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# Antimicrobial and antifungal potential of zinc oxide nanoparticles in comparison to conventional zinc oxide particles

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**ABSTRACT**

*Nanotechnology has become increasingly internalized into pharmaceuticals & cosmetics and is of great significance as an approach to killing or reducing the activity of numerous microorganisms. Natural antibacterial materials, such as zinc and silver, are being claimed to possess good antibacterial properties. Nano-sized particles of ZnO have been claimed to have pronounced antimicrobial activities than large particles. Antimicrobial/antifungal potential of ZnO on five pathogens (Escherichia coli MTCC 443, Staphylococcus aureus MTCC 3160, Bacillus subtilis MTCC 441, Aspergillus niger MTCC 281, Candida albicans MTCC 227) and the influence of particles size of these inorganic powder on its antimicrobial /antifungal efficacy was considered in the present study. Results indicated that zinc oxide nanoparticles do have strong antibacterial and good antifungal activity against selected strains of bacteria and fungus as compared to that of conventional zinc oxide particles.*

**Keywords:** Nanotechnology, antibacterial, antifungal, efficacy.

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## INTRODUCTION

Nanotechnology is being envisioned as a hurriedly developing field, it has potential to revolutionize pharmaceuticals and cosmetics. Nanotechnology, or the use of materials with constituent dimensions on the atomic or molecular scale, has become increasingly applied to pharmaceuticals & cosmetics and is of great interest as an approach to killing or reducing the activity of numerous microorganisms. Some natural antibacterial materials, such as zinc and silver, are being claimed to possess good antibacterial properties.

Microbial spoilage of cosmetic formulation has always been of special concern for cosmetic industry. The use of authorized preservatives of the new regulation 1123/209 is required to prevent product damage caused by micro-organisms and to protect the product from unwanted contamination by the consumer during its shelf –life. However since few years, the cosmetic industry is facing some restrictions regarding the use of some preservatives. So, there has been considerable interest in the development of new preservatives. Among raw materials exhibiting antimicrobial/antifungal properties, inorganic powders such as zinc oxide (ZnO) represent a promising alternative to these chemical preservatives [1, 2].

Zinc oxide is a non-toxic , II-VI semiconductor with wide band gap (3.37eV) and natural n-type electrical conductivity [3, 4]. Zinc oxide because of its interesting properties, such as optical transparency, electrical conductivity, piezoelectricity, near-UV emission [5, 6, 7, 8, 9, 10] and various morphologies, has become one of the most attractive nanomaterials for research objectives. Its significant properties has made it applicable in UV-light emitters, varistors, transparent high power electronics, surface acoustic wave devices, piezo-electric transducers, gas sensors, etc.[11].

Introduction of zinc oxide in cosmetic creams and gels makes them sunlight-protective and antibacterial [12]. The efficiency of their action largely depends not only on the concentration of the active substance, zinc oxide, but also

on the size of its particles, their modification, and the degree of polydispersity. Moreover, zinc oxide (ZnO) is listed as "generally recognized as safe"(GRAS) by the U.S. Food and Drug Administration (21CFR182.8991). Nano-sized particles of ZnO have been claimed to have pronounced antimicrobial activities than large particles, considering the fact that the small size (less than 100 nm) and high surface-to-volume ratio of nanoparticles may allow for better interaction with bacteria. Recent studies have shown that these nanoparticles have selective toxicity to bacteria but exhibit minimal effects on human cells [1, 13, 14, 15, 16, 17, 18, 19].

Even if ZnO has been used for a long time in cosmetic or pharmaceutical ointments, its antimicrobial properties had not been fully investigated in context of cosmetic preservation. A systematic and detailed study was designed, taking into account above claims, to investigate the enhanced antimicrobial and antifungal properties of nano-zinc oxide over conventional one.

Therefore, the purpose of the present paper is to demonstrate firstly the antimicrobial/antifungal potential of ZnO on five pathogens (Escherichia coli MTCC 443, Staphylococcus aureus MTCC 3160, Bacillus subtilis MTCC 441, Aspergillus niger MTCC 281, Candida albicans MTCC 227) that are used for challenge tests, and secondly to determine the influence of particles size of these inorganic powder on its antimicrobial /antifungal efficacy.

## EXPERIMENTAL SECTION

### Materials

Nanoparticles of ZnO with a diameter of either ~65 nm were synthesized and used in this study. A representative TEM image of the ~65 nm ZnO nanoparticles is shown in fig. 1**.** Conventional zinc oxide nanoparticles with a diameter ~1000nm were used for the comparison.

### Selection of Test Pathogens

Pathogenic microorganisms selected for the study include three bacteria, viz., Escherichia coli (MTCC 443), Staphylococcus aureus (MTCC 3160), Bacillus subtilis (MTCC 441) and two fungus, viz., Aspergillus niger (MTCC 281) and Candida albicans (MTCC 227).

### Preparation of dilutions of synthesized compounds

10 mg of the each particle (nano and conventional zinc oxide) was weighed accurately and dissolved in 10 ml DMSO giving a solution of 1mg/ml concentration. 1 ml of the above solution was again diluted to 10 ml with DMSO giving a solution of 100µg/ml concentration.

### Preparation of Agar nutrient broth (for bacteria)

5.6g of Agar was dissolved in 150ml distilled water and heated. The medium was the sterilized by autoclaving at 115$^{o}$C for 30 mins.

### Preparation of Sabouraud Dextrose Agar (for fungi)

9.20g of Sabouraud Dextrose Agar was dissolved in distilled water and heated. The medium was the sterilized by autoclaving at 115$^{o}$C for 30 mins.

### Preparation of nutrient broth medium

0.75 g of media (Bacterial/fungal) was dissolved in 30 ml of distilled water and heated. The medium was then sterilized by autoclaving at 115$^{o}$C for 30 mins.

### Preparation of bacterial and fungal slants

Five Nessler cylinders were sterilized by hot sterilization method in an oven at 160$^{o}$C for 30 min. Laminar air flow cabinet was wiped with cotton immersed in ethanol and UV was switched ON for 15mins. Sterlized bacterial and fungal media were poured into 5 sterilized Nessler cylinders (3 bacterial, 2 fungal) and were allowed to stand in slant position till the media in the cylinders was solidified. Sterlized loop wire was used to transfer bacterial and fungal strains to nessler cylinders. The nessler cylinders were then labelled and cotton plugs were fitted into their mouth and were incubated at 37$^{o}$C except aspergillus niger (which was incubated at 25 $^{o}$C) for 24 hr. From each of the strain, small portion was transferred to 3ml of nutrient broth media separately and incubated at 37$^{o}$C for 24hrs. 0.1 ml of the above five medias were transferred to five different stoppered conical flasks containing 0.9% NaCl solution.

**Antimicrobial activity: Determination of Minimum inhibitory concentration and Minimum Bactericidal/ Fungicidal concentration**

Minimum inhibitory concentration (MIC) was determined for conventional and nano-sized zinc oxide nanoparticles showing antimicrobial and antifungal activity against test pathogens by serial dilution method. Broth microdilution method was followed for determination of MIC values. 1ml of media was taken in a test tube, to which, 1ml of test solution (100µg/ml) was added. Thereafter, 0.1ml of the microbial strain (bacterial/ fungal) prepared in 0.9% NaCl was added to the test tube containing media and test solution. Serial dilution were done five times giving concentrations of 50, 25, 12.5, 6.25, 3.75, 1.5 µg/ml. The test tube were stoppered with cotton and incubated at $37^oC$. The time incubation time varied for different strains (bacteria/fungus), i.e., 24 hrs for bacteria and one week for fungus.

The MIC values were taken as the lowest concentration of the particles in the test tube that showed no turbidity after incubation. The turbidity of the contents in the test tube was interpreted as visible growth of microorganisms. The minimum bacterial/fungicidal concentration (MBC/MFC) was determined by subculturing 50µl from each test tube showing no apparent growth. Least concentration of test substance showing no visible growth on subculturing was taken as MBC/MFC.

## RESULTS AND DISCUSSION

Zinc oxide nanoparticles particles were fully characterized. A representative TEM image of the ZnO nanoparticles (~65 nm) is shown in figure 1**.**

Secondly, the antimicrobial properties of conventional ZnO particles and ZnO nanoparticles were studied. Both, the conventional particles and nanoparticles, showed antimicrobial activity against Escherichia coli MTCC 443, Staphylococcus aureus MTCC 3160 and Bacillus subtilis MTCC 441 with a size dependent effect. Figure 2 & figure 3 portray the behaviour of bacterial populations following the incubation with conventional ZnO particles and ZnO nanoparticles for 24 hrs. The Minimum inhibitory concentration of ZnO nanoparticles (as shown in table 1) against the three bacterias, viz., Escherichia coli MTCC 443, Staphylococcus aureus MTCC 3160 and Bacillus subtilis MTCC 441 was found to be 6.25µg/ml, 6.25µg/ml, 12.5µg/ml, respectively which is very less concentration as compared to that of conventional zinc oxide particles (25µg/ml, 12.5µg/ml, 12.5µg/ml, respectively). Similarly the Minimum bacterial count for ZnO nanoparticles was less in each case as compared to conventional ZnO particles (table 1 & 2). Figure 4 & figure 5 portray the intense bacterial growth on the plate in the presence of conventional zinc oxide particles and fewer bacterial growths on the plate in the presence of ZnO nanoparticles. The difference of sensitivity to same test substance between these three strains can be attributed to structural and chemical differences of their bacterial cell walls [20].

According to a study by Yamamoto et al., 2000 [21], the presence of reactive oxygen species (ROS) generated by ZnO nanoparticles is responsible for their bactericidal activity. Zhang et al., 2010 [22], further proposed that the antibacterial behaviour of ZnO nanoparticles could be due to chemical interactions between hydrogen peroxide and membrane proteins, or between other chemical species produced in the presence of ZnO nanoparticles and the outer lipid bilayer of bacteria. The hydrogen peroxide produced enters the cell membrane of bacteria and kills them. The study also showed that nano-sized ZnO particles are responsible for inhibiting bacterial growth [22]. Further, Padmavathy and Vijayaraghavan, 2008 [23], showed the bactericidal activity of ZnO nanoparticles. As per their findings, once hydrogen peroxide is generated by ZnO nanoparticles, the nanoparticles remains in contact with the dead bacteria to prevent further bacterial action and continue to generate and discharge hydrogen peroxide to the medium. The results of the present study correspond with the results of the authors above, showing that ZnO nanoparticles have an excellent antimicrobial activity**.**

Zin oxide also exhibited antifungal activity but in a minor extent than the antibacterial one since no fungicidal activity is reported. The ZnO nanoparticles did show activity against Aspergilllus Niger and Cadida ablicans at a concentration of 12.5µg/ml and 6.25µg/ml, respectively. Again these concentration were higher for conventional ZnO particles, i.e., 25 and 12.5, respectively (table 1). The Minimum fungal count for zinc oxide nanoparticles was found to be same as Minimum inhibitory concentration, i.e., 12.5µg/ml and 6.25µg/ml, respectively. Same was the case with conventional ZnO particles in case of Aspergillus niger, where MFC (25µg/ml) was same as MIC (25µg/ml). But the antifungal activity against candida albicans showed different pattern in view that MFC (25µg/ml) was more than MIC (12.5µg/ml). Figure 6 & figure 7 represent the intense fungal growth on the plate in the presence of conventional zinc oxide particles and fewer fungal growths on the plate in the presence of ZnO nanoparticles.

**Table 1 Determination of MIC and MBC for conventional zinc oxide particles**

| Pathogen | Concentration (µg/ml) | Observation | Minimum Inhibitory concentration (µg/ml) | Minimum bacterial concentration (µg/ml) |
|---|---|---|---|---|
| *Escherichia coli (MTCC 443)* | 50 | No turbidity | 12.5 | 12.5 |
| | 25 | No turbidity | | |
| | 12.5 | No turbidity | | |
| | 6.25 | Turbidity | | |
| | 3.75 | Turbidity | | |
| | 1.5 | Turbidity | | |
| *Staphylococcus aureus (MTCC 3160)* | 50 | No turbidity | 12.5 | 12.5 |
| | 25 | No turbidity | | |
| | 12.5 | No turbidity | | |
| | 6.25 | Turbidity | | |
| | 3.75 | Turbidity | | |
| | 1.5 | Turbidity | | |
| *Bacillus subtilis (MTCC 441)* | 50 | No turbidity | 25 | 25 |
| | 25 | No turbidity | | |
| | 12.5 | No turbidity | | |
| | 6.25 | Turbidity | | |
| | 3.75 | Turbidity | | |
| | 1.5 | Turbidity | | |
| *Aspergillus niger (MTCC 281)* | 50 | No turbidity | 25 | 25 |
| | 25 | No turbidity | | |
| | 12.5 | Turbidity | | |
| | 6.25 | Turbidity | | |
| | 3.75 | Turbidity | | |
| | 1.5 | Turbidity | | |
| *Candida albicans (MTCC 227)* | 50 | No turbidity | 12.5 | 25 |
| | 25 | No turbidity | | |
| | 12.5 | No turbidity | | |
| | 6.25 | Turbidity | | |
| | 3.75 | Turbidity | | |
| | 1.5 | Turbidity | | |

**Table 2 Determination of MIC and MBC for zinc oxide nanoparticles**

| Pathogen | Concentration (µg/ml) | Observation | Minimum Inhibitory concentration (µg/ml) | Minimum bacterial concentration (µg/ml) |
|---|---|---|---|---|
| *Escherichia coli (MTCC 443)* | 50 | No turbidity | 6.25 | 6.25 |
| | 25 | No turbidity | | |
| | 12.5 | No turbidity | | |
| | 6.25 | No turbidity | | |
| | 3.75 | Turbidity | | |
| | 1.5 | Turbidity | | |
| *Staphylococcus aureus (MTCC 3160)* | 50 | No turbidity | 6.25 | 6.25 |
| | 25 | No turbidity | | |
| | 12.5 | No turbidity | | |
| | 6.25 | No turbidity | | |
| | 3.75 | Turbidity | | |
| | 1.5 | Turbidity | | |
| *Bacillus subtilis (MTCC 441)* | 50 | No turbidity | 12.5 | 12.5 |
| | 25 | No turbidity | | |
| | 12.5 | No turbidity | | |
| | 6.25 | Turbidity | | |
| | 3.75 | Turbidity | | |
| | 1.5 | Turbidity | | |
| *Aspergillus niger (MTCC 281)* | 50 | No turbidity | 12.5 | 12.5 |
| | 25 | No turbidity | | |
| | 12.5 | No turbidity | | |
| | 6.25 | Turbidity | | |
| | 3.75 | Turbidity | | |
| | 1.5 | Turbidity | | |
| *Candida albicans (MTCC 227)* | 50 | No turbidity | 6.25 | 6.25 |
| | 25 | No turbidity | | |
| | 12.5 | No turbidity | | |
| | 6.25 | No turbidity | | |
| | 3.75 | Turbidity | | |
| | 1.5 | Turbidity | | |

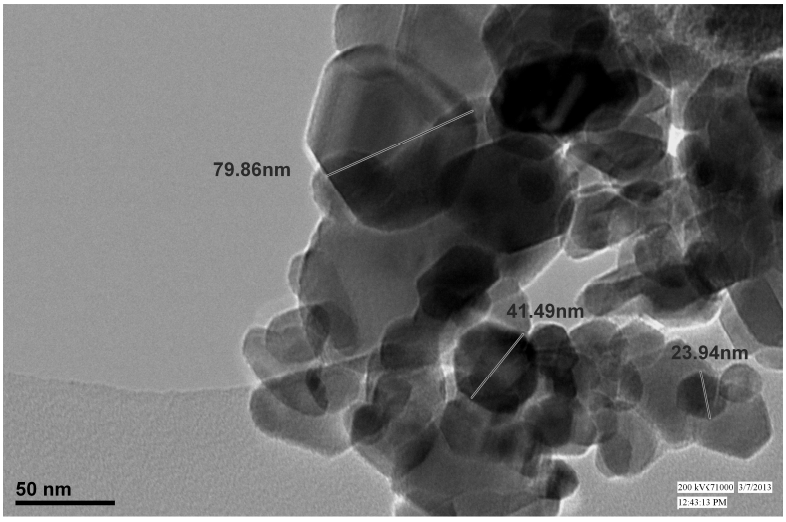


**Figure 1 TEM image of zinc oxide nanoparticles**

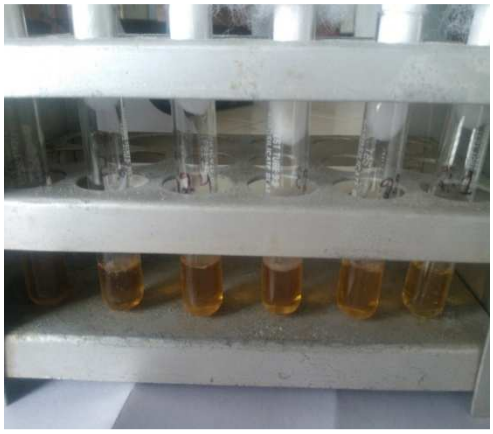

**Figure 2 Determination of MIC for conventional zinc oxide particles against bacterial strain**

**Figure 3 Determination of MIC for zinc oxide nanoparticles against bacterial strain**

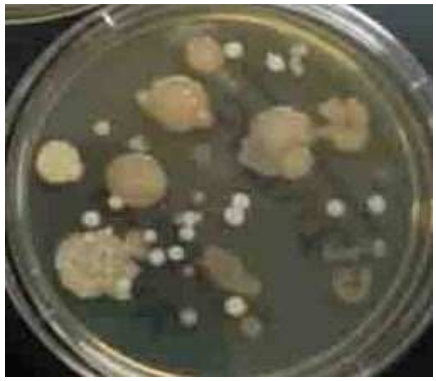

**Figure 4 Bacterial growth in the presence of conventional zinc oxide particles**

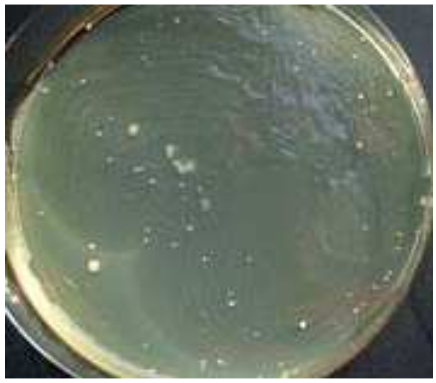

**Figure 5 Bacterial growth in the presence of zinc oxide nanoparticles**

**Figure 6 Fungal growth in the presence of conventional zinc oxide particles**

**Figure 7 Fungal growth in the presence of zinc oxide nanoparticles**

## CONCLUSION

Results in present study indicate that zinc oxide nanoparticles had strong antibacterial and good antifungal activity against selected strains of bacteria and fungus as compared to that of conventional zinc oxide particles. In summary,

the present study reveals that zinc oxide nanoparticles could potentially be an antibacterial and antifungal agent to treat infections caused by bacteria and fungus. In future, these nanoparticles might replace conventional preservatives in cosmetics. However, antibacterial/antifungal effects, safety, and detailed mechanisms of zinc oxide nanoparticles should be further studied in vitro and in vivo.

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