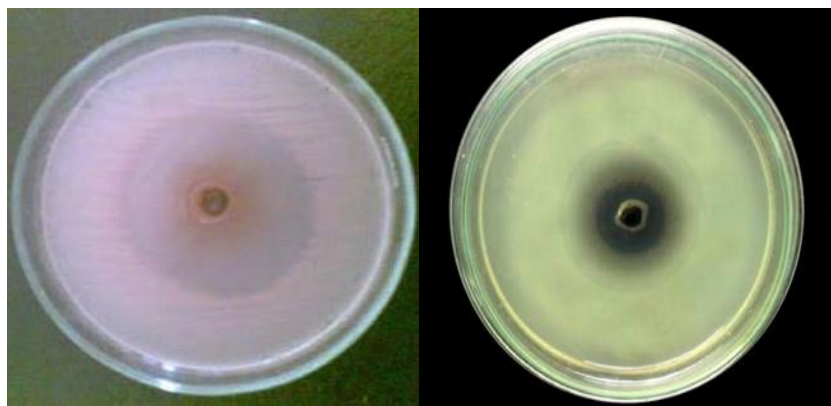


Figure 4(a): Zone of inhibition of Zinc and Copper nanoparticles on Gram Positive Bacteria.



Figure 4(b): Zone of inhibition of Zinc and Copper nanoparticles on Gram Negative Bacteria.

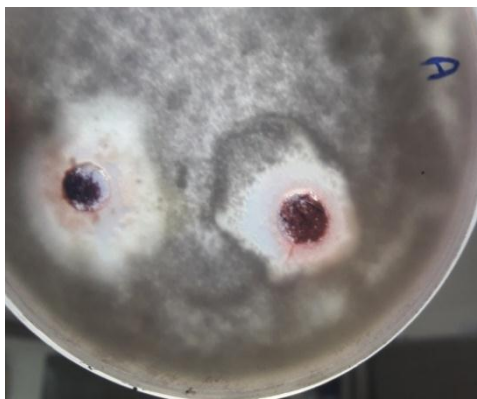


Figure 4(c): Zone of inhibition of Zinc and Copper nanoparticles on Aspergillus.

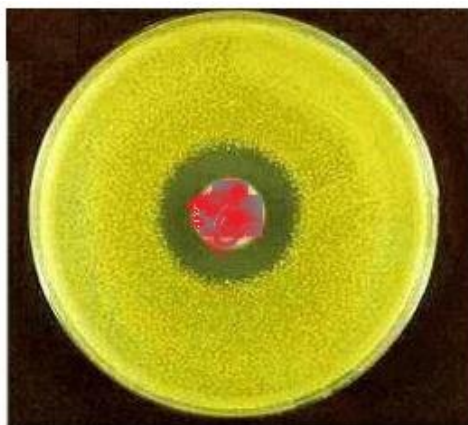


Figure 4(d): Zone of inhibition of Zinc and Copper nanoparticles on Pencillium.

Table 1: Zone of inhibition by Zinc and Copper nanoparticles on Microorganisms.

Microorganisms	Zone of inhibition	
	Zinc nanoparticles	Copper nanoparticles
Bacillus gram postive	0.7mm	0.5mm
E.coli gram negative	1.2mm	0.8mm
Aspergillus	1.3mm	0.8mm
Pencillium	1.1mm	0.7mm

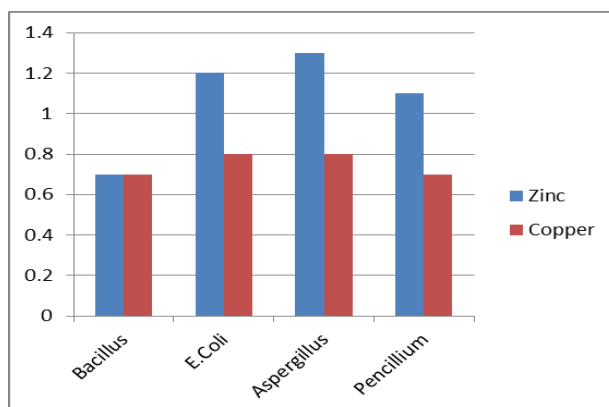


Figure 3: Zinc and Copper nanoparticles inhibition on Microorganisms.

Antimicrobial activity of Copper nanoparticles

In this study, the prepared Copper nanoparticles exhibited antimicrobial activity against *Bacillus* (0.7mm), *E-coli* (0.8mm) and fungal strains on *Aspergillus*(0.8mm), *Penicillium* (0.7mm) which is less comparative to Zinc. Figure 4(a,b,c,d) shows the photographic image of an inhibition zone produced by Copper nanoparticles on *Bacillus*, *E-coli* and fungal strains on *Aspergillus*, *Penicillium*. Azam *et al.*, (2012) research group showed that the antibacterial effect of CuO NPs was dependent on the particle size and a significant increase in antibacterial activities against both Gram-positive and Gram-negative bacterial strains was achieved using the highly stable minimum-sized monodispersed CuO NPs. Pang *et al.*, (2009) discussed that as the cuprous oxide crystals change from cubic to octahedral, the antibacterial activity changes from general bacteriostasis to high sensitivity.

Ruparelia *et al.*,(2008) investigated antimicrobial properties of silver and Cu NPs on *E. coli*, *B. subtilis* and *S. aureus*. Results of minimum inhibitory concentrations (MICs), minimum bactericidal concentrations (MBCs) and disk diffusion test revealed that the Cu NPs were more efficient compared to the silver particles against *B. subtilis* which is suggested to be due to more affinity of the Cu NPs to surface amines and carboxyl groups of *B. subtilis*.

Chaladar *et al.*,(2012) study showed that the zone of inhibition is 1.5mm for Copper and 0.8mm for Copper oxide on *Penicillium*. Current study showed that the copper nanoparticles showed inhibition both on *Penicillium*(0.8mm) and *Aspergillus*(0.8mm) is same(Table-1).

CONCLUSION

From the current study it is concluded that ZnO nanoparticles has more inhibition capacity compare with CuO nanoparticles both bacterial and fungal strains. Factors like the size of the, shape of the particles and type of nanoparticles also influences the antimicrobial activity.

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