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Exploring the Antibacterial Activity of Zinc Oxide Nanoparticles against Some Selected Gram-Positive and Gram-Negative Bacteria

Abiola Olanike Adesina[1*]

[1]Department of Chemical Sciences, Adekunle Ajasin University, P.M.B 001, Akungba-Akoko, Ondo State, Nigeria

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***Corresponding author:** Abiola Olanike Adesina
Department of Chemical Sciences, Adekunle Ajasin University, P.M.B 001, Akungba-Akoko, Ondo State, Nigeria
Email: abiola.adesina@aaua.edu.ng

Abstract

Increase in microbial resistance of commonly used antibiotics is a major health concern globally this has necessitated researchers to focus on cheaper alternative materials which could perform the role of antibiotics. This study aimed at investigating the synthesis of zinc oxide nanoparticles (ZnO Nps) and exploring the synthesized nanoparticles as sources of antimicrobials. The antimicrobial activity of the synthesized zinc oxide nanoparticles was tested against ten different bacteria namely; *Enterobacter agglumerans, Corynebacterium bovis, Pseudomonas aeruginosa, Acinetobacter baumannii, Escherichia coli, Micrococcus luteus, Klebsiella pneumoniae, Bacillus subtilis, Staphylococcus aureus,* and *Bacillus cereus.* The results from this study revealed that four out of the test organisms (*S. aureus*, *B. cereus*, *E. coli* and *Bacillus subtilis*) were susceptible to the ZnO nanoparticles. The diameter of the zones of inhibition (ZOI) ranging from 14.50 – 25.50 mm. The synthesized nanoparticles showed the highest activity with *S. aureus* (ZOI = 25.50 mm, followed by *B. cereus* (22.00 mm), *E. coli* and *B. subtilis* (14.50 mm). The minimum inhibitory concentration (MIC) results showed that ZnO NPs was most effective against *S. aureus* and *B. cereus* at 25% concentration while minimum bactericidal concentration (MBC) was at 50%. These findings revealed that zinc oxide nanoparticles have great potentials for inhibiting clinical isolates; thus, their use as an alternative means for new drug discovery should be encouraged.

Keywords: Antibacterial activity**;** Zinc oxide Nanoparticles; Antibiotics resistance; Bacteria.

1.0 INTRODUCTION

The emergence of new transmissible diseases and the sharp increase in antibiotic resistance in pathogenic microorganisms have made treating infectious diseases more difficult and significantly impacted treatment rates. Due to the fact that antimicrobial resistance poses a serious threat to human health, an increase in the rates of morbidity and mortality is of great concern (Franci *et al*., 2015)**.** The multidrug resistance of antibiotics to bacterial infections has also sparked interest in the use of nanoparticles as an antibiotic substitute. Recent developments in nanotechnology have also demonstrated the antibacterial properties of these nanoparticles (Wang and Hu, Chen, 2017)**.**

Researchers employed the use of nanoparticles because of their distinct physiochemical properties. These special qualities have made them useful in a variety of biomedical sciences, including clinical diagnostics, gene transfer, biomolecule detection, and sensing applications (Loo *et al*., 2018; Pandey *et al*., 2008)**.** Nanoparticles have attracted significant attention for use as antimicrobial agents in medical therapy. Due to their small size and large surface area, which enhance their contact with microbes, provide potent antimicrobial activities, and a great potency as antimicrobial agent (Rai *et al*., 2012)**.**

Several reports have shown that the antibacterial activity of nanoparticles is therefore ascribed to their large surface area, tiny size, and volume ratio (o-Martı *et al*., 2008)**.** Numerous industries, including pharmaceutical, food, health, electronics, energy research, cosmetics, space, drug-gene delivery, the chemical and optical industries, catalysis, and environmental sciences, are being profoundly impacted by the tremendously fast growth of nanotechnology (Ahmed *et al*., 2016). The science of producing, modifying, and applying nanoparticles with sizes ranging from 1 to 100 nanometers is known as nanotechnology. Because of their size, distribution, and shape, nanomaterials offer new and improved useful

properties compared to the bigger particles of the mass material from which they were made.

Recently, nanotechnology has emerged as a creative breakthrough that has the potential to improve human health and well-being (Khalil and Fouad, Hassan, T. Elsarnagawy, 2013). It has also been established that nanoparticles have antibacterial properties and are employed in medicine (Jemal *et al*., 2017). Metal oxide nanoparticles are seen to be the most promising among nanomaterials and nanoparticles employed for all of the aforementioned applications because of their exceptional antibacterial qualities. The emergence of antibiotic-resistant microbial strain has led to researchers showing great interest in this area (Khalil and Fouad, Hassan, T. Elsarnagawy, 2013).

A metal oxide nanoparticle like ZnO has been extensively studied for their antimicrobial activity. It has been found that nanoparticles with smaller particle sizes exhibit strong antibacterial action (Jones *et al*., 2008). Human pathogenic microorganisms including Staphylococcus aureus and Escherichia coli (Yoon *et al*., 2007) have been used extensively to study the antimicrobial properties of nanoparticles (Ruparelia *et al.*, 2008). Furthermore, it appears that these microorganisms are extremely susceptible to zinc oxide (ZnO) and copper oxide (CuO) nanoparticles (Heinlaan *et al*., 2008; Jones *et al.*, 2008). These nanoparticles' bactericidal action is partially determined by their size, stability, and concentration in the growing media *(Azam et al*., 2012).

Several studies have shown that inorganic metal oxide nanoparticles such as CaO, ZnO, MgO exhibited strong antibacterial activity which is ascribed to the ability of the nanoparticles to generate reactive oxygen species (ROS) on the surface of the oxides (Zhao *et al*., 2008). The generation of ROS is capable of causing both physical and mechanical damages to the microorganisms (Li *et al*., 2018; Park *et al*., 2019). The benefit of employing these inorganic oxides as antibacterial agents is that they contain vital minerals for human health and are highly active even at low dosages. Also, inorganic antibacterial compounds exhibit better durability, lower toxicity, higher selectivity, and stronger heat resistance (Zhao *et al*., 2008).

As one of the multifunctional inorganic nanoparticles among metal oxide nanoparticles, ZnO nanoparticles have a wide range of applications as semiconductors, sensors, transparent electrodes, solar cells. Other important properties of ZnO nanoparticles include intense ultraviolet and infrared adsorption, high catalysis activity, chemical and physical stability, and effective antibacterial activity (Emami-karvani and Chehrazi, 2012). Shantikumar *et al.*, (2009) investigated the selective toxicity of ZnO nanoparticles to cancer cells. ZnO nanostructures have demonstrated promising anticancer effects on human brain tumor U87 and cervical cancer Hela. These effects show encouraging activity that varies depending on the size and structure alterations. (Rizwan W, Nagendra KK, Akhilesh KV, Anurag M, Hwang IH, You-Bing Y, Hyung-Shik Sh, 2010).

Furthermore, due to their antimicrobial efficacy, ZnO nanoparticles have the potential to influence numerous facets of food and agricultural systems, particularly in light of the increasing need to develop new, safe, and affordable antibiotic formulation techniques to stop the spread of resistant pathogens in food processing environments (Wahab *et al*., 2010). In the present study, zinc oxide nanoparticles were successfully synthesized and characterized by Transmission electron microscopy (TEM), Energy dispersive x-ray spectroscopy (SEM/EDS) and X-ray diffraction (XRD). The synthesized nanoparticles were further investigated for their antimicrobial activity against some selected gram-positive and gram-negative organisms.

2.0. MATERIALS AND METHODS

2.1. Chemicals and Reagents

All chemicals and reagents used in this study Zinc Acetate dihydrate [$Zn(CH_3COO)_2.2H_2O$], Sodium hydroxide (NaOH), Dimethyl sulfoxide (DMSO) were of analytical grade and purchased from sigma Aldrich. All the reagents were utilized in their original form and were prepared and used according to manufacturer's instruction.

2.2. Synthesis of Zinc Oxide (ZnO) Nanoparticles

Zinc Oxide (ZnO) nanoparticles was synthesized following a method previously reported by (Narayanan and Wilson, 2012) with slight modifications. The synthesis involved the combination of zinc acetate dihydrate and sodium hydroxide at a mole ratio of 2:1 for $Zn(CH_3COO)_2.2H_2O$: $NaOH.Zn(CH_3COO)_2.2H_2O$ (26.5 mmol) and NaOH (0.1 mmol) were accurately measured and placed in a 100ml Crucible. The Crucible was then covered and introduced into a furnace set at a temperature of 550°C. Once the furnace reached the desired temperature, the reaction mixture was left for 2 hours, after which the furnace was turned off and the resulting zinc oxide nanoparticles were retrieved.

2.3. Characterization of Zinc Oxide Nanoparticles

The synthesized zinc oxide nanoparticles (ZnO Nps) were characterized using XRD, Energy dispersive X-ray spectroscopy (SEM /EDX), and Transmission electron microscopy (TEM).

Transmission Electron Microscopy:

The images were acquired using JEOL 2100F equipment and the copper grid coated (using drop-dry) with materials to be investigated.

Energy dispersive X-ray (EDX)

Analysis spectra were obtained using an X-ray microanalysis system added as a module on the Nova NanoSEM 200.

X-Ray Diffraction (XRD)

Analysis was performed on a Bruker D8 Discover diffractometer, equipped with a Lynx Eye detector, under Cu-K_α radiation (λ= 1.50405 Å). Data were collected in the range $2\theta = 10^o$ to 70^o, scanning at 1^o min^{-1} with a filter time-constant of 2.5 s per step and a slit width of 6.0 nm. The samples were placed on a zero-background silicon wafer slide.

2.4. Media Preparation

Nutrient agar medium was prepared according to manufacturer's specification. The medium was homogenized by boiling to fully dissolve all components. It was then autoclaved at 121^{o}C for 15 min. After autoclaving, the medium was allowed to cool to about 45^{o}C and poured into Petri-dish. The plates were allowed to set/solidify before inoculation.

2.5. Test Organisms

Ten different clinical were isolates collected from out-patients ward of the Federal medical Centre, Owo whose morphological and biochemical characteristics were confirmed according to Bergey's manual of determinative bacteriology 9th edition. The isolates used are *Enterobacter agglumerans, Corynebacterium bovis, Pseudomonas aeruginosa, Acinetobacter baumannii, Escherichia coli, Micrococcus luteus, Klebsiella pneumoniae, Bacillus subtilis, Staphylococcus aureus,* and *Bacillus cereus.* The bacterial cultures were maintained in nutrient broth and stored at 4^{o}C throughout the study period.

2.6. Determination of Antimicrobial Activity

The concentration of each bacterial culture was adjusted to inoculum size of 1.5 X10^8 cfu/ml and used to seed already solidified Petri plates of Mueller-Hinton Agar (MHA). The antibacterial activities of the synthesized zinc oxide nanoparticles (ZnO Nps) were determined using agar well diffusion method. A sterile 6 mm cork borer was used to make well on already solidified agar, the wells were filled with the metal complex dissolved in 30% DMSO ensuring that there was no spill on the agar surface surrounding the well. The plates were allowed to stand for about 2 hours to allow absorption of the metal complex and ligands dissolved in 30% DMSO into the medium after which they were incubated at 37^{o}C for 24 hours for bacterial.

Macro-broth dilution technique as modified by Ajibade *et al.*, (2012) was used in this research for minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). Those recorded as MIC were the lowest concentration of the tested metal complex dissolved in 30% DMSO that showed no visible growth of the tested isolate. Serial dilutions of the metal complex dissolved in 30% DMSO were carried out to give a concentration of 50 mg/ml, 25 mg/ml, 12.5 mg/ml, and 6.25 mg. For MIC, a sterile 6 mm cork borer was used to make four wells on already solidified and bacterial seeded agar plates; each well was filled with a concentration of the diluted metal complex and labeled appropriately. The plates were incubated at 37^{o}C for 24 hours and observed afterwards. For MBC, 2 ml of each diluted concentration was added to 18 ml of pre-sterilized molten MH medium, mixed properly and allowed to set, after which the standardized inoculum was seeded on each plate. The bacterial plates were incubated at 37^{o}C for 24 hours. The results were observed and recorded. Those recorded as MBC were the lowest concentration of the tested metal complex that showed no visible growth of the tested bacteria.

2.7. Killing Rate Dynamics

Fifty percent (50%) dilution of metal complex was used to observe the killing rate of the antibacterial agent against the test organisms. A 1 ml of the 50% diluted oil was added to a 9 ml broth containing 1 ml test organism with inoculum size of 1.5 x10^8 cfu/ml. The killing rate was measured using UV/Visible spectrophotometer at a wave length of 620 nm for 48 hours.

3.0. RESULTS

3.1. Characterization of Zinc Oxide (ZnO Nps) Nanoparticles

3.1.1. Energy dispersive X-ray Spectroscopy (EDX) Spectrum of ZnO Nps

The EDX spectrum is presented in Fig 3.1. It a non-destructive elemental analysis was used to characterized the synthesized zinc oxide nanoparticles to confirm the presence of the expected elements. The presence of the all the expected elements was an indication of the successfully synthesis of the nanoparticles. The percentage composition of the elements is shown with the EDX spectrum.

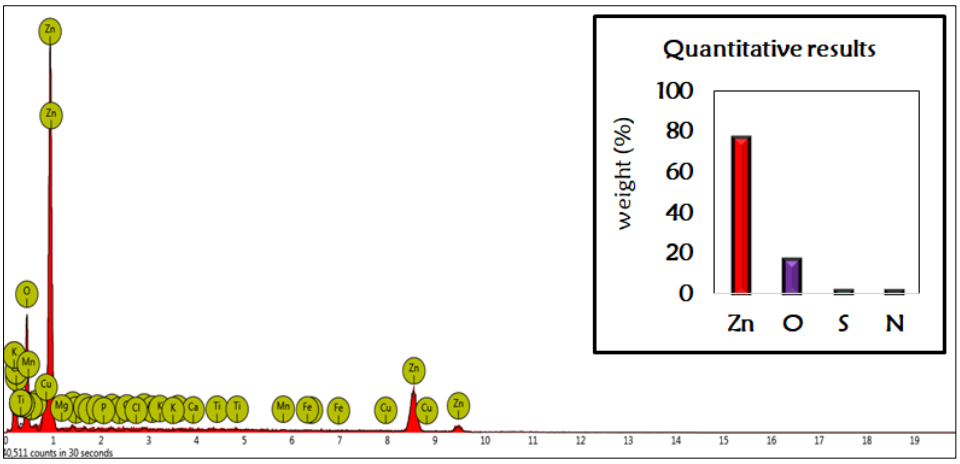

Fig. 3.1: EDX spectrum of zinc oxide nanoparticles

3.1.2. Transmission Electron Microscopy (TEM) Image of ZnO Nps

The TEM image of the synthesized nanoparticles revealed the morphology of the nanoparticles, particle size distribution, and the shape of the nanoparticles. From the TEM result, the nanoparticles were spherical in shape. The particle size distribution ranges from 2.70 nm – 17.83 nm with the majority of the nanoparticles between 2.70 – 7.30 nm having the highest distribution. The average particles size was found to be 6.4 ± 4.3 nm.

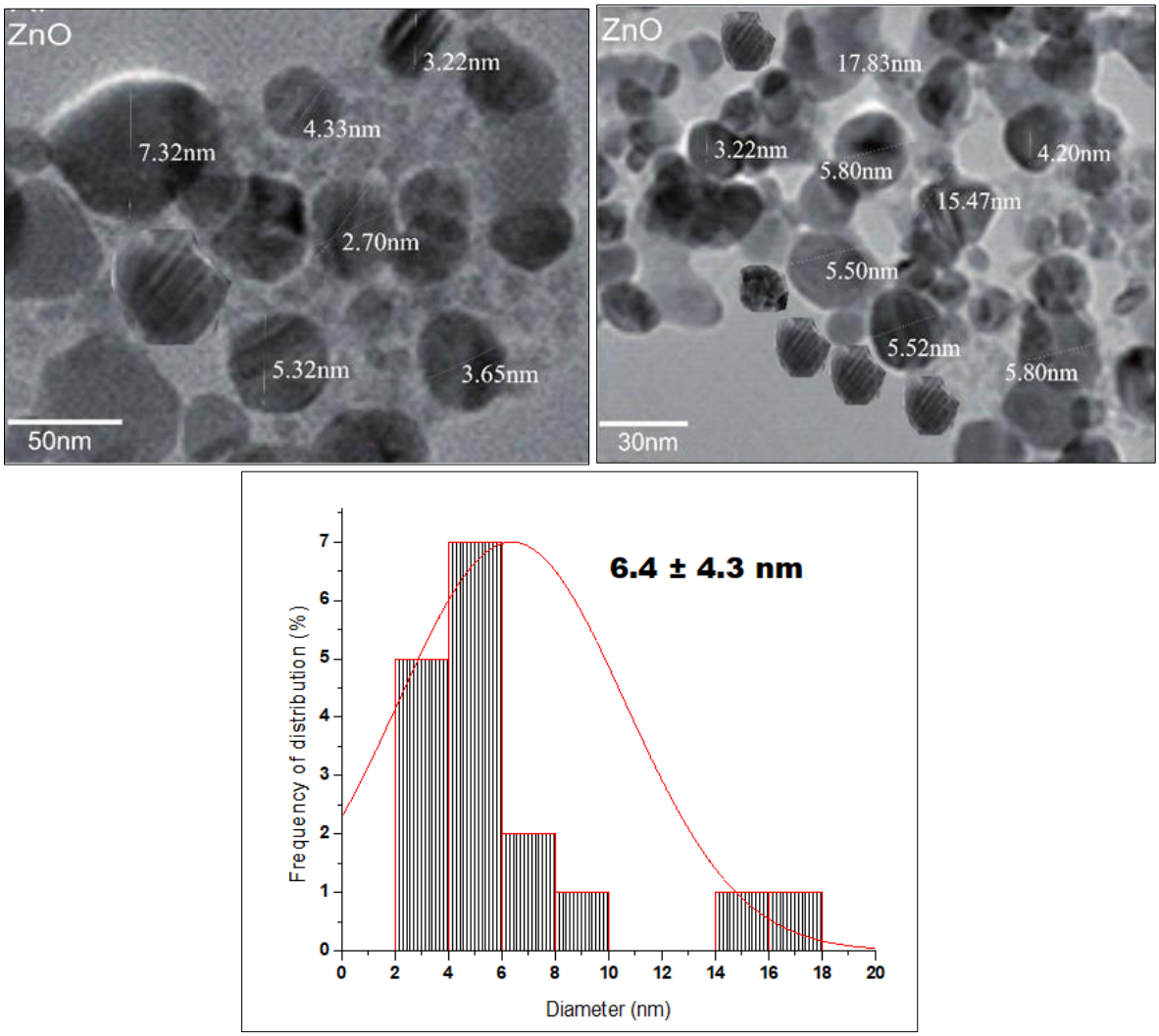

Fig. 3.2: TEM images of the ZnO Nps at different magnifications and the corresponding histogram

3.1.3. Scanning Electron Microscopic Image (SEM) of ZnO Nps

The SEM image of the synthesized nanoparticles shows the morphology of the zinc oxide nanoparticles. The nanoparticles were not uniformly distributed and there of different sizes and spherical in shape.

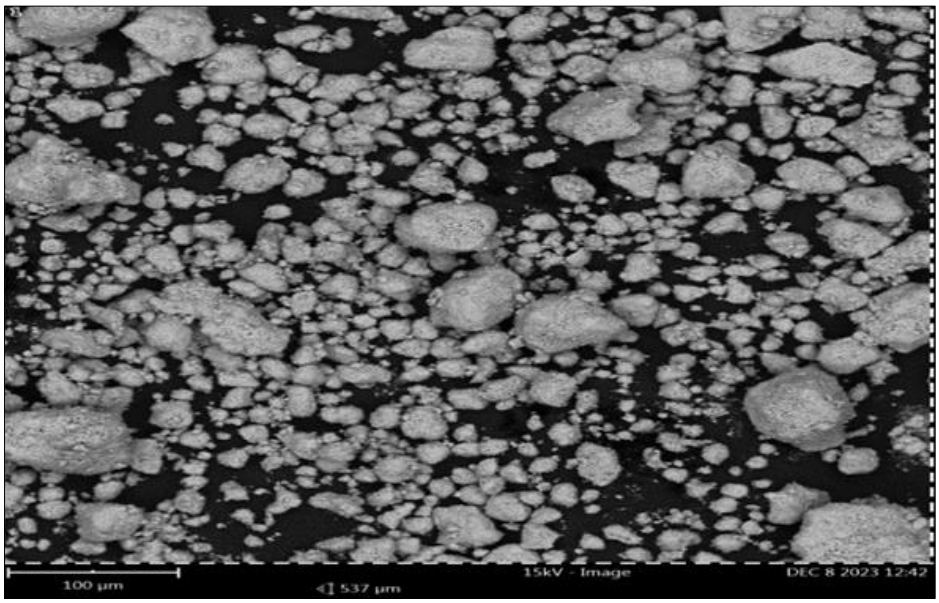

Fig. 3.3: SEM image of ZnO Nps

3.1.4. X-ray Diffraction (XRD) Patterns of Zinc Oxide Nanoparticles (ZnO Nps)

X-ray diffraction was used to confirm phase of zinc oxide nanoparticles. XRD diffractogram of synthesized zinc oxide nanoparticles is shown in Fig. 3.4, seven peaks were observed in diffractogram at 2θ equals to 31.8, 34.6, 36.3,47.8, 58, 62.4 and 68.5 with Miller indices value of (100), (002), (101), (102), (110), (103), and (112) respectively. These strong peaks indicated the crystalline nature of zinc oxide nanoparticles.

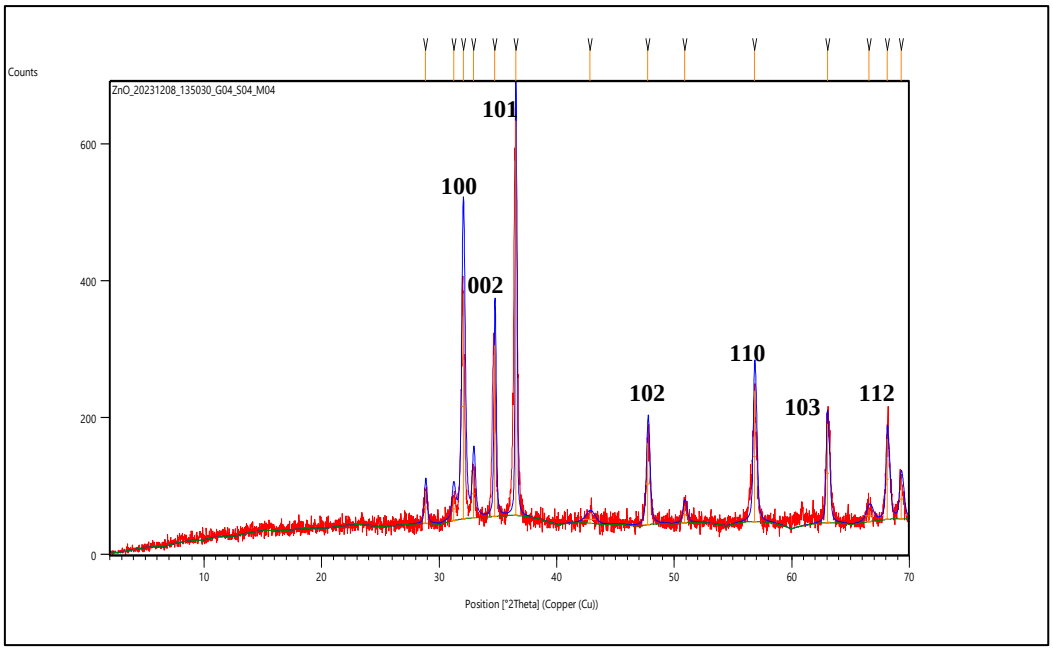

Figure 3.4: XRD diffratogram of ZnO Nps

3.2 Antimicrobial Susceptibility Test (AST)

The results from this study revealed that four out of the test organisms were susceptible to the ZnO nanoparticles. These organisms were (*S. aureus*, *B. cereus*, *E. coli*, and *B. subtilis*). The zone of inhibition ranged from 14.50 – 25.50 mm. The synthesized nanoparticles showed the highest activity with *S. aureus* (ZOI= 25.50 mm, followed by *B. cereus* ZOI = 22.00 mm

E. coli and *B. subtilis* ZOI= 14.50 mm). The remaining six organisms, *E. agglumerans C. bovis P. aeruginosa*, *A. baumannii*, *M. luteus* and *K. pneumoniae* showed ZOI less than 14.00 mm (Table 1). The MIC results further showed that synthesized nanoparticles had highest value of ZOI against *S. aureus* and *B. cereus* (Table 2). The minimum inhibitory concentration for the two organisms ranged from 50% - 25%. No activity was observed at a concentration lesser than 25% for all the test organisms. The MBC results presented in Table 3 revealed that the ZnO had MBC at 50%.

Table 1: Antimicrobial zones of inhibition (ZOI) using 100mg/ml of zinc oxide (ZnO Nps) against bacterial isolates

Organism	Zinc oxide (mm)	Ampicillin (mm)
Enterobacter agglumerans	11.50	23.50
Corynebacterium bovis	10.00	26.50
Pseudomonas aeruginosa	11.00	38.50
Acinetobacter baumannii	11.00	16.00
Escherichia coli	14.50	20.00
Micrococcus luteus	13.50	31.50
Klebsiella pneumoniae	12.00	20.50
Bacillus subtilis	14.50	17.00
Staphylococcus aureus	25.50	40.00
Bacillus cereus	22.00	25.00

Table 2: Result of Minimum Inhibitory Concentration (MIC) of zinc oxide nanoparticles (ZnO Nps) against various bacterial isolates

Organism	50%	25%	12.5%	6.25%	3.125%
Staphylococcus aureus	+	+	_	_	_
Bacillus cereus	+	+	_	_	_

Key: + = Growth; - = no growth

Table 3: Result of Minimum Bactericidal Concentration (MBC) of zinc oxide nanoparticles (ZnO Nps) against various bacterial isolates

Test organisms	50%	25%	12.5%	6.25%	3.125%
Staphylococcus aureus	+	_	_	_	_
Bacillus cereus	+	_	_	_	_

Table 4: The killing time of 50% dilutions of Zinc oxide synthesized against bacterial isolates

Organism	0hr	6hrs	12hrs	18hrs	24hrs	30hrs	36hrs	42hrs	48hrs
Staphphylococcus aureus	0.234	0.164	0.092	0.059	0.025	0.007	0.000	0.000	0.000
Bacillus cereus	0.227	0.186	0.089	0.047	0.021	0.004	0.000	0.000	0.000

4. DISCUSSION

Zinc oxide nanoparticles are widely known for their antibacterial properties. The antibacterial activity of zinc oxide nanoparticles (ZnO Nps) was tested by agar well diffusion method. The presence of inhibition zone clearly indicated the antibacterial effect of ZnO Nps. The results of the study revealed that zinc oxide nanoparticles (ZnO Nps) exhibited excellent and significant antimicrobial activity against four out of the ten bacterial isolates. A sample exhibiting ≥ 14 mm inhibition zone is considered significant (Mustopa *et al*., 2016). The obtained result indicated that some of the organisms were susceptible to the synthesized ZnO Nps. The excellent antimicrobial activity of the nanoparticles against some of the tested organisms could be attributed to the fact that the synthesized ZnO Nps, could penetrate the cell wall of the organisms. ZnO Nps may generate reactive oxygen species (ROS) in bacterial cells resulting from strong affinity of the nanoparticles for the bacterial cell membrane (Li *et al.,* 2018). This may significantly reduce the ability of bacteria to survive and grow and caused some bacteria to die due to damaged cell wall.

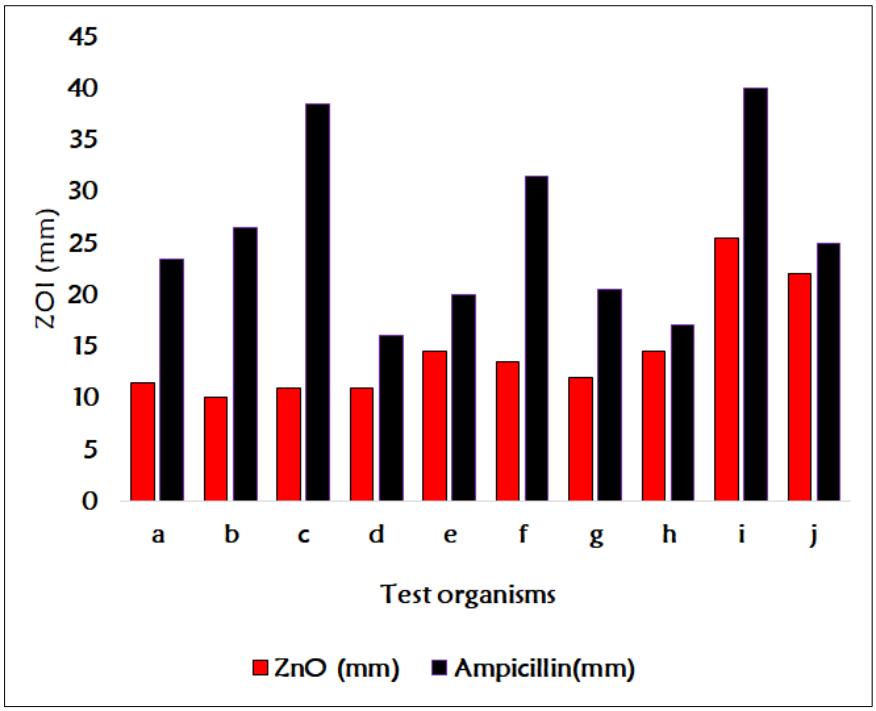

Figure 4.1: Graphical presentation of antimicrobial activity of ZnO Nps against some organisms (a: *Enterobacter agglumerans* b: *Corynebacterium bovis* c: *Pseudomonas aeruginosa*, d: *Acinetobacter baumannii*, e: *Escherichia coli*, f: *Micrococcus luteus*, g: *Klebsiella pneumoniae*, h: *Bacillus subtilis*, i: *Staphylococcus aureus* and j: *Bacillus cereus*

The antimicrobial activity of ZnO Nps observed in the present study is consistent with the findings of previous studies. For instance, Ragunathan *et al*., (2022) reported that ZnO Nps exhibit significant antimicrobial activity against various bacterial strains, including *E. coli* and *S. aureus.* The obtained result from this present study showed a better antimicrobial activity. Similarly, Chaudhary *et al*., (2019) investigated the antimicrobial potency of ZnO nanoparticles against *S. aureus* and *E. coli.* The present study showed a better activity for *S. aureus* compared to the work reported by Chaudhary *et al*., (2019).

The potential antibacterial activity of zinc oxide nanoparticles was assessed by Wahab *et al*., (2008) against *B. subtilis* and *E. coli* growth using disc diffusion approach. The potency of zinc oxide nanoparticles was evaluated and reported to have significant effect against the tested organisms. Further investigation was carried out in 2011 by Mami-Karvani and Chehrazi about the antibacterial activity of ZnO nanoparticles against *E. coli* and *S. aureus.* The obtained result from the present study is in agreement with what was previously reported.

Moreso, results obtained from this study indicated the high sensitivity of *S. aureus* to ZnO than *E. coli* which is similar to what has been previously reported by Reddy *et al*., (2007). Also, Padmavathy and Vijayaraghavan reported that higher concentrations of negatively charged free radicals, such as peroxide anions and superoxide radical, cause *S. aureus* cells to be damaged and die, even at low concentrations (Padmavathy and Vijayaraghavan, 2008)

The mechanism of antimicrobial action of ZnO Nps has been attributed to their ability to disrupt the bacterial cell membrane, leading to cell death (Arshad *et al*., 2017). Also, ZnO Nps can induce oxidative stress by generating reactive oxygen species (ROS) that damage bacterial DNA and proteins (Mudshinge *et al*., 2011) thereby causing cell death.

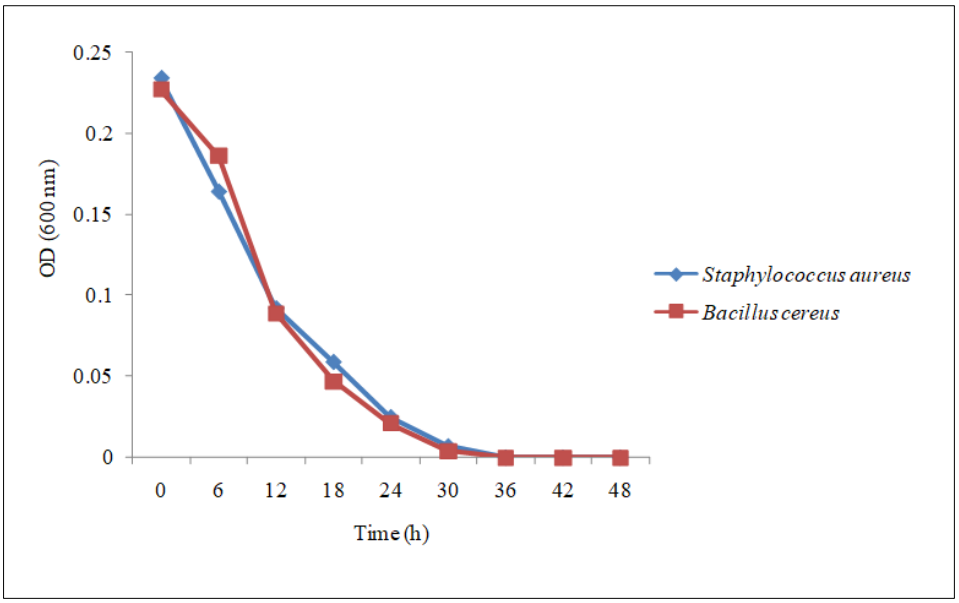

Figure 4.2: Time-kill plots of 50% dilution of ZnO Nps synthesized against *Staphylococcus aureus and Bacillus cereus*

4.2 CONCLUSION

In conclusion, this study successfully synthesized and characterized ZnO NPs using TEM, SEM, and XRD techniques. The antimicrobial activity of the synthesized nanoparticles was evaluated, demonstrating their strong inhibitory effects against microorganisms. These findings suggest that zinc oxide nanoparticles are promising lower-cost alternatives that can inhibit the growth of some microorganisms and can provide alternative means of overcoming the challenge of multi drug resistance to antibiotics. Also, the potential applications of zinc oxide nanoparticles may lead to valuable findings in various fields such as such as medicine, pharmaceuticals, and materials science.

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