

EFFECT OF ZINC OXIDE NANOPARTICLE PRODUCED BY *ZINGIBER OFFICINALE* AGAINST PATHOGENIC BACTERIA

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ABSTARCT

The necessity of producing nanoparticle without the involvement of toxic chemicals as capping agents had paved way for the development of Green Nanotechnology. Many nanoparticles have been synthesized using this method. Nowadays, much research has been focussed on the inorganic nanoparticles due to their lesser toxicity and better antimicrobial activity for treating various infectious diseases. Due to the resistance offered by the microbial strains a newer search of antimicrobials is required. In this aspect the present study was undertaken in order to show the antibacterial activity of Zinc Oxide (ZnO) nanoparticle synthesized using root extracts of *Zingiber officinale*. The production of nanoparticle was confirmed using Scanning Electron Microscope (SEM) and the antibacterial activity was performed with varying concentrations (0.5 mg/ml, 1 mg/ml, 1.5 mg/ml, 2 mg/ml, 2.5 mg/ml, 3 mg/ml and 4 mg/ml) against gram negative (*Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*) and gram positive (*Staphylococcus aureus*, *Bacillus subtilis*) bacteria. The size of the nanoparticle was found to be 30nm and they were spherical in shape. The antibacterial activity yielded a higher zone of inhibition at 2mg/ml. Thus ZnO nanoparticle can be used as an antibacterial agent in treating ailments caused by the pathogenic bacteria.

Keywords: Zinc Oxide Nanoparticles, Ginger, antibacterial activity,

INTRODUCTION

For the past several decades, thousands of antimicrobial agents were developed, but still there is a necessity of developing an efficient antimicrobial agent that prevents the microorganisms getting resistant to it. This necessity made the researchers constantly to search a newer agent that can inhibit the growth or kill the resistant organisms (Trivedi and Hotchandani, 2004). Currently, research in nanotechnology opens a new gateway in producing new antibacterial agents. Nanotechnology has a wide application in pharmaceutical and cosmetics and has its great significance by killing or reducing the activity of several pathogens (Singh and Nanda, 2013). It also serves as a platform for the development and modification of nanoparticles thereby enhancing their properties at nanoscale (Singh *et al.*, 2014; Sangeetha and Kuppusamy, 2013). Nanoparticles may be defined as a cluster of atoms having a size range of 100nm and are classified as metal nanoparticles and metal oxide nanoparticles. Metal nanoparticles have attained more importance because of their intrinsic properties which are determined by their shape, size, morphology, etc. (Yadav *et al.*, 2006). However, in the recent years transition metal oxide nanoparticles having semiconductor properties like ZnO, CuO, TiO₂ have attracted researchers to focus on their synthesis (Hasna *et al.*, 2014). Of these ZnO nanoparticle production has been concentrated more due to its diversifying properties which includes catalysis, electrical conductivity, antibacterial activity, cytotoxicity etc. (Gnanasangeetha and Thambavani, 2014; Shanthikumar *et al.*, 2009). Though there are several conventional physical and chemical methods available for their synthesis, biological method have attained a growing interest due to their advantages (Raj and Jayalakshmy, 2015). The biological method of synthesizing metal oxide nanoparticles may be of three ways, which includes the use of microorganisms, plant or with enzymes (Vidhya *et al.*, 2013). The use of plants or their extracts, for the synthesis of nanoparticles has been focussed due to its simplicity, lower cost and also it involves proteins as capping or reducing agent (Sangeetha and Kuppusamy, 2013; Vidhya *et al.*, 2013).

Zingiber officinale commonly known as ginger is widely used as a cooking spice, condiment and herbal remedy. For centuries it has been a one of the important ingredients in Chinese, Ayurvedic and Unani herbal medicine (Banerjee *et al.*, 2011). In tradition, ginger was treated for various ailments, including as a digestive aid, anti-nausea agent, to treat bleeding disorders, rheumatism etc (Chandan *et al.*, 2011). It has a wide variety of pharmacological properties such as anti-inflammatory, antioxidant, analgesic, hepatoprotective, antiproliferative etc., which is due to the presence of volatile oils such as sesquiterpene alcohol, zingiberol, sesquiterpene hydrocarbons, monoterpene, etc (Banerjee *et al.*, 2011, Behera *et al.*, 2012). Recent studies show that ginger has been used for the synthesis of various nanoparticles (Ipsa and Nayak, 2013; Priyaa and Kumidini, 2014; Chandan *et al.*, 2011). In the concept of using plants for the synthesis of nanoparticles the present study was undertaken in producing Zinc Oxide nanoparticles using *Zingiber officinale* which may have increased medical and pharmaceutical applications.

MATERIALS AND METHODS:

Synthesis of ZnO nanoparticles: ZnO nanoparticles were synthesized using the root extracts of *Zingiber officinale*. The precursor for ZnO nanoparticle was Zinc acetate dihydrate, which was purchased from Fischer Scientific. The protocol for preparing the root extracts and the synthesis of ZnO nanoparticles using them were referred from the earlier studies led by Singh *et al.*, (2011) and Gnanasangeetha and Thambavani (2013).

The bacterial strains such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Staphylococcus aureus* and *Bacillus subtilis* were used to check the antibacterial activity. The cultures were maintained in the nutrient agar medium as slant cultures and stored at 4°C. The same medium was used for sub culturing (Saraswathy *et al.*, 2013).

Preparation of Ginger extract: Ginger rhizome was purchased from local market. They were washed well with double distilled water in order to remove the mud present on it. The ginger was dried and was cut into small pieces weighing approximately 5 grams and dried in hot oven at 60°C for an hour. The cut pieces were then mashed well using mortar and pestle by slowly adding required quantity of water to it. The solution was filtered using Whatman filter paper and the extract was stored at 4°C.

Biosynthesis of ZnO nanoparticles: 0.1M of Zinc acetate dihydrate was prepared in a beaker containing 50ml of deionized water. 500µl of the ginger extract was added to the solution. Sodium Hydroxide was added to the solution drop wise in order to maintain the pH at 12. The solution was then stirred for 2 hours continuously after which they were centrifuged at 10,000 rpm for 10 minutes. The supernatant was discarded and the pellet was washed with deionized water and dried in hot air oven at 80°C overnight. The resulting white powder was collected and characterized.

Structural Characterization of Zinc Oxide Nanoparticle: The synthesized ZnO nanoparticle was structurally analyzed using Scanning Electron Microscope Carl Zeiss, Germany in order to confirm the presence of the nanoparticle.

Antibacterial Test: The antibacterial activity was carried out by the modified Kirby – Bauer disc diffusion method by performing the test with five different microorganisms such as *E. coli*, *P. aeruginosa*, *K. pneumonia*, *S. aureus* and *B. subtilis*. The cultures were grown in shake flasks containing 100 ml of nutrient broth and were left overnight in the shaker at 120rpm (Sangeetha *et al.*, 2012). The antibacterial assay was performed by the agar well diffusion method (Haritha *et al.*, 2011) by spreading a suspension of bacteria (100 µl) in a nutrient agar plate. The ZnO nanoparticle of 200 µl were poured in the agar wells at different concentrations of 0.5 mg/ml, 1 mg/ml, 1.5 mg/ml, 2 mg/ml, 2.5 mg/ml, 3 mg/ml and 4 mg/ml and were incubated at 37°C for 24 hours. The Zone of inhibition of the ZnO nanoparticle at varying concentration towards each microorganism was measured. The experiment was repeated thrice in order to confirm the reproducibility.

RESULTS AND DISCUSSION

Characterization of Zinc Oxide Nanoparticle: The Scanning Electron Microscopic (SEM) images confirm the size and shape of the synthesized ZnO nanoparticle. Figure 1 & 2 represents the SEM images of ZnO nanoparticles. The obtained powder of ZnO nanoparticle was spherical and was well agglomerated with a particle size range of 30-50nm (Sangeetha *et al.*, 2011). Earlier studies have reported that the average size of the ZnO nanoparticle was found to be 50nm and these results were in accordance with our results (Sangeetha *et al.*, 2011; Aswathi *et al.*, 2012).

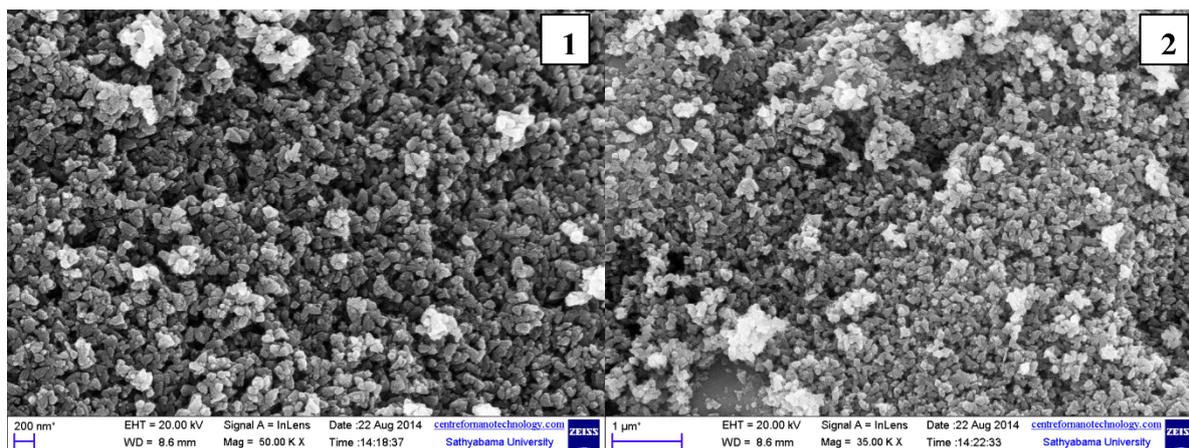


Figure 1 & 2: SEM images of ZnO nanoparticles produced using *Zingiber officinale*.

Antibacterial Studies: The ZnO nanoparticles synthesized using the root extracts of *Zingiber officinale* showed antibacterial activity against Gram negative (*E. coli*, *P. aeruginosa*, *K. pneumonia*) and Gram positive bacteria (*S. aureus*, *B. subtilis*). The antibacterial activity was performed with varying concentrations of ZnO nanoparticle, such as 0.5 mg/ml, 1 mg/ml, 1.5 mg/ml, 2 mg/ml, 2.5 mg/ml, 3 mg/ml and 4 mg/ml. It was found that the zone of inhibition increased with increase in concentration of the ZnO nanoparticle. The zone of inhibition was found to be 1 mm at 0.5 mg/ml, 1- 2.5 mm at 1 mg/ml, 2-3.5 mm at 1.5 mg/ml, 4-5 mm at 2 mg/ml, 2.5-3.5 mm at 2.5 mg/ml, 2-3 mm at 3 mg/ml and 2-3 mm at 4 mg/ml. According to Vani *et al.*, (2011) the concentration of nanoparticles increases with increase in antibacterial activity and this may be due to the destructive action of the ZnO

nanoparticles. Figures 3A, B, C, D & E shows the zone of inhibition of ZnO nanoparticles (Azam *et al.*, 2012). Figure 4 displays the graph for the zone of inhibition (mm) of different microorganisms.

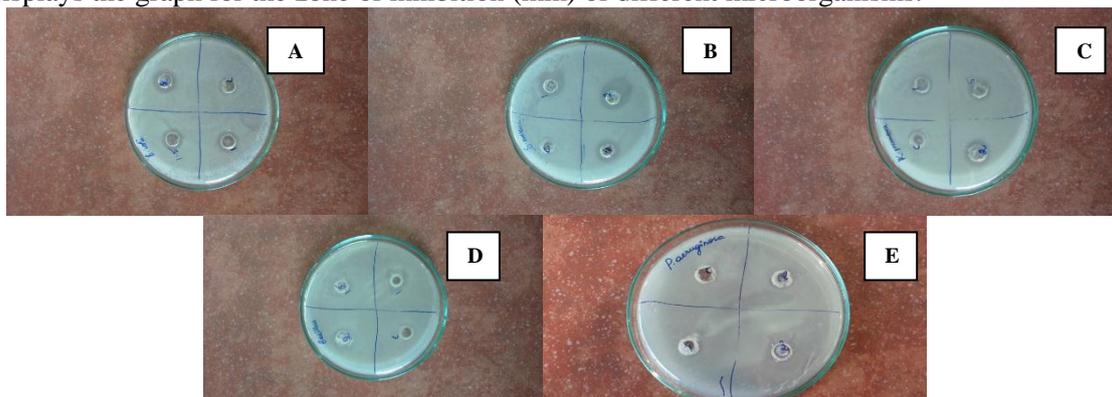


Figure 3: Zone of inhibition in (mm) produced by ZnO nanoparticles against different microbial strains (A) *E. coli*, (B) *S. aureus*, (C) *K. pneumonia*, (D) *B. subtilis*, (E) *P. aeruginosa*.

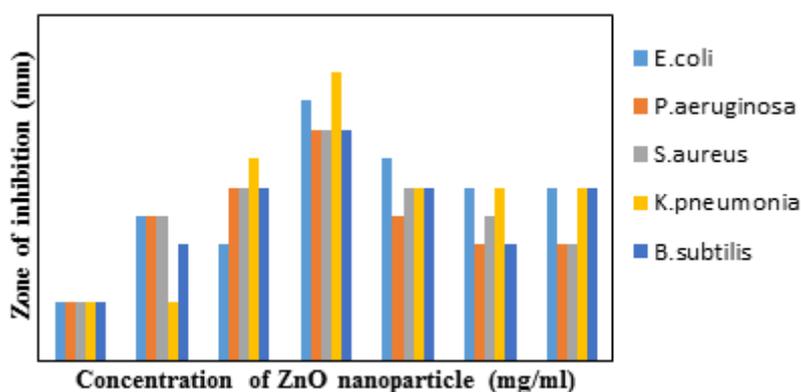


Figure 4. Bar graph showing the zone of inhibition (mm) of different microorganisms

The zone formation is a clear indication of the bactericidal activity of ZnO nanoparticles by generating a high rate of surface oxygen species which lead to the death of pathogens. (Sangeetha *et al.*, 2012) The antibacterial activity of ZnO nanoparticles may be influenced by the concentration and also depends on the size of the nanoparticle. The lesser the size of the nanoparticle the greater is the antibacterial activity and is also found to be more efficient in producing reactive oxygen species which helps in inhibiting the bacterial growth. Since the ZnO nanoparticle obtained in the study is 30nm which is of a lesser nanometer that may be responsible for the antibacterial activity (Azam *et al.*, 2012). Earlier studies suggest that the antibacterial activity of ZnO nanoparticle was due to electrostatic interaction between the negative charges of the pathogen with the positive charge of metal oxide this interaction oxidizes and kills the microorganism (Hosseinkhani *et al.*, 2011).

CONCLUSION

The present study dealt with the biosynthesis of Zinc Oxide nanoparticles using the root extracts of *Zingiber officinale* that were characterized using Scanning Electron Microscope. These results reveal that the nanoparticle were spherical in shape and had an average size range of 30nm. The synthesized ZnO nanoparticles were checked for antibacterial activity which may be due to the reactive oxygen species produced by the nanoparticle against bacteria as well as due to the electrostatic interaction between nanoparticles and bacterial species. Moreover the size of the nanoparticle also influenced the nanoparticle's antibacterial activity. The ZnO nanoparticle shows higher activity against gram negative bacterial strains than the gram positive strains. Thus, by using the greener method for producing ZnO nanoparticle can have a potential impact on treating ailments pertaining to pathogenic bacteria and hence can be applied in pharmaceuticals.

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