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Research Article

Zinc Oxide Nanoparticles

Zinc Oxide Nanoparticles as The Anti-Bacterial Tool: *In Vitro* Study

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Abstract: A new class of metal oxide nanoparticles has recently been found to be widely used in various health-related research. In particular, Zinc Oxide (ZnO) nanoparticles have occupied a prominent status due to their unique chemical and electrical properties. In our study, we aim to reveal the anti-bacterial effect of these nanoparticles. The antibacterial activity of zinc oxide (ZnO) nanoparticles against isolated pathogenic Escherichia coli (strain MTCC 723) was determined from our study. The antimicrobial properties of ZnO were also determined by adjusting the concentration of ZnO nanoparticles. By sonicating ZnO nanoparticles in water, a homogenized suspension was created, and the infusion was made at 50 – 150g/ml concentrations. Anaerobic conditions were used to culture the pathogenic strain of microbial species, and DNA was extracted using an extraction kit. For testing, equalized standard dilutions of cultivated bacteria were utilized. The anti-bacterial capabilities of zinc oxide (ZnO) nanoparticles against bacteria were measured using spectroscopic and diffusion experiments. ZnO nanoparticles with a diameter of 50 nm and a concentration of 150 g/ml lysed Escherichia coli DNA and cells. According to the findings, ZnO nanoparticles with a concentration of 150 g/ml had significantly more vigorous activity against Escherichia coli. The agar diffusion method was used to quantify the anti-bacterial activity of zinc oxide (ZnO) and the quality and amount of DNA extraction in the presence of ZnO nanoparticles (ZnO). The pathogenic Escherichia coli cells and the gene DNA are killed at the optimal dose. This study aims to know the anti-bacterial properties of ZnO nanoparticles against pathogenic Escherichia coli cells. The optimization of ZnO nanoparticles concentration against its effect on Escherichia coli cells is the next aim of this study.

Keywords: Zinc oxide, Antimicrobial activity, *Escherichia coli*, DNA extraction

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1. INTRODUCTION

Nanotechnology is becoming increasingly important in a variety of fields of study. Many fundamental features of nanostructure materials (optical, electrical, mechanical, and so on) may be described as a function of their size, composition, and structural order, as is widely understood. Meanwhile, diverse morphologies of nanostructures are essential components of functional nanostructure devices[1-5]. ZnO nanoparticles have been demonstrated to have anti-bacterial properties against a wide range of Gram-positive and Gram-negative bacteria, including significant foodborne pathogens such as E. coli O157:H7, Salmonella, Listeria monocytogenes, and Staphylococcus aureus[6-10]. Ultrasonic irradiation is used in various production procedures for nanocrystalline ZnO particles, and the particle sizes are regulated using different solvents throughout the sonication process. When seen as a whole, it is clear that the distinctive features (i.e., small size) are the most important. (small size and huge specific surface area) of small nanometer-scale ZnO particles impose various mechanisms that govern its anti-bacterial action[11-13]. Because of their large surface area (surface/volume ratio), nanomaterials produce better results than other water treatment procedures. It is claimed that they could be employed for large-scale water filtration in the future[14-17]. Silver, like ZnO, is a safe and effective anti-bacterial metal because it is harmless to animal cells but very poisonous to numerous bacteria, including E. coli, Staphylococcus aureus, and Staphylococcus epidermidis[18-21]. When embedded and coated on surfaces, metal nanoparticles with antimicrobial activity have a wide range of uses in water treatment, synthetic textiles, biomedical and surgical equipment, food processing, and packaging[22-26]. One of the more recent studies on the anti-bacterial impact of ZnO nanoparticles is a study of bacterial DNA destruction with the ZnO nanoparticle effect. The DNA fragment bands were seen on gel electrophoresis; this activity could be related to surface charge interactions between particles and cells. The particles' free radical scavenging characteristics may have aided cell wall breakdown and anti-bacterial action[27-30]. Due to its unusual optical, electrical, and chemical properties, ZnO has attracted significant interest recently as a wide band-gap semiconductor (3.36eV) with potential electronic applications[31]. Nanostructures are helpful in various applications, including nanoscale optoelectronics[32], piezoelectric nanogenerators [33], and biotechnology[34]. ZnO, like metal nanoparticles CaO, MgO, and Au, appears resistant to microbes[35]. A conductance approach was used to determine the activity of ZnO powder slurries. The anti-bacterial action of ZnO increased with decreasing particle size, which was associated with the change in electrical conductivity with bacterial growth[36]. This study's objective was to determine: 1. what influence ZnO has on bacterial DNA degradation. 2. In the presence of nanoparticles ZnO, the quality and quantity of DNA isolated by chemical techniques. 3. The agar diffusion method was used to test ZnO's anti-bacterial properties. ZnO nanoparticles having a diameter of 50 nm and a concentration of 150 g/ml degraded the quality and quantity of recovered DNA from E. coli MTCC 723. The study aims to see the anti-bacterial activity of ZnO nanoparticles. This activity could be due to surface charge interactions between the particles and the cells. The mechanism behind the antimicrobial properties of ZnO nanoparticles is the free radical scavenging characteristics that have aided in cell wall breakdown and anti-bacterial action. The concentration of ZnO nanoparticles in this paper was adjusted to 150 g/ml for use as an anti-bacterial agent. It functions as an anti-bacterial agent for cells and a gene labelling tool. The peculiarity of the study is that we consider the concentration suitable for the human body and commercial application.

2. MATERIALS AND METHODS

2.1 Chemicals and Synthesis of ZnO-NPs

Materials

Sigma-Aldrich Ltd provided zinc acetate, zinc nitrate, and hexamethylenetetramine. All the compounds employed were research-grade, and the experiments were conducted with double distilled water. The curing agent (Sylgard 184 Silicon Elastomer Kit, Dow Corning, Midland, MI). Polydimethylsiloxane (PDMS).

2.2 Synthesis of ZnO nanoparticle

2.2.1 Preparation and characterization:

The sol-gel-based hydrothermal process generated zinc oxide nano-rods on the PDMS substrate. The following three stages make up the process.

a) *PDMS bed preparation*

A 10:1 solution of PDMS and curing agent was made and continually mixed until a homogeneous mixture was obtained. The mixture was then desiccated to remove any air bubbles. It's then spun coated at 3000 rpm on a transparent polycarbonate sheet. Finally, it is cured 48 hours in a hot air oven at 80°C.

b) *Formation of a seed layer*

A 0.01 M solution dissolving 0.2195 g of zinc acetate dihydrate [Zn (CH3COOH)2.2H2O] in 100 ml ethanol. This seed solution is sprayed over the PDMS substrate, which is then baked for 1 hour at 900 degrees Celsius. As a result, a seed layer with homogeneous and uniform interstitial sites forms. Because of the lattice mismatch, seed-free development of well-ordered ZnO nano-rods is challenging on many surfaces. Therefore, a seed layer is required to achieve well-aligned nano-rods on the substrate.

c) *Growth of ZnO nanorods*

In a beaker, 1.4875 g of zinc nitrate hexahydrate [Zn(NO3)2.6H2O] is mixed with a small amount of DI water. To produce the solution 200 ml, more DI water is added, resulting in a 0.025 M solution. The obtained solution is then given 0.7 g of Hexamethylenetetramine (HMTA) [C6H12N4]. The resulting solution was agitated for 20 minutes with a magnetic stirrer to dissolve the Zinc nitrate and HMTA completely. Next, 50ml of the prepared solution is poured into Teflon containers, with the seed-covered beds at the bottom. The vessels are sealed and heated in a muffle furnace for 3 hours at 900°C.

2.3 Characterization of ZnO.

SEM was used to determine the morphology of ZnO. Scanning electron microscopy (SEM, FEI ESEM Quanta 200)

was used to examine the surface morphology of uncoated and coated beads. Energy dispersive X-ray analysis was used to determine their elemental composition (EDX, JEOL-3010 electron microscope). Determination of nanoparticles concentration Elemental concentrations of the nanoparticle solutions were measured using inductively coupled plasma-mass spectrometry (ICP-MS). Milli-Q water and nitric acid were used in all samples, with basic standards for ICP-MS measurements from CPI International (Amsterdam, The Netherlands). Samples were digested in nitric acid using a microwave discover SP-D system (CEM Microwave Technology, Germany) with parameters: temperature 200 °C; ramp time 4 min; hold time 6 min; maximum power 300 W. Digested samples were diluted with Milli-Q water to give nitric acid concentrations lower than 3% and zinc and silver concentrations lower than 20 ng/g. ICP-MS measurements were performed according to Teiner et al.using an ICP-quadrupole MS instrument Agilent 7500ce (Agilent Technologies, Waldbronn, Germany) equipped with a CETAC ASX-520 autosampler (Nebraska, USA) and a MicroMist nebulizer, at a sample uptake rate of approx. 0.25 mL/min. The instrument was tuned daily, and rhenium served as an internal standard for zinc and silver. The ICP-MS was equipped with nickel cones and operated at an RF power of 1550 W. Argon was used as plasma gas with a flow of 15 L/min and as a carrier gas with a flow of ~1.1 L/min. The dwell time was set to 0.3 s, and replicates of 10 measurements were taken. Agilent Mass Hunter® (Workstation Software, Version B.01.01, 2012) was used for data processing.

2.4 Test microorganisms

MTCC723 is the pathogenic genetic stock (H-10407 collected from National Centre for Cell Science). For the subsequent studies, Escherichia coli was grown in nutrient broth flasks. First, the nutrient broth medium was created by dissolving 28 g of nutrient agar in 1000 ml of Mili-Q water. After that, the solution was autoclaved for 30 minutes at 121°C, 15 lbs.

2.5 Preparation and incubation of test inoculums:

MTCC723 is the pathogenic genetic stock (H-10407). For the subsequent studies, *Escherichia coli* were grown in nutrient broth flasks. First, the nutrient broth medium was created by dissolving 28 g of nutrient agar in 1000 ml of Mili-Q water. After that, the solution was autoclaved for 30 minutes at 121°C, 15 lbs.

2.6 Determination of zone of Inhibition

The antibacterial activity of ZnO nanoparticles was determined using the agar well diffusion method. Mueller Hinton agar media was autoclaved at 120 °C (15 pounds), and 30 ml was placed into each Petri plate. A 24-hour broth culture was distributed aseptically onto solidified Mueller Hinton agar plates using a sterile cotton swab. A sterilized gel borer was used to create wells of uniform length and diameter (10 mm). Each well was filled with 50 l of varied concentrations of ZnO nanoparticles (50 g/ml, 75 g/ml, 100 g/ml, 120 g/ml, 150 g/ml). The plates were incubated for 24 hours at 37 degrees Celsius. The test organisms' sensitivity to varying doses of ZnO Nanoparticles was demonstrated by a clear zone around the wells (figure 2). The average diameter of the area was measured in various directions.

3. RESULTS AND DISCUSSION

3.1 Zinc oxide nanoparticle characterization

3.1.1 Fourier transform infrared spectroscopy.

Fig. 1 shows the FTIR spectra of chemically synthesized ZnONPs. The absorption at 450-540 cm^{-1} corresponded to the ZnONPs. One weak absorption peak occurred at 2926 cm^{-1} due to-C-H stretching. Two weak absorption peaks at 1684 cm^{-1} and 1404 cm^{-1} were due to C=C alkene and C=C aromatic bonds, respectively. The two firm absorption peaks at 1340 cm^{-1} and 1025 cm^{-1} were due to NO2 stretching and C-OH stretching, respectively, while the absorption peaks at 932 cm^{-1}, 832 cm^{-1}, 679 cm^{-1} and 617 cm^{-1} indicated C-H bending (monosubstituted), C-H bending (para), C-H bending (ortho) and acetylenic C-H bending, respectively. The absorption at 444 cm^{-1} corresponded to the ZnO-NPs. The structural changes in the FTIR spectra indicated the capping and stabilization of the ZnO-NPs via coordination with N-H, C=C, CO, and NO2. The FT-IR spectra The strong intensity band at around 505 cm^{-1} indicates the Zn-O stretching vibration.

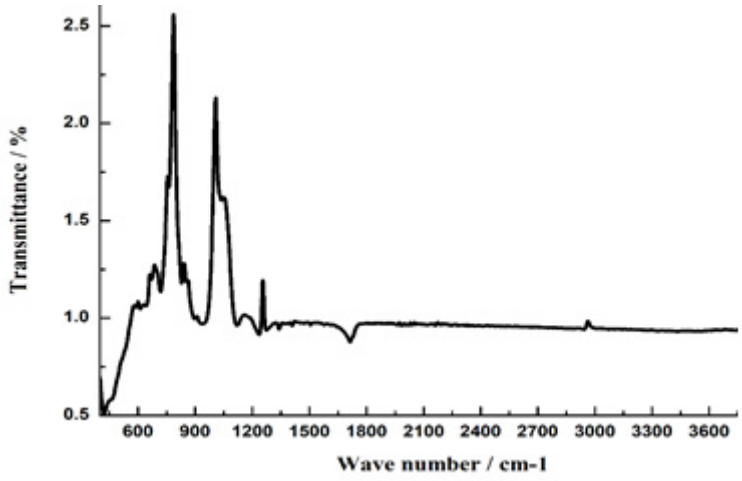


Fig 1. FT-IR Spectra of ZnO Nanoparticle.

The intensity band at different peaks shows the ZnO nanoparticles Stretching vibration.

3.1.2. X-ray diffraction

XRD was used to determine the zinc oxide phase of the NPs. Figure 2 and Table 1 (a and b) show the XRD patterns of ZnO-NPs fabricated by chemical and green methods. The XRD peaks for the chemically synthesized particles were at 2.83, 2.59, 1.92, 1.63, 1.48 and 1.38, while the green-synthesized particles were at 4.08, 3.14, 2.69, 2.48 and 1.56. The limited and strong diffraction peaks indicate the good crystalline nature of zinc oxide. The powder diffraction patterns were indexed, and the Miller indices (h k l) for each peak were assigned in the first step. The strength of the peaks revealed the high crystallinity of the ZnO-NPs. However, the diffraction peaks were broad, illustrating that the crystallites were small[37]. The crystallite size in 99 the ZnO-NPs synthesized by the chemical method was greater than that of ZnO-NPs synthesized by the green method, ranging from 3-78.2 nm and 3-23.6 nm, respectively. The XRD results indicated that the ZnO-NPs formed are crystalline. The size of the ZnO-NPs based on the Debye–Scherrer formula ranged from 3-78.2 nm in chemical synthesis and 3-23.6 nm in green synthesis[39]. ZnO nano-rods were characterized by the X-ray diffraction (XRD) technique, and the diffraction pattern is shown in Fig. The measured diffraction peaks include (100), (002), (101), (102), (110), (103), (200), (112), and (201), which are in accordance with under-reported work and the Joint Committee on Powder Diffraction Standards (JCPDS) card no. 75-0956. The peak intensities were measured in the 2θ range of 30° to 70°. The intensity of the (002) peak should be high relative to the other peaks, indicating that the rods are grown perpendicular to the substrate surface. But since the rods are at an inclination and not all have preferred c-axis orientation, the intensity of (002) is relatively lesser compared (100).

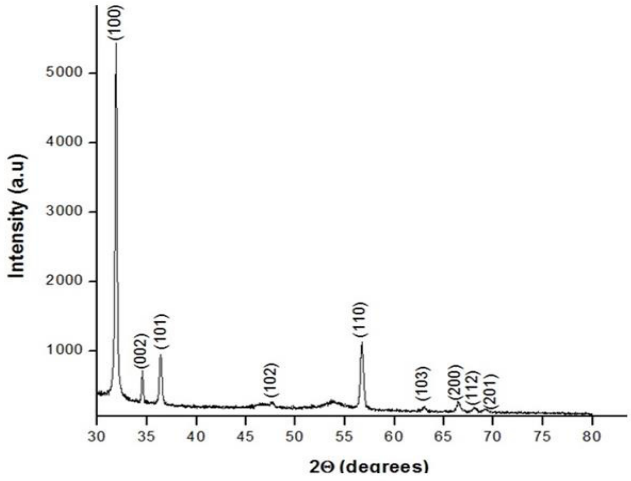
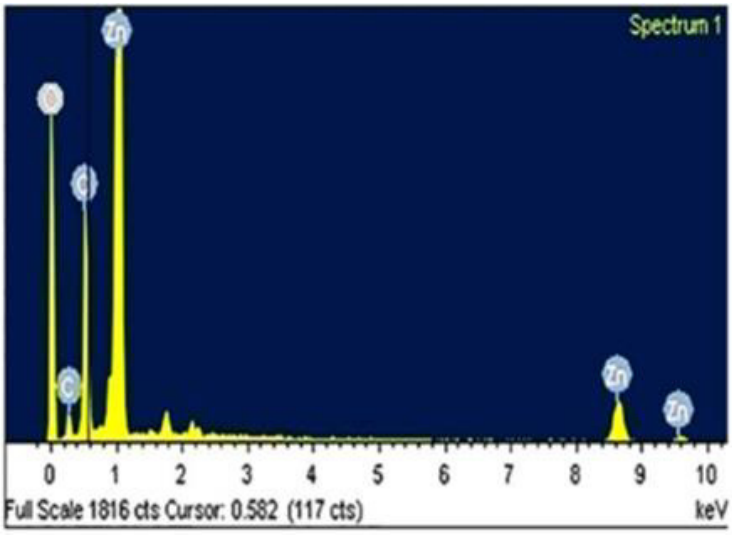


Fig 2. XRD Spectra of ZnO Nanoparticle

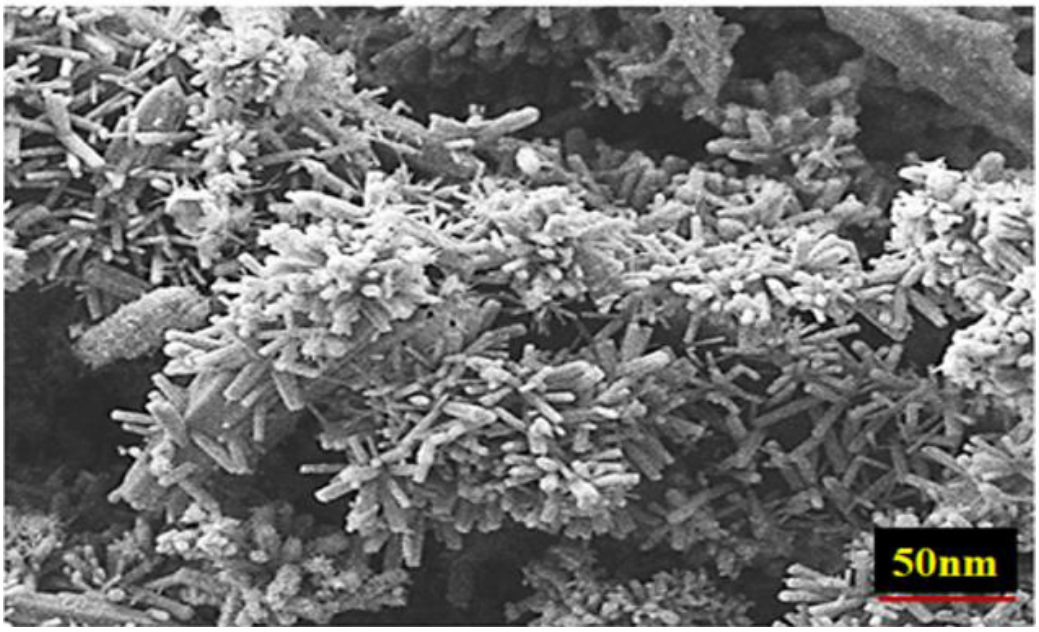


(a)

Table 1: Showing elements and their corresponding composition percentages

Element	Weight%	Atomic %
Carbon(C)	9.38	23.52
Oxygen (O)	24.46	46.01
Zinc (Zn)	66.16	30.47

(b)



(c)

Fig 3. (a) EDX of ZnO nano-rods (b) Table showing elements and their corresponding composition percentages(c) SEM of synthesized ZnO nanoparticles.

The SEM analyses also revealed the presence of agglomerates in the nanoparticles. Scanning Electron Microscope (SEM) image of ZnO (fig. 3) depicts that ZnO nanoparticles are connected to make large network systems with irregular pore sizes and shapes. Pore formation in the combustion-derived products is due to many escaping gases, resulting in a high surface area of the nanoparticles. Occur during ZnO fabrication and post-treatment. These flaws are usually found on the surface of ZnOnanostructures[39,40]. The imperfections could affect the band edge emission's position and the luminescence spectrum's form. The type of the ligand, the relative concentration of reagents, the solvent, the overall concentration of reagents, the reaction time, the evaporation time, and the reaction/evaporation temperature were shown to influence the shape, size and homogeneity of the as-synthesized products. From the experimental procedure based on the same synthetic route with different experimental parameters, i.e., the effects of solvent, ligand, concentration, time, and temperature. The optical absorption spectra of ZnO nanoparticles showed a sharp absorption band at 362 nm (fig.4).

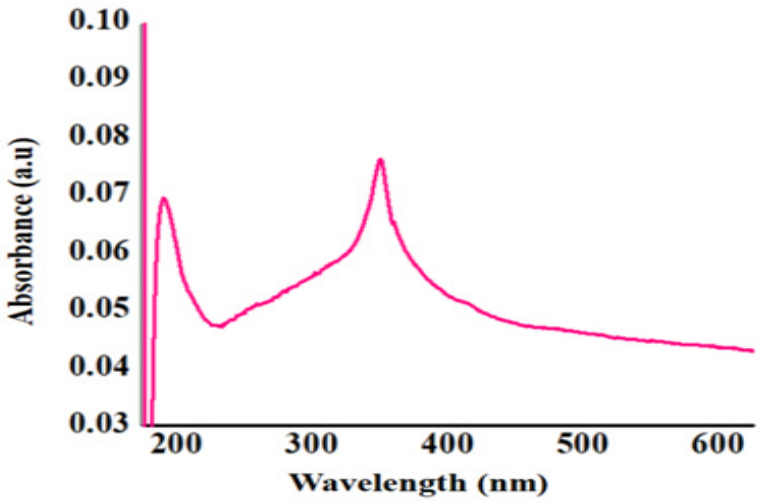


Fig 4: UV-visible spectrum of ZnO nanoparticles

3.2 Anti-bacterial assay

3.2.1 Diffusion method and UV-Vis study

According to the standard reduction of bacteria criterion, a reduction of less than 0–20 per cent indicates no bactericidal effect; a decrease of 20–50 per cent indicates a low bactericidal impact; a decline of 50–70 per cent suggests an expressive bactericide; and a reduction of greater than 70 per cent means a powerful bactericidal effect[22-25]. (Fig. 5) 50 g/ml ZnO has no bactericidal effect according to this criterion. However, plates with 75 g/ml, 100 g/ml, and 125 g/ml have a 30 per cent and 50 per cent bactericidal impact, respectively, and plates with 150 g/ml concentrations have a robust bactericidal effect. According to these findings, ZnO nanoparticles exhibit a potent anti-bacterial influence against

gram-negative E. coli strains. The anti-bacterial impact of manufactured ZnO nanoparticles at various concentrations was investigated on *Escherichia coli* isolates (fig. 5), and (table 2) depicted the inhibition zone of different metal nanoparticle concentrations. For 50 g/ml, 75 g/ml, 100 g/ml, 125 g/ml, and 150 g/ml of zinc nanoparticles concentrations, respectively, ZnO displayed inhibitory zones (mm) of roughly 18, 20, 25, 28, and 30 mm in diameter. The harmful effect of ZnO nanoparticles was clearly understood from the absorbance analysis (fig. 6 and fig.7). The absorbance of the *Escherichia coli* cell with ZnO is lower than that of the bacterial cell alone. The binding qualities of ZnO nanoparticles with bacterial cells are yet unknown due to the mechanism of tuning ZnO with bacterial cells. Still, the primary explanation could be: 1.Zn2+ emitted in the broth significantly contributed to zinc oxide nanoparticles' overall anti-bacterial action[24]. 2. ZnO comes into direct touch with the bacterial cell walls[25]. The tailored nanoparticles destroy bacterial cell integrity[26,27] and produce reactive oxygen species (ROS)[28]. Soluble zinc species and ZnO powders with a greater specific area exhibited microbial strain specificity[29].

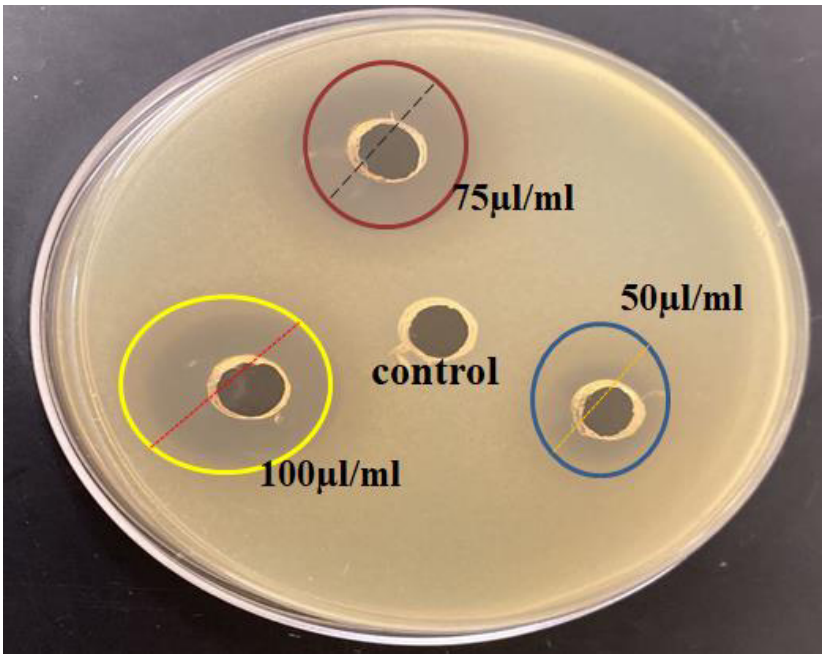


Fig 5: Anti-bacterial activity of different concentrations of ZnO nanoparticles against Escherichia coli cells

Table 2: Zone of inhibition of ZnO nanoparticles against *Escherichia coli* cells.

ZnO Nanoparticle µg/L	Zone of Inhibition (mm)
50	18
75	20
100	25

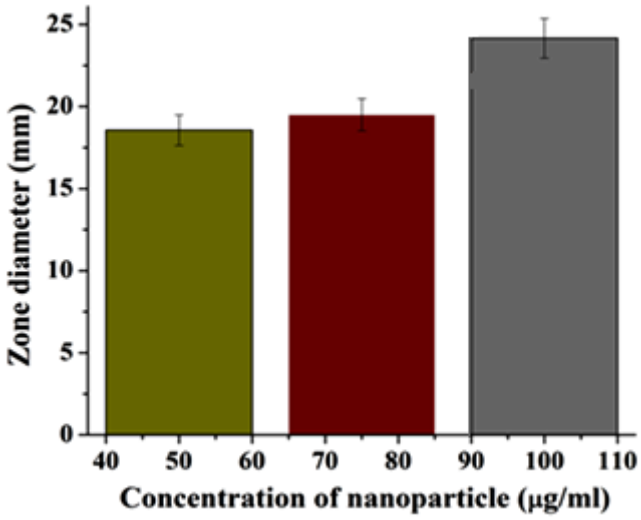


Fig 6: Zone of Inhibition of ZnO nanoparticle against *Escherichia coli* cells

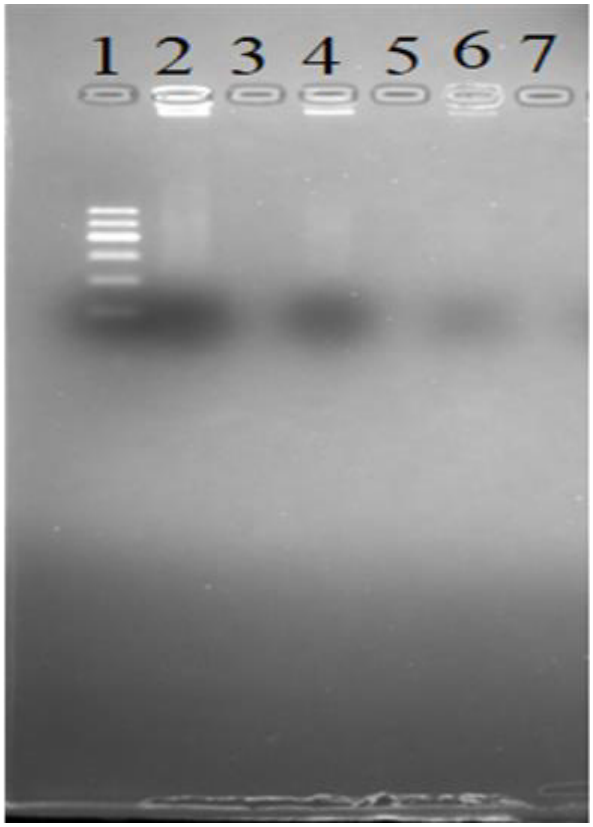


Fig 7: The amount of DNA from normal *Escherichia coli* cells (Lane 2) and bacterial cells treated by Nano ZnO (Lane4 (50µg/ml) and Lane 6(150µg/ml))

4. CONCLUSION

Based on the preceding in vitro investigation, it was established that ZnO NPs have high anti-bacterial activity. It shows anti-bacterial efficacy against pathogenic Gram-negative Escherichia coli bacteria at higher concentrations. When DNA was extracted in the presence of ZnO NPs, the quality and quantity of the DNA were degraded from an experimental standpoint, it is clear that ZnO NPs are an effective anti-bacterial weapon against pathogenic microbes. Because of the favourable effects of metal nanoparticles on microbes and their ability to regulate and prevent the spread of many infectious diseases, many experts predict that nanoparticles will usher in a new era in health.

5. ACKNOWLEDGMENT

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6. AUTHORS CONTRIBUTION STATEMENT

Gargibala Satpathy (corresponding author): Designed the purpose of the study, conception, and Collection of samples. Jayanti Rebecca (Second author): Supervised the subject's details, inclusion and exclusion criteria. Umabati Sahu(Third author), Ipsita Das (fourth author) and Dr E.Manikandan (Fifth author): Done the data analysis.

7. CONFLICTS OF INTEREST

Conflict of interest declared none.

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