International Scholars Journals

Advanced Journal of Microbiology Research ISSN 2241-9837 Vol. 13 (2), pp. 001-006, February, 2019. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Antibacterial activity of ZnO nanoparticle on gram-positive and gram-negative bacteria

Zarrindokht Emami-Karvani* and Pegah Chehrazi

Islamic Azad University, Falavarjan Branch, Iran.

Accepted 10 January, 2019

The aim of the present study is to determine the antimicrobial activity of ZnO nanoparticles against Gram-negative and Gram-positive bacteria. *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*) were used as test microorganisms. The effects of particle size and concentration on the antibacterial activity of ZnO nanoparticles was studied using bacteriological tests such as disc and well diffusion agar methods, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). These tests were performed in nutrient broth and nutrient agar following standard methods. In addition, the effect of different concentrations of ZnO nanoparticles on the growth of *E. coli* and *S. aureus* was measured with respect of time. The minimum inhibitory concentration was determined using seven different concentrations of ZnO nanoparticles including 16, 8, 4, 2, 1 and 0.5 mg/ml. The MIC value for *E. coli* and *S. aureus* was 1 and 0.5 mg/ml, respectively. The results showed that ZnO nanoparticles have antibacterial inhibition zone of 29 and 19 mm at the concentration of 10 mg/ml against *E. coli* and *S. aureus,* respectively. Gram-negative bacteria seemed to be more resistant to ZnO nanoparticles than Gram-positive bacteria. It was found that the antibacterial activity of ZnO nanoparticles increased with decreasing particle size and increasing powder concentration. The antibacterial effect of ZnO nanoparticles was time dependent and takes effect gradually. ZnO bulk powder showed no significant antibacterial activity.

Key word: ZnO nanoparticle, *Escherichia coli, Staphylococcus aureus*, minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC).

INTRODUCTION

Nanoparticlesare is a special group of materials with unique features and extensive applications in diverse fields (Matei et al., 2008) . Studying these particular features has always been of great interest to many scientists. In fact, nanoparticles display completely unique properties in comparison with their bulk size counterparts (Priyanka et al., 2009). A large number of materials which were considered to be safe develop toxicity at nano size ranges (Reddy et al., 2007) which is mainly related to the increased specific surface area and high reactivity of nano size materials (Nagarajan and Rajagopalan, 2008; Laura et al., 2006). A larger surface area (as in case of nanoparticles) ensures an increased range of probable interaction with bio- organics present on the viable cell surface (Rizwan et al., 2010c) . The considerable antimicrobial activities of inorganic metal oxide nanoparticles such as ZnO, MgO, TiO_2, SiO_2 and their selective toxicity to biological systems suggest their potential application as therapeutics, diagnostics, surgicaldevices 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). The advantages of using these inorganic oxides nanoparticles as antimicrobial agents are their greater effectiveness on resistant strains of microbial pathogens, less toxicity and heat resistance. In addition, they provide mineral elements essential to human cells and even small amounts of them exhibit strong activity

*Corresponding author. E-mail: emami@iaufala.ac.ir, zarrindokht4@gmail.com. Tel: +98-913-2034159. Fax: +98-312-3120136.

(Nagarajan and Rajagopalan, 2008; Toshiaki et al., 2008; Zakaria et al., 2010) Among metal oxide nanoparticles, ZnO nanoparticles as one of the multifunctional inorganic nanoparticles has many significant features such as chemical and physical stability, high catalysis activity, effective antibacterial activity as well as intensive ultraviolet and infrared adsorption with broad range of applications as semiconductors, sensors, transparent electrodes, solar cells, etc. (Matei et al., 2008; Kalyani et al., 2006). Also in recent years ZnO has received considerable attention because of its unique optical, piezoelectric, and magnetic properties (Marcus and Paul, 2007). In addition ZnO nanoparticles has the potential to impact many aspects of food and agricultural systems because of its antimicrobial efficacy especially with the growing need to find alternative methods for formulating new type of safe and cost-effective antibiotics in controlling the spread of resisted pathogens in food processing environment (Jin et al., 2009; Rizwan et al., 2010a). Some data suggest the selective toxicity of the ZnO nanoparticles toward cancer cells (Shantikumar et al., 2009). The anticancer effects of ZnO nanostructures on human brain tumor U87 and cervical cancer Hela were obtained and indicate promising activity that varies with the changes in the structure and the size (Rizwan et al., 2010b). Therefore, the present investigation was aimed to determine the antibacterial activity of ZnO nanoparticles toward *E coli* as Gram-negative bacteria and *S. aureus* as Gram-positive bacteria in laboratory condition.

MATERIALS AND METHODS

Preparation of the materials and bacterial cultures

ZnO nanoparticles powder which were prepared by Amiri followed by Nosaka method were used in this experiment (Nosaka et al., 1998). The size of prepared ZnO nanoparticles was 3 nm.

Staphylococcus auerus PTCC 1431 and *Escherichia coli* PTCC 1399 were obtained from Persian Type Culture Collection. All these strains were grown aerobically in nutrient broth for 24 h at 37°C before using as target organisms. The density of bacterial isolates was adjusted to an optimal density of 0.5 McFarland standards.

Antibacterial activity assay

In order to examine the antibacterial activity of the ZnO nanoparticles on these microorganisms, ZnO nanoparticles were suspended in sterile normal saline and constantly stirring until a uniform colloidal suspension was formed to yield a powder concentration of 1000 mg/ml. To assess toxicity range of ZnO nanoparticles against *E coli* and *S. aureus,* an appropriate volume of test bacteria were inoculated in nutrient broth medium supplemented with serially diluted ZnO nanoparticles with two various particle size and bulk suspensions, from 100 to 0.78 mg/ml. After these experiments, the best range was proposed from 0.5 to 16 mg/ml of nanoparticle-free medium and bacteria-free medium were used as control positive and control negative respectively. Colony forming units (cfu) were quantified after an overnight incubation at 37°C.

Determination of zone of Inhibition

0.05 and 0.1 ml was added of various concentrations of two different ZnO nanoparticle sizes and bulk ZnO in discs and wells, respectively. After inoculation and cultivation of different target bacteria on top of nutrient agar, discs and wells were placed in selected area on different plates. The zone of inhibition (ZOI) was measured after 24 h incubation. The antibacterial activity of two different particle sizes and bulk ZnO were compared. To gain different nanoparticle size, equal amount of synthesized ZnO nanoparticles were dried at two different temperatures, 40 and 70°C. Increasing temperature resulted in bigger particle size.

Determination of minimum inhibitory concentration

MIC and MBC were measured using agar dilution tests After inoculation of target bacteria on nutrient agar with various concentrations of ZnO nanoparticles, the growth rates of bacteria were determined by counting colony forming unit (cfu) in each plate. The plates which show no growth after 24 h incubation were selected 0 1 ml of sterile distilled water was added to these plates and transferred to fresh medium which had not any ZnO nanoparticles. The lowest concentration from which the bacteria do not grow when transferred to fresh medium is MBC and MIC is the lowest concentration from which the colonies appeared on top of fresh medium

Time dependent test

Time dependent tests were performed in nutrient broth supplemented with different concentrations of ZnO nanoparticles inoculated with the same amounts of test bacteria. Following incubation at 37°C, 0.1 ml of different cultures was spread separately on nutrient agar with respect of time. After 24 h, cfu was quantified for each plate and compared with cfu in control plates. All experiments were performed in triplicate and the averages were obtained.

RESULTS AND DISCUSSION

The antibacterial activity of ZnO nanoparticles was tested by the disc and well diffusion agar methods (Tables 1 and 2). The presence of an inhibition zone clearly indicated the antibacterial effect of ZnO nanoparticles. As it was also shown in the study of Rizwan et al. (2010c) it has been seen in this study that by increasing the concentration of ZnO nanoparticles in wells and discs, the growth inhibition has also been increased. The size of inhibition zone was different according to the type of bacteria, the size and the concentrations of ZnO nanoparticles.

Number of colony forming unit (cfu) of *E. coli* and *S. aureus* after overnight incubation at the presence of different concentrations of ZnO nanoparticles was shown in Figure 1. The minimum concentration of ZnO nanoparticles which inhibited the growth of bacteria was 3.1 mg/ml for *E. coli* and 1.5 mg/ml for *S.aureus*. This is in agreement with previously published reports on the antibacterial properties of ZnO nanoparticles which showed that the minimum concentration at which the

Table 1. Zone of inhibition (ZOI) for *S. areus.*

ZnO concentration in wells (mg/ml)	ZOI (mm)	ZnO concentration in discs(mg/ml)	ZOI (mm)
10	29	5	22
5	27	2.5	19
2.5	25	1.25	16
1.25	21	0.625	14
0.625	17	0.312	12
0.312	15	0.156	10
0.156	14	0.078	9
0.078	*14	0.039	*9
0.039	0	0.0195	0
0.0195	0	0.00975	0
Control	0	Control	0

* Minimum concentrations of ZnO nanoparticles at which zone of inhibition started to appear.

Table 2. Zone of inhibition (ZOI) for *E.coli.*

ZnO concentration in wells(mg/ml)	ZOI (mm)	ZnO concentration in discs(mg/ml)	ZOI (mm)
10	19	5	28
5	16	2.5	24
2.5	14	1.25	21
1.25	12	0.625	19
0.625	*10	0.312	*14
0.312	0	0.156	0
0.156	0	0.078	0
0.078	0	0.039	0
0.039	0	0.0195	0
control	0	Control	0

* Minimum concentrations of ZnO nanoparticles at which zone of inhibition started to appear.

growth of *E. coli* and *S. aureus* was inhibited was 3.4 and 1 mM, respectively (Reddy et al., 2007). The results of MIC and MBC for *E. coli* and *S. aureus* were summarized (Tables 3 and 4). Based on the results obtained from MIC, MBC, disc and well agar diffusion methods, it can be suggested that in comparison with Gram-positive bacteria, the growth of gram-negative bacteria is inhibited at higher concentrations of ZnO nanoparticles (Figure 2). Reddy et al. (2007) have reported the same results, emphasizing on the higher susceptibility of Gram-positive bacteria in comparison with Gram-negative bacteria. In the study done by Selahattin et al. (1998), 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. The results of time-dependant antibacterial activity of ZnO nanoparticles showed that cfu of the tested bacteria for each concentration decreased gradually during 72 h, whereas colony formation of control solution remained uncountable (Figures 3 and 4).

Significant differences was observed between antibacterial activity of bulk ZnO, yellow ZnO nanoparticles dried at 70°C and white ZnO nanoparticles dried at 40°C. The antibacterial efficacy increased with decreasing particle size from bulk ZnO to white ZnO nanoparticles (Figure 5). Particle concentration seems to be more effective on the inhibition of bacterial growth than particle size under the condition of this work (Figure 5) (Lingling et al., 2006). 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 antibacterial agents both on Gram-positive and Gram-negative bacteria. The same results were confirmed in the study of Zhongbing et al. (2008) in which Gram-negative membrane and Gram-positive membrane disorganization was approved by transmission electron microscopy of bacteria ultrathin sections. In order to use ZnO nanoparticles in *in vivo* condition, further studies should be performed investigating the toxic effect of ZnO nanoparticles on eukaryotic cells. The study done by Alok and Vyom (2010)

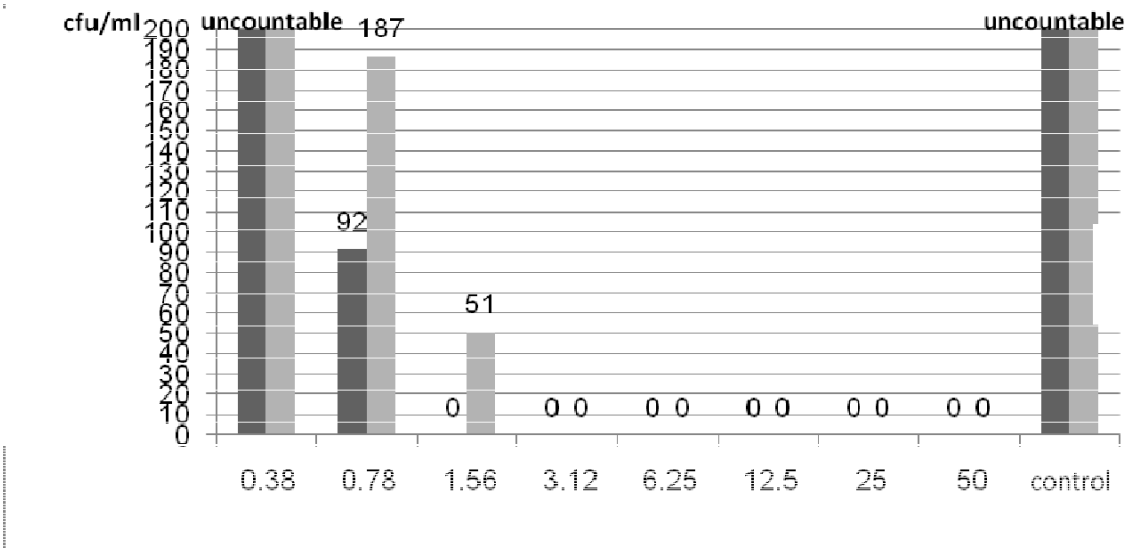

Figure 1. Number of colony forming units (cfu) of *E. coli* and *S. aureus* after overnight incubation at the presence of different concentrations of ZnO nanoparticles.

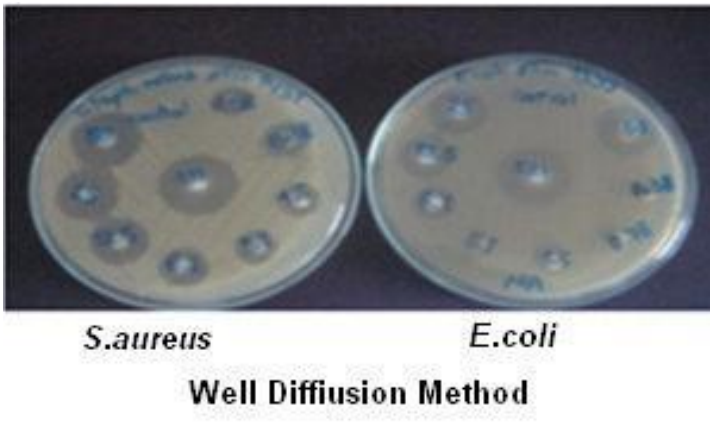

Figure 2. Comparison of antibacterial activity of ZnO nanoparticles on *E. coli* and *S. Aureus*

Table 3. Determination of MIC and MBC for *E. coli.*

Mode of effect	Concentration (mg/ml)
Growth	0.125
Growth	0.25
Growth	0.5
Bacteriostatic	1(MIC)
Bacteriostatic	2
Bacteriostatic	4
Bacteriostatic	8
Bactericidal	16(MBC)

Table 4. Determination of MIC and MBC for *S. aureus.*

Mode of effect	Concentration (mg/ml)
Growth	0.125
Growth	0.25
Bacteriostatic	0.5(MIC)
Bacteriostatic	1
Bacteriostatic	2
Bacteriostatic	4
Bactericidal	8(MBC)
Bactericidal	16

emphasized on the necessity of additional experiments on the safety/toxicity properties of nanoparicles, due to the many experimental challenges encountered when assessing the toxicity of them. The results of this study

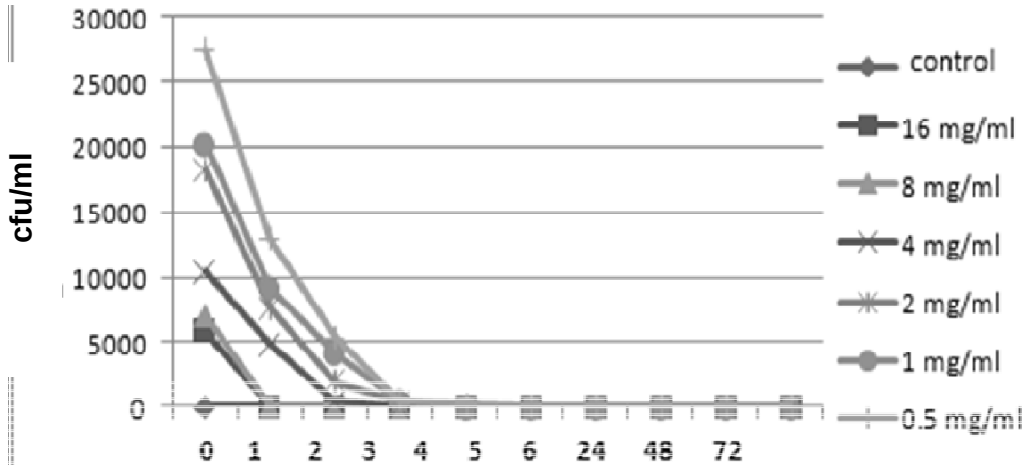

Figure 3. Number of colony forming units (cfu) of *E. coli* with respect of time.

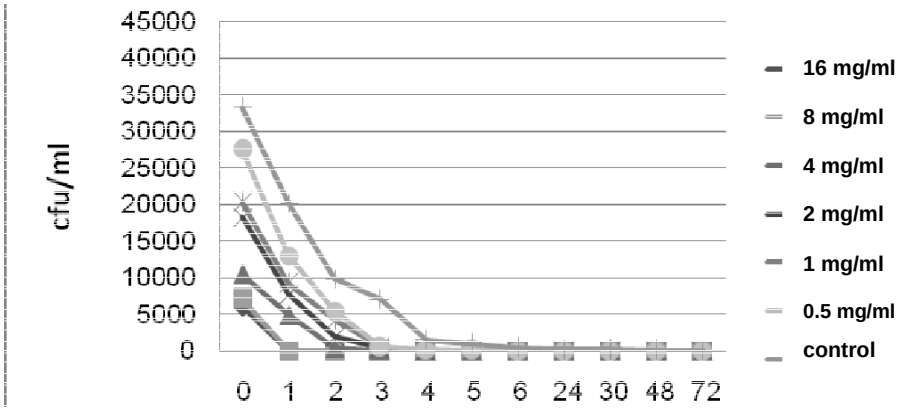

Figure 4. Number of colony forming units (cfu) of *S. aureus* with respect of time.

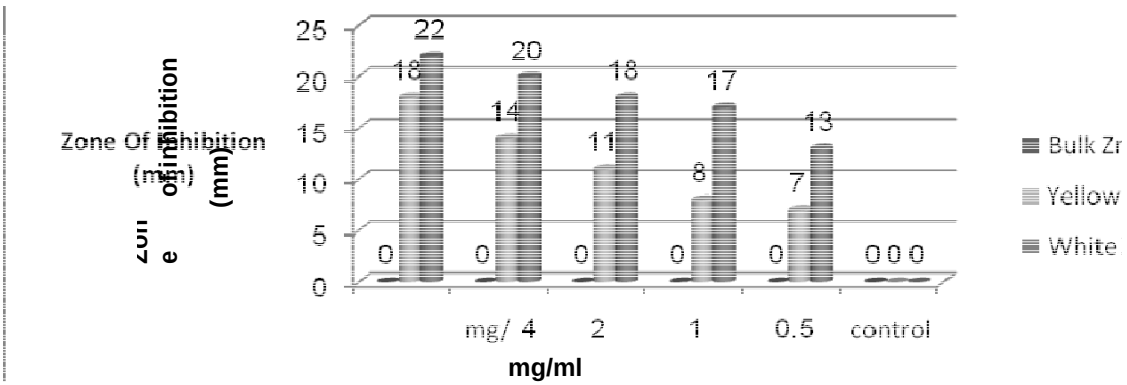

Figure 5. Comparison of zone of inhibition for different particle size in *E. coli.*

also highlighted the need for understanding how ZnO nanoparticles affect the bacterial cell and furthermore the mechanism by which ZnO nanoparticles affect viable cell.

It is proposed to work on the resistance mechanism of resistant strains which were encountered in contact with ZnO nanoparticles. It could be performed by studying the

plasmid profile and identification of the resistant gene.

ACKNOWLEDGEMENT

We thank Dr. Gholamreza Amiri for preparing ZnO nanoparticles for this study.

REFERENCES

Alok D, Vyom S (2010). Toxicity assessment of nanomaterials: methods and challenges. Anal. Bioanal. Chem., 398(2): 589-605.

Jin T, Sun D, Su JY, Zhang H, Sue HJ (2009). Antimicrobial efficacy of zinc oxide quantum dots against *Listeria monocytogenes*, *Salmonellaenteritidis*, and *Escherichia coli* O157:H. J. Food. Sci., 74(1): 46-52.

Kalyani G, Anil VG, Bo-Jung C, Yong-Chien L (2006). Preparation and characterization of ZnO nanoparticles coated paper and its antibacterial activity study. J. Green Chem., 8: 1034-1041.

Laura KA, Delina YL, Pedro JJA (2006). Comparative eco-toxicity of nanoscale TiO2, SiO_2, and ZnO water suspensions. J. Water Res., 40: 3527–3532.

Lingling Z, Yunhong J, Yulong D, Malcolm P, David Y (2006). Investigation into the antibacterial behavior of suspensions of ZnO nanoparticles (ZnO nanofluids). J. Nanoparticle Res., 9(3): 479-489.

Marcus CN, Paul AW (2007). ZnO tetrapod nanocrystals. J. Mater. today., 10(5): 50-54.

Matei A, Cernica I, Cadar O, Roman C, Schiopu V (2008). Synthesis and characterization of ZnO – polymer nanocomposites. Int. J. Mater. Form., 1: 767-770.

Mohsen J, Zahra B (2008). Protein nanoparticle: A unique system as drug delivery vehicles. Afr. J. Biotechnol., 7(25): 4926-4934.

Nagarajan P, Rajagopalan V (2008). Enhanced bioactivity of ZnO nanoparticles—an antimicrobialstudy. J. Sci. Technol. Adv. Mater., 9(3): 035004.

Priyanka G, Brian P, David WB, Wenjie H, William PJ, Anne JA (2009). Antimicrobial activities of commercial nanoparticles against an environmental soil microbe, *Pseudomonas putida* KT2440. J. Bio. Eng., 3(9): 1-13.

Reddy KM, Kevin F, Jason B, Denise GW, Cory H, Alex P (2007). Selective toxicity of zinc oxide nanoparticles to prokaryotic and eukaryotic systems. J. Appl. Phys. Lett., 90(21): 1-3.

Rizwan W, Amrita M, Soon-Il Y, Young-Soon K, Hyung-Shik Sh (2010a). Antibacterial activity of ZnO nanoparticles prepared via non-hydrolytic solution route. J. Appl. Microbial. Biotechnol., 87(5): 1917-1925.

Rizwan W, Nagendra KK, Akhilesh KV, Anurag M, Hwang IH, You-Bing Y, Hyung-Shik Sh, Young-Soon K (2010b). Fabrication and growth mechanism of ZnO nanostructures and their cytotoxic effect on human brain tumor U87, cervical cancer HeLa, and normal HEK cells. J. Biol. Inorg. Chem., 16(3): 431-442.

Rizwan W, Young-Soon K, Amrita M,Soon-Il Y, Hyung-Shik Sh (2010c). Formation of ZnOmicro-flowers prepared via solution process and their antibacterial activity. J. Nanoscale Res. Lett., 5(10): 1675–1681.

Sawai J, Yoshikawa T (2003). Quantitative evaluation of antibacterial activities of metallic oxide powders ZnO, MgO and CaO by conductimetric assay. J. Microb. Meth., 54(2): 177-182.

Selahattin A, Kadri G, Ramazan (1998). The effect of zinc on microbial growth. Tr. J. Med. Sci., 28: 595-597.

Shantikumar N, Abhilash S, VVDivya R, Deepthy M, Seema N, Manzoor K, Satish R (2009). Role of size scale of ZnO nanoparticles and microparticles on toxicity toward bacteria and osteoblast cancer cells. J. Mater. Sci: Mater Med., 20: 235–241.

Sobha K, Surendranath K, Meena V, Jwala KT, Swetha N, Latha KSM (2010). Emerging trends in nanobiotechnology. J. Biotech. Mol. Bio. Rev., 5(1): 001-012.

Toshiaki O, Osamu Y, Yasuhiro I, Zenbe-e N (2008). Antibacterial activity of ZnO powder with crystallographic orientation. J. Mater. Sci. Mater. Med., 19(3): 1407-1412.

Zakaria ZA, Mat Desa A, Ramasamy K, Ahmat N, Mohamad AS, Israf DA, Sulaiman MR (2010). Lack of antimicrobial activities of Dicranopteris linearisextracts and fractions. Afr. J. Microbial. Res., 4(1): 071-075.

Zhongbing H, Xu Zh, Danhong Y, Guangfu Y, Xiaoming L, Yunqing K, Yadong Yao, Di Huang, Baoqing H (2008). Toxicological effect of ZnO nanoparticles based on bacteria. Langmuir, 24(8): 4140–4144.