

Review Paper

MECHANISM OF ACTION OF ANTIBACTERIAL ACTIVITY OF BIOSYNTHESIZED ZINC OXIDE NANOPARTICLES - A REVIEW

ABSTRACT

Now-a-days, nanotechnology has become increasingly important in the biomedical and pharmaceutical areas as alternative antimicrobial strategy due to their wide spectrum of action at nano-scale level. Antibacterial agents are very important in the textile industry, water disinfection, medicine, food packaging etc. This review focuses on the synthesis and mechanism of antibacterial activity of zinc oxide nanoparticles (ZnO NPs). The antibacterial activity of ZnO NPs is determined by the mode of synthesis, organisms used, size of the nanoparticles etc. The variations in the antibacterial activity of ZnO NPs are mostly attributed to their high specific surface area-to-volume ratios and their distinctive physicochemical properties. Overall, the review suggest that ZnO nanoparticles can be used externally to control the variety of bacterial pathogens, hence it can be useful in many fields.

Keywords: Nanoparticles, biosynthesis, ZnO, antibacterial, mechanism

1. NANOPARTICLES

Nanoparticles are microscopic particles with at least one dimension less than 100nm. Nanoparticles are of great scientific interest as because they bridge the gap between bulk materials and atomic or molecular structures. Nanoparticles can be broadly grouped in two categories like namely: organic and inorganic nanoparticles. Organic nanoparticles include carbon nanoparticles (fullerenes) and while the inorganic nanoparticles include magnetic nanoparticles, noble metal nanoparticles like gold and silver and semiconductor nanoparticles like titanium oxide and zinc oxide. Inorganic nanoparticles have created great interest among the researchers, as they provide superior material properties with functional versatility [1].

Nanoparticle research is an area of intense scientific research due to the wide variety of potential applications such as in biomedical, optical and electrical fields. These Nanoparticles have mostly can only be been formed in chemical reactions either by combustion or by condensation [2]. Nanoparticles were are expected to form the basis of many of the technological and biological innovations of this century, exhibiting distinct advantageous physical, chemical and biological and properties, and hybrid techniques. The synthesis of nanoparticles with specific morphologies and properties has been was one of the most important aspects of nanoscience which studies material whose size lies within the nanometer range (1

Formatted: Font color: Auto

Formatted: Font color: Red

Formatted: Font color: Red

Formatted: Highlight

Formatted: Font color: Red

Formatted: Highlight

Formatted: Font color: Red

Formatted: Highlight

Formatted: Font color: Red

Formatted: Highlight

Formatted: Font color: Red

Formatted: Font color: Red

Formatted: Font color: Red

Formatted: Font color: Red

Formatted: Highlight

Formatted: Font color: Red

Formatted: Highlight

Formatted: Font color: Red

Formatted: Highlight

Formatted: Font color: Red

Formatted: Highlight

Formatted: Font color: Red

Formatted: Highlight

Formatted: Font color: Red

Formatted: Font color: Red

Formatted: Highlight

36 | nm=10⁻⁹ m). Applications of these These nanostructures were seen have been used in catalysis,
37 | sensors, water purification, antibacterial and nano-electronics. The noble metals such as gold;
38 | silver and platinum were have been widely applied in products that directly come in contact with
39 | the human body such as detergent, soaps, cosmetic products and toothpaste besides medical and
40 | pharmaceutical applications [3].

41 | 2. ZINC OXIDE NANOPARTICLES

42 | Zinc oxide nanoparticles (ZnO NPs) are unstable, and easily agglomerates and are very difficult to recycle
43 | after use. Therefore, preventing the agglomeration of these nanostructures is the key factor for
44 | preserving the high photoactivity of nanoscale photocatalysts. low level or even absence of
45 | agglomerations suggest that the Critical Coagulation Concentration of the nanoparticles will correlates
46 | positively with the specific surface area, i.e. particles with higher specific surface area exhibited higher
47 | stability. Furthermore, the strong dependence on the specific surface area indicates that, it is the
48 | surface chemistry rather than the bulk properties that dominated in the microstructure [XXX1].

49 | To reduce or overcome the problem of agglomeration, one of the most efficient methods is to
50 | the use of semiconductor-based hetero structures with desirable compositions and/or
51 | morphologies. The properties of these semiconductors can be have been modified by adding
52 | noble metals with complementary properties to form core-shell, hetero, and/or doped structures
53 | [4]. Zinc oxide nanomaterials are non-toxic with wide band gap has been identified as a potential
54 | semiconducting material for exhibiting room temperature ferromagnetism when it is doped with
55 | transition metals [XXX2]. Doping of transition metals into zinc oxide nanoparticles have been
56 | are considered to be an effective method to fine-tune the energy level structure of the host which
57 | can be further improved by the different concentrations of the dopants leading refining the
58 | optical properties [5].

59 | Highly ionic nanoparticulate metal oxides (ZnO NPs) are unique in that they can be produced
60 | with high surface areas and with unusual crystal structures. Compared to organic
61 | materials nanomaterial, inorganic nanomaterials such as ZnO possess superior durability, greater
62 | selectivity, and heat resistance. It is known that the green synthesis of ZnO nanoparticles is much
63 | safer and environmentally friendly as compared to chemical synthesis [6]. The size of the
64 | synthesized ZnO nanoparticles was in the range of 60-70 nm. The larger nanoparticles of ZnO
65 | resulted from the agglomeration of smaller nanoparticles [6]. Generally, Biological synthesis
66 | methods of ZnO nanoparticles -have shown more attractive benefits relative to the conventional
67 | chemical synthesis methods.

68 | 3. BIOLOGICAL SYNTHESIS OF ZnO NPs

69 | Biological methods have emerged as an alternative to the conventional methods for synthesis of
70 | NPs. Synthesis of inorganic nanoparticles by biological systems makes nanoparticles more
71 | biocompatible and environmentally benign. Moreover, the process is cost effective too. Many

Formatted: Highlight

Formatted: Font color: Red

Formatted: Font color: Auto, Highlight

Formatted: Font color: Red

Formatted: Highlight

Formatted: Font color: Red

Formatted: Font color: Red

Formatted: Highlight

Formatted: Font color: Red

Formatted: Highlight

Formatted: Font color: Red

Formatted: Font color: Red

Formatted: Font color: Red

Formatted: Highlight

Formatted: Font color: Red

Formatted: Font color: Red

Formatted: Highlight

Formatted: Font color: Red

Formatted: Highlight

Formatted: Font color: Red

Formatted: Font color: Red

Formatted: Highlight

Formatted: Highlight

Formatted: Font color: Red

Formatted: Font color: Red

Formatted: Font color: Red

Formatted: Highlight

Formatted: Font color: Red

Formatted: Font color: Red

Formatted: Font color: Red

Formatted: Font color: Red

Formatted: Highlight

bacterial as well as fungal species have been used for zinc oxide nanoparticles synthesis. This synthesis always takes longer reaction times and **also** demands subsequent extraction and recovery steps. On the contrary, in plant extract mediated synthesis **such as the modified oxalate method [XXX2] and the mangosteen leaf extract [8]**, the reaction times have **also** been reported to be very short compared to that of microbial synthesis. Most importantly, the process can be suitably scaled up for large scale synthesis of NPs [7]. Green synthesis provided advancement over chemical and physical method as it **was is** cost effective, environment friendly, easily scaled up for large-scale synthesis, **and furthermore**, there **was is** no need to use high pressure, energy, temperature and toxic chemicals. Using plants for nanoparticle synthesis **was is** advantageous over other biological processes because it eliminates the elaborate process of maintaining cell cultures and can also be suitably scaled up for large-scale synthesis of nanoparticles under non-aseptic environment [8].

Formatted: Highlight

Formatted: Highlight

Formatted: Highlight

Formatted: Highlight

Formatted: Highlight

Formatted: Highlight

The “bio - synthesis” of metallic nanoparticles receives more attention due to their uncommon optical, photo-chemical, chemical and electrical properties [9]. The bio - methods of nanoparticle synthesis using biological entities like bacteria, yeast, fungi and plants are stated to be clean, nonhazardous, in-expensive and environmentally tolerable when compared to chemical methods. The use of microorganisms or plant-based biomimetic methods consumes very little energy, generate very little pollution, and mostly operate under conditions of normal temperature and pressure [10]. Among the several biological methods of nanoparticle synthesis, microbe facilitated synthesis of nanoparticle is, however, not commercially viable as they involve maintenance of highly hygienic conditions and very complex processes of maintaining microbial cultures. Additionally, the microorganisms require a relatively longer incubation time for the reduction of metallic ions as compared to plants which attained such education in a much shorter time due to the presence of water soluble phytochemicals which act as reducing agents [11,12,13].

4. ANTIBACTERIAL ACTIVITY OF ZnO NPs

The antimicrobial activity of nanoparticles has been studied largely with human pathogenic bacteria, mainly *E.coli* and *S. aureus*. **[reference]. The** ZnO NP is currently being considered as an antibacterial agent in both micro as well as nano scale formulations**[reference]**. Results have specified that ZnO nanoparticles show antibacterial action apparently better than micro particles. **While However**, the exact mechanisms of the antibacterial action have not yet been clearly understood, **though** it has been advised that the rule of reactive oxygen species (ROS) produced on the surface of the particles, **(zinc ion) release, membrane dysfunction, and nanoparticles** internalization are the main reason of cell swelling [14]. Disc diffusion method was used for the assessment of antibacterial activity of ZnO nanoparticles against *Bacillus subtilis* **[15]**. The zone of inhibition was more for both ZnO nanoparticles and antibiotics like nitrofurantoin, tetracycline, nalidixic acid, gentamicin, and methicillin **[15]**.

Formatted: Highlight

Formatted: Highlight

Formatted: Highlight

Formatted: Highlight

Formatted: Highlight

109 Zinc oxide exhibited the antibacterial activity like many other metal oxide groups and like the
110 others only few have been scaled down to the nano size. The advantage of using inorganic oxides
111 such as zinc oxide as antimicrobial agents is that they contain mineral elements essential to
112 humans and exhibit strong activity even when administered in small amounts. ZnO nanoparticles
113 exhibited strong antibacterial activities on a broad spectrum of bacteria [16]. The antibacterial
114 activity of antibiotics along with ZnO nanoparticles was investigated against pathogenic
115 bacterium, *Proteus vulgaris* using disc diffusion technique [17]. *Proteus vulgaris* was resistant
116 against penicillin and ampicillin but the highest increase in the inhibition zones (antibiotic with
117 ZnO nanoparticles) were observed for erythromycin. The moderate increase in inhibition zones
118 were against penicillin followed by Ampicillin in combination with ZnO nanoparticles [17].

Formatted: Highlight

119 Antibacterial properties of ZnO nanoparticle of *E. coli*, *P. aeruginosa* and *S. aureus* was reported
120 by [18]. Antibacterial properties of nanoparticle increased with increase in concentration of
121 nanoparticles [19]. In both Gram positive and Gram negative bacteria, cell wall and cell
122 membrane are crucial in maintaining the osmotic balance and integrity of cell. Damage of
123 bacterial cell integrity could be the reason in present study [20]. Based on the results obtained
124 from disc and well agar diffusion methods, it can be suggested that in comparison with Gram-
125 positive bacteria, the growth of gram-negative bacteria is inhibited at higher concentrations of
126 ZnO nanoparticles. It has been proposed that the higher susceptibility of Gram-positive bacteria
127 could be related to differences in cell wall structure, cell physiology, metabolism or degree of
128 contact [21]. The results of time-dependant antibacterial activity of ZnO nanoparticles showed
129 that cfu of the tested bacteria for each concentration decreased gradually during 72 h, whereas
130 colony formation of control solution remained uncountable [22].

Formatted: Highlight

131 Significant differences were observed between antibacterial activity of bulk ZnO. The
132 antibacterial efficacy increased with decreasing particle size from bulk ZnO to white ZnO
133 nanoparticles. Particle concentration seems to be more effective on the inhibition of bacterial
134 growth than particle size under the condition of this work [23]. The enhanced bioactivity of
135 smaller particle probably is attributed to the higher surface area to volume ratio [24]. ZnO
136 nanoparticles are effective antibacterial agents both on Gram-positive and Gram-negative
137 bacteria. The same results were confirmed in the study of [25] in which Gram-negative
138 membrane and Gram positive membrane disorganization was approved by transmission electron
139 microscopy of bacteria ultrathin sections.

Formatted: Font color: Light Blue

140 5. MECHANISM OF ANTIBACTERIAL ACTIVITY of ZnO NPs

141 The mechanisms of antibacterial activity of ZnO NPs were direct contact of ZnO NPs with
142 bacterial cell walls, resulting in destructing bacterial cell integrity, liberation of antimicrobial
143 ions mainly Zn^{+2} ions and ROS formation. However, the toxicity mechanism varies in various
144 media as the species of dissolved Zn may change according to the medium components besides
145 the physicochemical properties of ZnO NPs [26]. Antibacterial activity of biological synthesized

Formatted: Font color: Text 2

146 ZnO nanoparticles was seen against Gram negative (*Pseudomonas aeruginosa*, *Klebsiella*
147 *pneumoniae*, *E.coli*, *Proteus vulgaris*) and Gram positive (*Staphylococcus aureus*) bacteria[27].
148 Further the zone of inhibition of ZnO nanoparticles against Gram negative and Gram positive
149 bacteria was measured, and The results indicated that ZnO nanoparticles synthesized from
150 *Murraya koenigii* extract showed effective antibacterial activity both in Gram negative and Gram
151 positive bacteria. Therefore, the mechanism of inhibition of bacterial growth by ZnO
152 nanoparticles could be attributed to damage of the bacterial cell membrane and extrusion of the
153 cytoplasmic contents thereby resulting in the death of the bacterium [27].

Formatted: Font color: Auto, Highlight

Formatted: Font color: Auto, Highlight

Formatted: Font color: Auto, Highlight

154 The antimicrobial activity of the ZnO nanoparticles was tested against gram negative bacteria
155 *Salmonella typhi* and *Klebsiella pneumoniae*[28]. The results for the antimicrobial activity of
156 ZnO (chemical) and green ZnO indicated that green ZnO showed excellent antibacterial activity
157 against *Salmonella typhi* and *Klebsiella pneumoniae*. The remarkable antimicrobial activities of
158 green ZnO nanoparticles are due to the generation of surface oxygen species which leads to the
159 killing of the pathogens [28]. while The enhanced antibacterial activity is attributed to surface
160 defects on ZnO abrasive surface texture. One functional application of the ZnO antibacterial
161 bioactivity was discussed in food packaging industry where ZnO NPs are used as an antibacterial
162 agent toward food borne diseases[29]. Proper incorporation of ZnO NPs into packaging materials
163 can cause interaction with food borne pathogens, thereby releasing NPs onto food surface where
164 they come in contact with bad bacteria and cause the bacterial death and/or inhibition [29].

Formatted: Font color: Auto, Highlight

Formatted: Font color: Auto

Formatted: Font color: Auto, Highlight

165 Antibiotics such as Amikacin, Gentamicin and ciprofloxacin were impregnated with Zinc oxide
166 nanoparticles synthesized from *Ficus carica* which showed good activities against
167 *Staphylococcus aureus*, *Proteus*, *Acinetobacter*, *Pseudomonas aeruginosa* and *Escherichia*
168 *coli*[30]. Antimicrobial susceptibility showed that most of the bacterial isolates were resistant
169 towards antibiotics that became sensitive after nanoparticles application [30]. Nano-sized ZnO
170 exhibited varying morphologies and showed a significant antibacterial activity over a wide
171 spectrum of bacterial species explored by a large body of researchers. ZnO is currently being
172 investigated as an antibacterial agent in both microscale and nanoscale formulations. ZnO
173 exhibited significant antimicrobial activities when particle size is reduced to the nanometer
174 range, then nano-sized ZnO interacted with bacterial surface and/or with the bacterial core where
175 it entered inside the cell, and subsequently exhibited distinct bactericidal mechanisms [31].

Formatted: Font color: Auto, Highlight

176 The properties of ZnO nanoparticle is are strongly influenced by the particle size and mechanism
177 of cell inhibition. The properties include disruption of cell membrane, altering the permeability,
178 electrostatically binding to the cell surface and accumulation of nanoparticle in cytoplasm [32].
179 Nanoparticles of pure and copper - nitrogen co - doped ZnO were used for studying their
180 antibacterial and antifungal activity. *E.coli*, *Pseudomonas* and *Aspergillus* were used for bacterial
181 and fungal strains[33]. The bacterial strain as well as fungal strain was injected to form the active
182 strain by using well diffusion method. The oxy radicles were highly accountable for the death of

Formatted: Font: 12 pt, Highlight

183 bacteria and also prevent from the further attack of bacteria. The released peroxides sheltered the
184 ZnO nanoparticles [33].

Formatted: Font: 12 pt, Highlight

185 Antimicrobial activity of zinc oxide nanoparticles was known from the very distant past and has
186 many applications in disinfecting medical devices, water purification, and wound healing,
187 creams, lotions and antibacterial creams. Zinc oxide is one of the nanoparticles which are used in
188 an industrial scale in many countries. The mechanism of action of zinc oxide is similar to other
189 nanoparticles, but it acts mostly through destruction of bacterial walls. Zinc oxide nanoparticles
190 have been widely used against Gram-positive and Gram negative bacteria [34]. Nano-sized ZnO
191 exhibits varying morphologies and shows significant antibacterial activity over a wide spectrum
192 of bacterial species explored by a large body of researchers [35,36,37,38,39]. ZnO is currently
193 being investigated as an antibacterial agent in both microscale and nanoscale formulations. ZnO
194 exhibits significant antimicrobial activities when particle size is reduced to the nanometer range,
195 then nano-sized ZnO can interact with bacterial surface and/or with the bacterial core where it
196 enters inside the cell, and subsequently exhibits distinct bactericidal mechanisms [40]. The
197 interactions between these unique materials and bacteria are mostly toxic, and which have been
198 exploited for antimicrobial applications such as in food industry.

Formatted: Font color: Text 2

Formatted: Font color: Text 2

Formatted: Font color: Auto, Highlight

199 Interestingly, ZnO NPs are reported by several studies as non-toxic to human cells [41], this
200 aspect necessitated their usage as antibacterial agents, noxious to microorganisms, and hold good
201 biocompatibility to human cells. The various antibacterial mechanisms of nanomaterials are
202 mostly attributed to their high specific surface area-to-volume ratios [42], and their distinctive
203 physicochemical properties. However, the precise mechanisms are yet under debate, although
204 several proposed ones are suggested and adopted. Investigations on antibacterial nanomaterials,
205 mostly ZnO NPs, would enhance the research area of nanomaterials, and the mechanisms and
206 phenomenon behind nanostructured materials. The functional activities of nanoparticles are
207 heavily influenced by the size of the particles against *Staphylococcus aureus*. The data clearly
208 suggest that nanoparticles with smaller particle sizes showed more than 95% growth inhibition at
209 1 mM of ZnO nanoparticles with concentration, whereas larger particles showed only 40–50%
210 growth inhibition as compared with the control [43].

211 Synthesis and growth techniques lead to holding numerous active facets in NP. Rod-structures of
212 ZnO have (111) and (100) facets, whereas spherical nanostructures mainly have (100) facets.
213 High-atom-density facets with (111) facets exhibit higher antibacterial activity [44]. The facet-
214 dependent ZnO antibacterial activity has been evaluated by few studies, and nanostructured ZnO
215 with different morphologies have different active facets, which may lead to enhanced
216 antibacterial activity [45]. In this regard, the shape of ZnO nanostructures can influence their
217 mechanism of internalization such as rods and wires penetrating into cell walls of bacteria more
218 easily than spherical ZnO NPs [46]. Whereas, flower-shaped have revealed higher biocidal
219 activity against *S. aureus*, and *E. coli* than the spherical and rod-shaped ZnO NPs [47]. In
220 addition to the enhancement of internalization, it has been suggested regarding the contribution

of the polar facets of ZnO nanostructured to the antibacterial activity, that the higher number of polar surfaces possess higher amount of oxygen vacancies. Oxygen vacancies are known to increase the generation of ROS and consequently affect the photocatalytic of ZnO [48].

6. CONCLUSION

The review is aimed to discuss and analyze research works carried out by Researchers with references to the nature and potential use of Zinc oxide Nano particles (ZnO NPs) for antibacterial activity. The extensive discussion is focused on the mechanism of antibacterial activity of ZnO NPs against various bacterial species specifically pathogenic strains. Further, the review also connected with a number of factors that influencing the nature of antibacterial activity. The influencing factors include the size and shape of the ZnO NPs that is coupled with the mode of action of antibacterial activity. The present review will helpful in the various fields / industries for betterment use of ZnO NPs as a best antibacterial agent.

REFERECES

- [1] Ganesan V, Deepa B, Nima P, Astalakshmi. Bio-inspired of silver nanoparticles using leaves of *Millingtonia hortensis* L.f. Int. J. Adv. Biotechnol. Res. 2014; 5(2): 93-100.
- [2] Obaid AY, Al-Thabaiti SA, Al-Harbi LM, Khan Z. Extracellular bio-synthesis of silver nanoparticles. Global Advan. Res. J. Microbiol. 2015; 3(8): 119-126.
- [3] Simi CK, Abraham TE, Hydrophobic grafted and cross-linked starch nanoparticles for drug delivery. Bioprocess Biosyst. Eng. 2007; 30: 173-180.
- [4] Lupan O, Chai G, Chow L, Emelchenko G, Heinrich H, Ursaki V, Gruzintsev A, Tiginyanu I, Redkin A. Ultraviolet photoconductive sensor based on single ZnO nanowire. Phys. Status Solidi A 2011; 207: 1735–1740.
- [5] Alwan RM, Kadhim QA, Sahan KM, Ali RA, Mahdi RJ, Kassim NA, Jassim AN. Synthesis of zinc oxide nanoparticles via Sol – Gel route and their characterization. Nanosci. Nanotechnol. 2015; 5(1): 1-6.
- [6] Padmavathy N, Vijayaraghavan R. Enhanced bioactivity of ZnO nanoparticles - An antimicrobial study. Sci. Technol. Adv. Mater. 2008; 9: 432-438.
- [7] Shankar SS, Ahmad A, Sastry Geranium M. Leaf assisted biosynthesis of silver nanoparticles. Biotechnol. Prog. 2003; 3: 1627-1631.
- [8] Veerasamy R, Xin TZ, Gunasagaran S, Xiang TFW, Yang EFC, Jeyakumar N. Biosynthesis of silver nanoparticles using mangosteen leaf extract and evaluation of their antimicrobial activities. J. Saudi Chem. Soci. 2011; 15: 113-120.
- [9] Mohanpuria P, Rana NK, Yadav SK. Biosynthesis of nanoparticles: technological concepts and future applications. J. Nanoparticle Res. 2008; 10: 507-517.
- [10] Nazeruddin GM, Prasad NR, Waghmare SR, Garadkar KM, Mulla IS. Extracellular biosynthesis of silver nanoparticle using *Azadirachta indica* leaf extract and its anti-microbial activity J. Alloys Compd. 2014; 583: 272–277.
- [11] Ghaffari-Moghaddam M, Hadi-Dabanlou R, Khajeh M, Rakhshanipour M, Shameli K. Green synthesis of silver nanoparticles using plant extracts. Korean. J. Chem. Engineer. 2014; 31(4): 548–557.
- [12] Bar VV, Dipak KR, Bhui Gobinda P, Sahoo M, Sarkar P, Santanu Pyne, Ajay Misra. Green synthesis of silver nanoparticles using seed extract of *Jatropha curcas*. Colloids Surfaces: Physicochem. Engin. Aspects 2009; 348: 212–216.

[13] Chung Hsin, Lu. Surfactant effects on the microstructure and electrochemical properties of emulsion-derived lithium cobalt oxide powders. *Mat. Sci. Enginee. B.* 2013; 102: 627–634.
 [14] Ashe BA. Detail investigation to observe the effect of zinc oxide and silver nanoparticles in biological system [Dissertation]. Nit, Orissa, India. 2011.
 [15] Meruvu S, Hugendubler L, Mueller E. Regulation of adipocyte differentiation by the zinc finger protein ZNF638. *J. Biol. Chem.* 2011; 286: 26516 – 26523.
 [16] Jones EW, Berget PB, Burnette Iii JM, Anderson C, Asafu-Adjei D, Avetisian S, Barrie F, Chen R, Chu B, Conroy S. The spectrum of Trp(-) mutants isolated as 5-fluoroanthranilate-resistant clones in *Saccharomyces bayanus*, *S. mikatae* and *S. paradoxus*. *Yeast* 2008; 25(1): 41-46.
 [17] Ravichandrika T. Synthesis, characterization and antibacterial activity of ZnO nanoparticles. *Int. J. Pharm. Pharm. Sci.* 2012; 4(4): 336- 338.
 [18] Premanathan M, Karthikeyan K, Jeyasubramanian K, Manivannan G. Selective toxicity of ZnO nanoparticles toward gram-positive bacteria and cancer cells by apoptosis through lipid peroxidation, nanomedicine nanotechnology. *Biol. Med.* 2011; 7(2): 184-192.
 [19] Xia Y, Yang P, Sun Y, Wu Y, Mayers B. One-dimensional nanostructures: Synthesis, characterization and applications. *Adv. Mater.* 2003; 15(5): 353-389.
 [20] Espitia PJP, Soares NDF, Coimbra JSJR, De andrade NJ, Cruz RS, Medeiros EAA. Bioactive peptides: Synthesis, properties, and applications in the packaging and preservation of food. *Comprehensive Reviews in Food Science and Food Safety.* New Jersey. 2012; 11(2): 187-204.
 [21] Reddy KM, Feris K, Bell J, Wingett DG, Hanley CA, Punnoose, Selective toxicity of zinc oxide nanoparticles to prokaryotic and eukaryotic systems. *Appl. Phys. Lett.* 2007; 90(21): 213902.
 [22] Selahattin A, Kadri G, Ramazan. The effect of zinc on microbial growth. *Tr. J. Med. Sci.* 1998; 28: 595-597.
 [23] Lingling Z, Yunhong J, Yulong D, Malcolm P, David Y. Investigation into the antibacterial behaviour of suspensions of ZnO nanoparticles (ZnO nanofluids), *J. Nanopart. Res.* 2007; 9(3): 479–489.
 [24] Nagarajan P, Rajagopalan V. Enhanced bioactivity of ZnO nano-particles—an antimicrobial study. *Environ. Sci. Technol.* 2008; 9 (035004): 7-15.
 [25] Zhongbing H, Xu Z, Danhong Y, Guangfu Y, Xiaoming L, Yunqing K, Yadong Y, Di H, Baoqing H. Toxicological effect of ZnO nanoparticles based on bacteria. *Langmuir.* 2008; 24(8): 4140–4144.
 [26] Li M, Zhu L, Lin D. Toxicity of ZnO nanoparticles to *Escherichia coli*: Mechanism and the influence of medium components. *Environ. Sci. Technol.* 1977; 45(5): 1977–1983.
 [27] Gupta AK, Joshi MB, Philippova M, Erne P, Hasler P, Hahn S. Activated endothelial cells induce neutrophil extracellular traps and are susceptible to netosis mediated cell death. *Inter. Confer. on Recent Advan. in Mat.* 2010; 584: 3193–3197.
 [28] Cai S, Sing RB. A distinct utility of the amide III infrared band for secondary structure estimation of aqueous protein solutions using partial least squares methods. *Biochem.* 2004; 43: 2541–2549.
 [29] Sirelkhatim A, Mahmud S, Seeni A, Haida N, Kaus M, Ann L, Mohd Bakhori SK, Habsah H, Mohamad D. Review on zinc oxide nanoparticles: Antibacterial activity and Toxicity mechanism. *Nano-Micro. Lett.* 2015; 7(3): 219–242.
 [30] Ehsan S, Sajjad M. Bioinspired synthesis of zinc oxide nanoparticle and its combined efficacy with different antibiotics against multidrug resistant bacteria. *J Biomat. Nanobiotechnol.* 2017; 8: 159-175.
 [31] Seil JT, Webster TJ. Antimicrobial applications of nanotechnology: methods and literature. *Int. J. Nanomed.* 2012; 7: 2767–2781.
 [32] Stoimenov PK, Klinger RL, Marchin GL, Klabunde KJ. Metal oxide nanoparticles as bactericidal agents. *Langmuir* 2002; 18(17): 6679- 6686.
 [33] Sivakumar N, Saranya S. Synthesis, Characterization and evaluation of ZnO and Copper – nitrogen Co doped zinc oxide (Cu-N-ZnO) nanoparticles for its biological applications. *J. Chem. Pharma. Sci.* 2017; 8: 16-20.
 [34] Emami S, Akya A, Hossain Zadeh A, Barkhordar S. Bacterial contamination of traditional ice creams in Kermanshah. *Iran J Med Microbiol.* 2013; 7(2): 59–62.

312 [35] Buzea C, Pacheco II, Robbie K. Nanomaterials and nanoparticles: sources and toxicity.
 313 Biointerphases. 2007; 2(4): 17–71.

314 [36] Brayner R, Ferrari-Iliou R, Brivois N, Djediat S, Benedetti MF, Fiévet F. Toxicological impact
 315 studies based on *Escherichia coli* bacteria in ultrafine ZnO nanoparticles colloidal medium. Nano Lett.
 316 2006; 6(4): 866–870.

317 [37] Jones N, Ray B, Ranjit KT, Manna AC. Antibacterial activity of ZnO nanoparticle suspensions on a
 318 broad spectrum of microorganisms. FEMS Microbiol. Lett. 2008; 279(1): 71–76.

319 [38] Jalal R, Goharshadi EK, Abareshi M, Moosavi M, Yousefi A, Nancarrow P. ZnO nanofluids: green
 320 synthesis, characterization, and antibacterial activity. Mater. Chem. Phys. 2010; 121(1): 198–201.

321 [39] Raghupathi KR, Koodali RT, Manna AC. Size-dependent bacterial growth inhibition and mechanism
 322 of antibacterial activity of zinc oxide nanoparticles. Langmuir. 2011; 27(7): 4020–4028.

323 [40] Emami-Karvani Z, Chehrizi P. Antibacterial activity of ZnO nanoparticle on gram-positive and
 324 gram-negative bacteria. Afr. J. Microbiol. Res. 2011; 5(12): 1368–1373

325 [41] Colon G, Ward BC, Webster TJ. Increased osteoblast and decreased *Staphylococcus epidermidis*
 326 functions on nanophase ZnO and TiO₂. J. Biomed. Mater. Res. 2006; 78(3): 595–604.

327 [42] Seil JT, Taylor EN, Webster TJ. Reduced activity of *Staphylococcus epidermidis* in the presence of
 328 sonicated piezoelectric zinc oxide nanoparticles, in 2009 IEEE 35th Annual Northeast Bioengineering
 329 Conference, Boston, MA, USA, 3–5 April 2009 (IEEE, 2009), pp. 1–2.

330 [43] Rosi NL, Mirkin CA. Nanostructures in biodiagnostics. Chem Rev. 2005; 105: 1547–1562.

331 [44] Pal Y, Tak K, Song JM. Does the antibacterial activity of silver nanoparticles depend on the shape of
 332 the nanoparticle? A study of the gram-negative bacterium *Escherichia coli*. Appl. Environ. Microbiol.
 333 2007;73(6): 1712–1720.

334 [45] Ramani M, Ponnusamy S, Muthamizhchelvan C, Marsili E. Amino acid-mediated synthesis of zinc
 335 oxide nanostructures and evaluation of their facet-dependent antimicrobial activity. Colloids Surf. 2014;
 336 B 117: 233–239.

337 [46] Yang H, Liu C, Yang D, Zhang H, Xi Z. Comparative study of cytotoxicity, oxidative stress and
 338 genotoxicity induced by four typical nanomaterials: the role of particle size, shape and composition. J.
 339 Appl. Toxicol. 2009; 29(1): 69–78.

340 [47] Talebian N, Amininezhad SM, Doudi M. Controllable synthesis of ZnO nanoparticles and their
 341 morphology-dependent antibacterial and optical properties. J. Photochem. Photobiol. 2013; 120: 66–73.

342 [48] Tong G.-X, Du F.-F, Liang Y, Hu Q, Wu R.-N, Guan J.-G, Hu X. Polymorphous ZnO complex
 343 architectures: selective synthesis, mechanism, surface area and Zn-polar plane-codetermining
 344 antibacterial activity. J. Mater. Chem. 2013; B 1(4): 454–463

345 -[XXX1] Zhou D, Ji Z, Jiang X, Dunphy DR, Brinker J, et al, (2013) Influence of Material Properties on
 346 TiO₂ Nanoparticle Agglomeration. PLoS ONE 8(11):e81239.doi:10.1371/journal.pone.0081239

347 [XXX2] Ekane Peter Etape, Josepha Foba-Tendo, Lambi John Ngolui, Beckley Victorine Namondo,
 348 Fomogne Cyrille Yollande, and Marius Borel Nguefack Nguimezong “Structural Characterization and
 349 Magnetic Properties of Undoped and Ti-Doped ZnO Nanoparticles Prepared by Modified Oxalate Route”
 350 Journal of Nanomaterials Volume 2018 (2018), Article ID 9072325, 9 pages,
 351 <https://doi.org/10.1155/2018/9072325>

Comment [EPE1]:

Formatted: Font: (Default) Times New Roman, Font color: Black

Formatted: Font: 11 pt, Not Bold, Font color: Auto

Formatted: Font: 11 pt, Font color: Auto