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A Comparative Study of Antibacterial Activity of ZnO and TiO_2 Nanoparticles Against Gram-Positive and Gram-Negative Bacteria

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HIGHLIGHTS

- Two types of Nanoparticles, ZnO and TiO_2, were prepared utilizing the sol-gel technique.
- Nanoparticle characterization was studied using XRD, SEM, and FTIR.
- Antibacterial activity of the Nanoparticles was compared against E.coli and Staph.aureus bacteria.
- Results showed ZnO NPs affected the two bacteria more than TiO_2 NPs.

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ABSTRACT

Using the sol-gel technique, this study successfully synthesized two types of nanoparticles, ZnO and TiO_2. The Fourier-transform infrared (FTIR) spectrum exhibited a broad peak, providing insights into crucial chemical bonds. The average grain sizes, 18.6 nm for ZnO and 12.6 nm for TiO_2 were determined through X-ray diffraction (XRD). Scanning electron microscopy (SEM) images of the (ZnO & TiO_2) powder revealed the presence of pores and agglomeration. The antimicrobial efficacy of these nanoparticles was evaluated against Gram-negative bacteria (E. coli and Proteus) and Gram-positive bacteria (Staph. aureus). The results demonstrated the capability of both ZnO and TiO_2 to impact bacterial survival rates, with ZnO nanoparticles exhibiting a superior effect compared to TiO_2 nanoparticles. This research contributes valuable insights into the antimicrobial properties of ZnO and TiO_2 nanoparticles, emphasizing their potential applications in combating bacterial infections.

1. Introduction

In recent years, nanotechnology has experienced significant growth, with the development of nanomaterials being a crucial aspect of its advancement. Nanostructured materials, characterized by their unique properties and geometric dimensions at the nanoscale, have garnered substantial interest, particularly in biological and pharmaceutical applications [1]. While antibiotics have traditionally been effective against specific disease-causing organisms, the emergence of nanomaterials has displayed their ability to eliminate a broader spectrum, targeting up to 650 cells [2,3].

Among nanomaterials, nano oxides, specifically zinc oxide (ZnO), have attracted considerable attention due to their antimicrobial properties and perceived safety for humans and animals [4]. Nanoparticle laboratory experiments have demonstrated the rapid eradication of bacteria, viruses, and fungi upon contact. Understanding the impact of nanoparticles on bacteria is crucial, given their role at the lowest level of the ecosystem's food cycle [5].

In contrast to using organic and inorganic chemicals in the food sector as antibacterial agents, inorganic antibacterial materials like ZnO have gained prominence due to their stability under challenging processing conditions [6]. Notably, nanoparticles formed of metal oxides, especially those smaller than 100 nm, exhibit unique antimicrobial activities attributed to their distinctive properties, such as small particle size and large surface area [7]. Research has shown that certain metal oxide nanoparticles, like ZnO, selectively harm bacteria without adverse effects on human cells [7].

The antibacterial mechanisms of ZnO nanoparticles are still under investigation, with proposed actions including damage to the cell membrane, the production of reactive oxygen species like hydrogen peroxide, and interactions with intracellular contents [8,9]. Titanium dioxide (TiO_2), a photocatalyst known for its nontoxicity, photo-induced superhydrophobicity, and antifogging qualities, plays a significant role in environmental purification [10,11]. Its applications range from self-cleaning

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and self-disinfecting surfaces in medical centers to water and air purification from germs and harmful chemical compounds [12,13].

Various morphologies of one-dimensional TiO_2 nanostructures, such as nanorods, nanowires, and nanotubes, have been successfully synthesized using different techniques [14,15]. This study aims to investigate ZnO and TiO_2 nanoparticles' antibacterial effects against gram-positive and gram-negative bacteria across different concentrations. Exploring these nanomaterials' antibacterial properties holds promise for diverse applications, especially in fields requiring effective antimicrobial agents.

2. Materials and Methods

2.1 Preparation of Nanomaterial

2.1.1 Preparation of ZnO NPs

The sol-gel method is employed to synthesize zinc oxide nanoparticles (ZnO NPs). Initially, materials were accurately weighed using a balance, and distilled water was measured with a graduated cylinder. Specifically, 2g of zinc acetate dehydrate was dissolved in 10ml of distilled water, and 7g of sodium hydroxide was added to the solution. The mixture was stirred continuously for approximately five minutes to ensure thorough blending. Upon achieving a homogeneous solution, the zinc acetate solution was stirred with a magnetic stirrer for five more minutes while the sodium hydroxide solution was carefully added drop by drop. As the reaction progressed, a white precipitate became evident. Subsequently, the solution underwent filtration, and the resulting substance was subjected to drying at 65°Cfor one day. Post-drying, the substance was ground into a fine white powder.

2.1.2 Preparation of TiO_2 nanoparticles

In the synthesis of TiO_2 nanoparticles, the sol-gel method is employed. Specifically, 8 ml of titanium isopropoxide (TTIP), 140ml of ethanol, and 12ml of deionized water meticulously combined under constant stirring. The mixture was maintained at 80°Cfor 5 hours, facilitated by a paraffin oil bath. Subsequently, the gel formed was dried in a hot air oven set at 70˚C. The next step involved calcination, in which the dried gel was heated at 500°C for three hours. This critical step aimed to bring about the transformation of the gel into TiO_2 nanoparticles. The carefully controlled conditions during the synthesis process play a pivotal role in determining the characteristics and properties of the resulting nanoparticles, making them suitable for various applications in scientific research and technology.

2.2 Preparation of Bacterial Culture

Two types of bacteria (E.coli and Staph. aureus) were activated by culturing on nutrient agar at 37˚C for 24 hours. Then diluted by normal saline to obtain 107 CFU/ml in suspension (McFarland no.5). These were cultured with ZnO and TiO_2 in different concentrations (0.4, 0.8, and 1.6 mg/ml) and incubated in shaker incubation at 37°C for 24 hours at 160 rpm. Made serial dilutions three times, and 100 l of suspension, spread on a molar Hinton agar plate and incubated at 37°C for 24 hours. The formula to calculate the bacteria's survival rate is as follows:

$$\text{Number of Colonies } [(\text{CFU})/\text{ml}] = (\text{Number of colonies for each dilution}) \times (\text{dilution 103 factors})/\text{ sample volume}) \qquad (1)$$

As well as evaluated the percentage decreasing of bacteria by bacteria survival rate K [16]. The formula to calculate the bacteriostatic rate [17] is as follows:

$$\text{Bacteriostatic rate } 100\%) = (1\text{- colonies of test groups/ colonies of control group}) \times 100 \qquad (2)$$

3. Results and Discussion

3.1 Fourier Transformation Infrared Results

The functional groups present in the synthesized titanium dioxide particles were characterized using FTIR analysis. The essential chemical bonds of ZnO powder are elucidated through the FTIR spectrum presented in Figure 1. The graph displays a prominent peak at 466.77 cm^{-1} and a shoulder at 551.64 cm^{-1}, both indicative of Zn-O bonds [17]. In Figure 2, the FTIR spectrum of Titanium dioxide particles reveals a broad band between 500 and 600 cm^{-1}, attributed to the vibrations of Ti-O bonds within the TiO_2 lattice. Additionally, peaks observed between 1620 and 1630 cm^{-1} and 3100 and 3600 cm^{-1} are attributed to the vibrations of hydroxyl groups (-OH) [18,19].

3.2 XRD Results

X-ray Diffraction (XRD) is a robust and non-destructive method employed to analyze crystalline materials, especially when grain size significantly influences the intensity and shape of diffraction peaks in the patterns [20,21]. The XRD patterns of the synthesized ZnO nanoparticles are depicted in Figure 3, where intensity measurements were conducted over a 10-90° range. The average grain size of the produced ZnO powder was calculated using the Scherrer formula and the strongest peak intensity. The diffraction peaks at 2θ values of 31.65°, 34.3°, 36.14°, 47.45°, 56.43°, and 62.65° corresponded to the hexagonal

ZnO particles' (100), (002), (101), (102), (110), (103), (112), and (201) planes, respectively (JCPDS 36-1451). The absence of additional peaks signifies the purity of the ZnO powder [22].

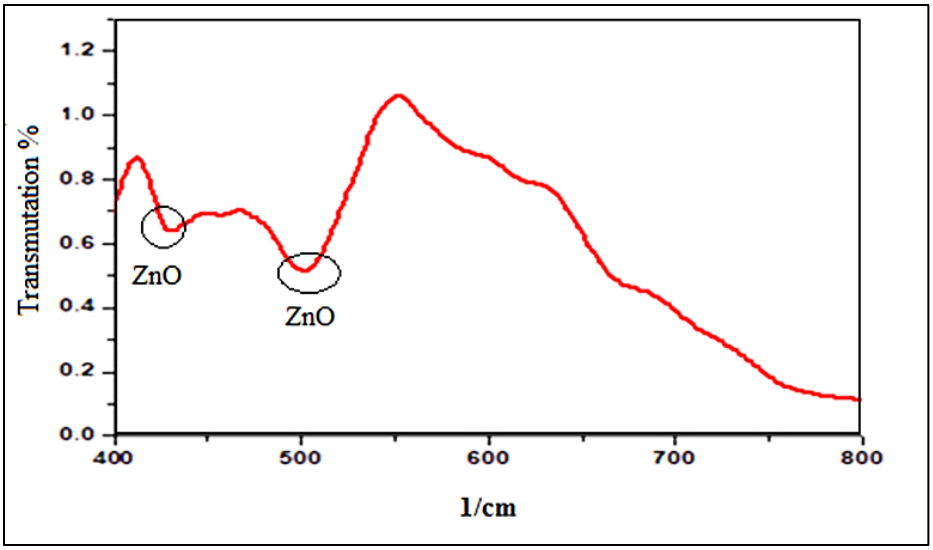


Figure 1: FTIR spectra of Zinc oxide particles, illustrating a large peak and a shoulder, both consistent with Zn-O bonds

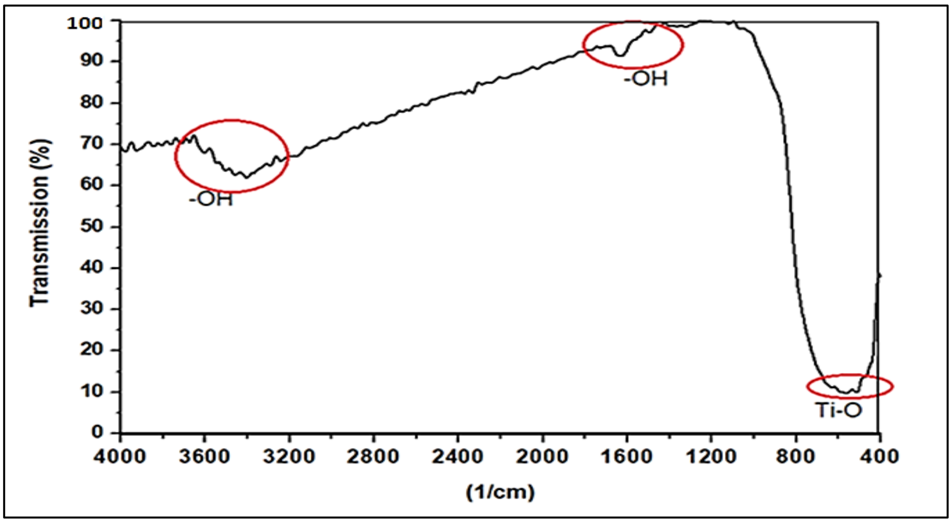


Figure 2: FTIR spectra of Titanium dioxide particles, highlighting a broad band between 500 and 600 cm^{-1} attributed to Ti-O bonds vibrating within the TiO_2 lattice

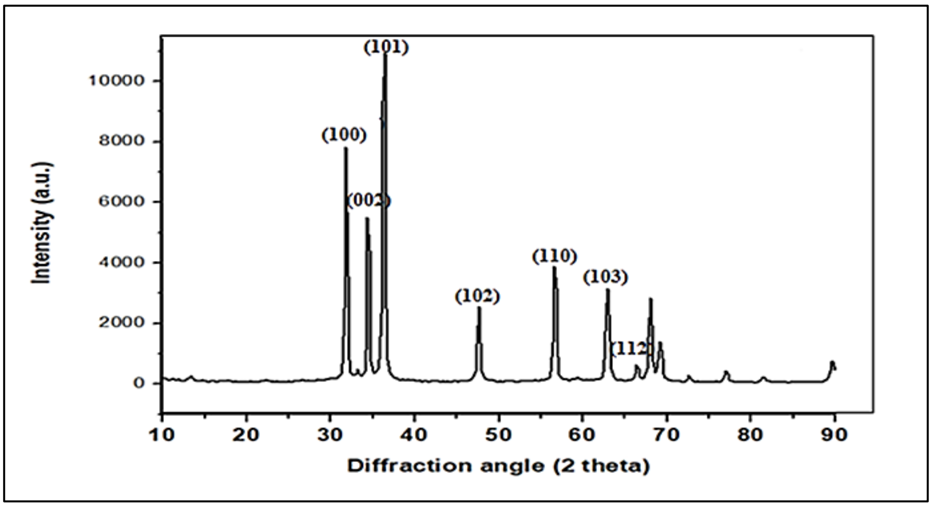


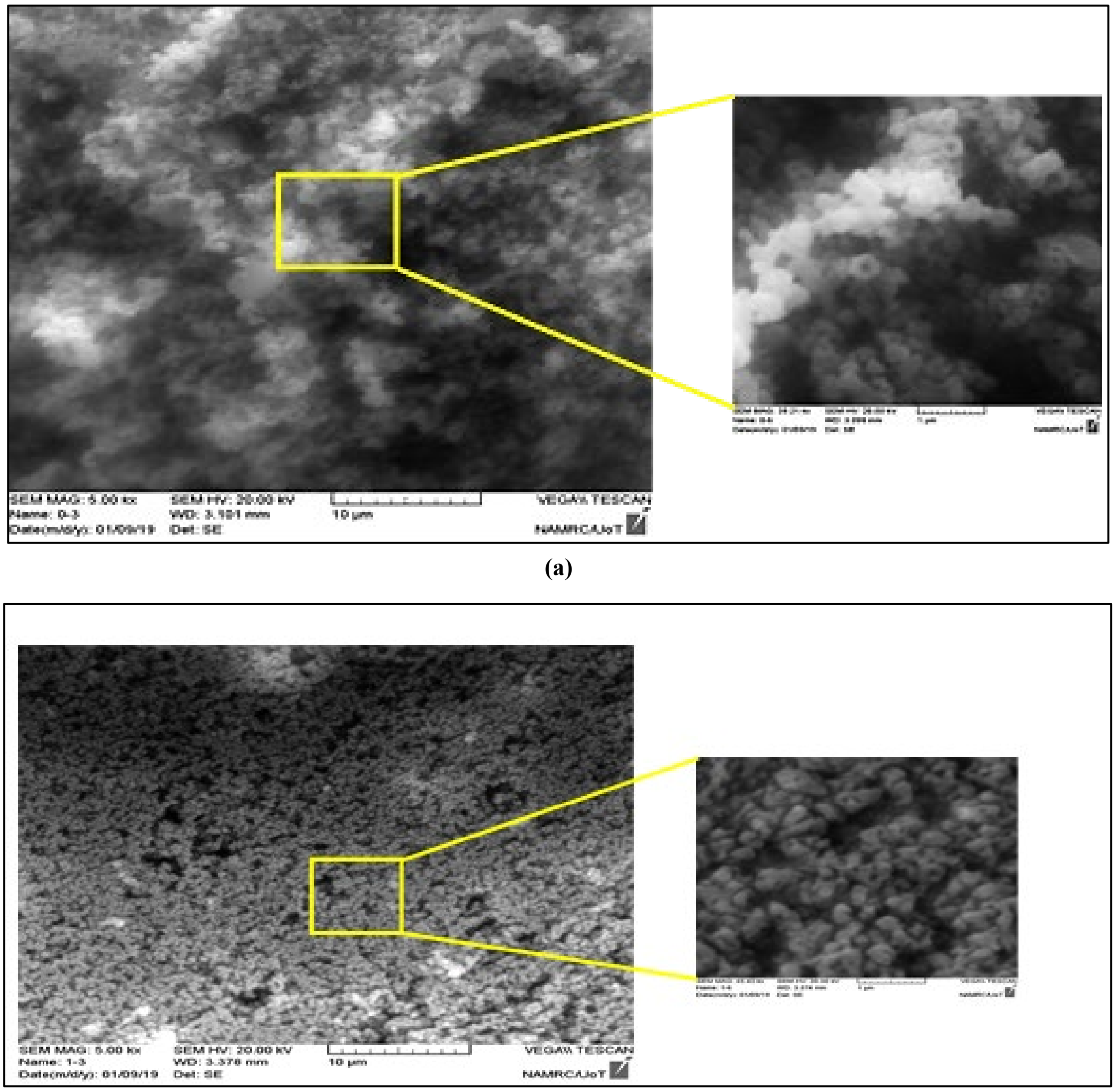
Figure 3: XRD peaks of ZnO particles, the hexagonal ZnO particles' No more peaks seen, demonstrating this purity of the powder

Titanium exists in three crystalline forms: anatase, rutile, and brookite. Rutile is the most prevalent and stable form characterized by superior UV absorption, lower photo reactivity, and reduced hazards compared to anatase [23–26]. Figure 4 illustrates the XRD diffraction of TiO_2 particles, revealing that the anatase phase is predominant in this synthesis, as evidenced by the main peaks at 25.3 (101), 38 (004), 48 (200), 54 (105), and 62.4 (204) [27].

The produced particles' mean grain size (D) was determined using the Scherrer Equation 3 from the XRD line broadening measurement. The grain sizes of Titania and zinc oxide were 12.6 nm and 18.6 nm, respectively.

$$D = 0.89\lambda/(\beta \mathrm{Cos}\theta) \tag{3}$$

where: λ is the wavelength (Cu Kα), β is the full width at the half-maximum (FWHM) of the ZnO (101) line, and θ is the diffraction angle.



(a)

(b)

Figure 4: SEM image of (a) synthesized TiO_2 particles, (b) synthesized ZnO particles

3.3 SEM Results

This study investigated the topography of powders derived from two materials, ZnO and TiO_2, using scanning electron microscopy (SEM). The examination revealed that ZnO particles exhibited an average size of less than 1000 nm, as illustrated in Figure 5, while titania particles displayed an average size of less than 500 nm, as depicted in Figure 5. The SEM images indicated the presence of pores within the observed powders. Furthermore, the images highlighted the occurrence of agglomeration in both ZnO and TiO_2 particles. This agglomeration phenomenon is attributed to the tiny particles' high surface area, which encourages the particles to come together.The agglomeration phenomenon is evident in the SEM images, emphasizing the need for careful consideration of particle size and morphology in applications where dispersion and uniformity are crucial.

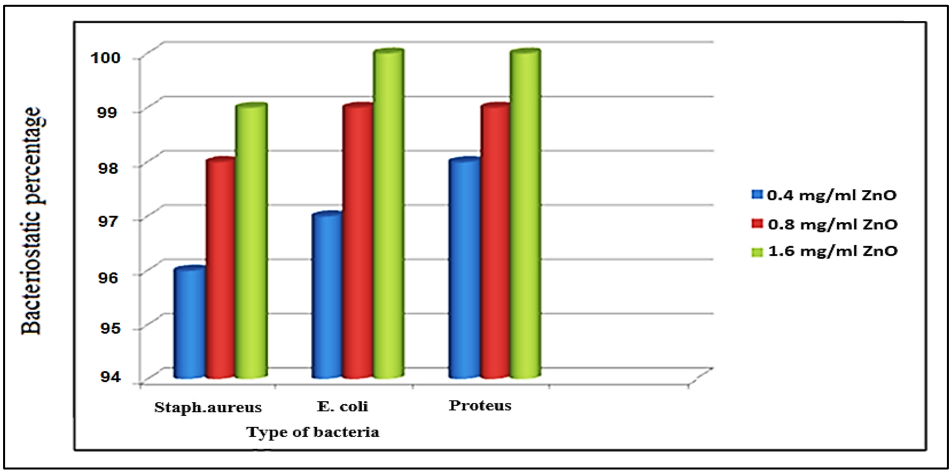


Figure 5: Bacteriostatic percentage of ZnO Nanoparticles against Staph.aureus, E.coli, and Proteus

3.4 Antibacterial Test

In this research, the results compared the two types of Nanoparticles, ZnO, and TiO_2, against two types of gram-positive bacteria (Staph. Aureus) and gram-negative bacteria (E.coli, Proteus) in different concentrations (0.4,0.8,1.6) mg/ml, and the results explain the ability of two types of Nanoparticles to effected on these types of bacteria in all concentration and different percentage by assessing the antibacterial activity of these materials.

The effect of TiO_2 Nanoparticles is less than the effect of ZnO Nanoparticles on all types of bacteria used in this research. The bacteriostatic of ZnO Nanoparticles in concentration (0.4, 0.8,1.6) mg/ml against (Staph. aureus) was (96, 98, 99)%, against (E.coli) was (97, 99,100)% and against (Proteus) was (98, 99,100)% respectively, as shown in Table 1. The results of TiO_2 Nanoparticles as bacteriostatic were (60,70, and 90)% against (Staph. Aureus), (94.5, 96.3, and 97)% against (E.coli) and (97, 98, and 100)% respectively in the same concentration, as shown in Figures (6 -8).

Table 1: The antibacterial activity of TiO2 &ZnO affected two types of bacteria with different percentages of kills

Material	Cont.	Gram-negative bacteria		Gram-positive bacteria
		E.coli	Proteus%	Staph.Aureus%
Zno	0.4	97	98	96
	0.8	99	99	98
	1.6	100	100	99
Tio_2	0.4	94.5	97	60
	0.8	96.3	98	70
	1.6	97	100	90

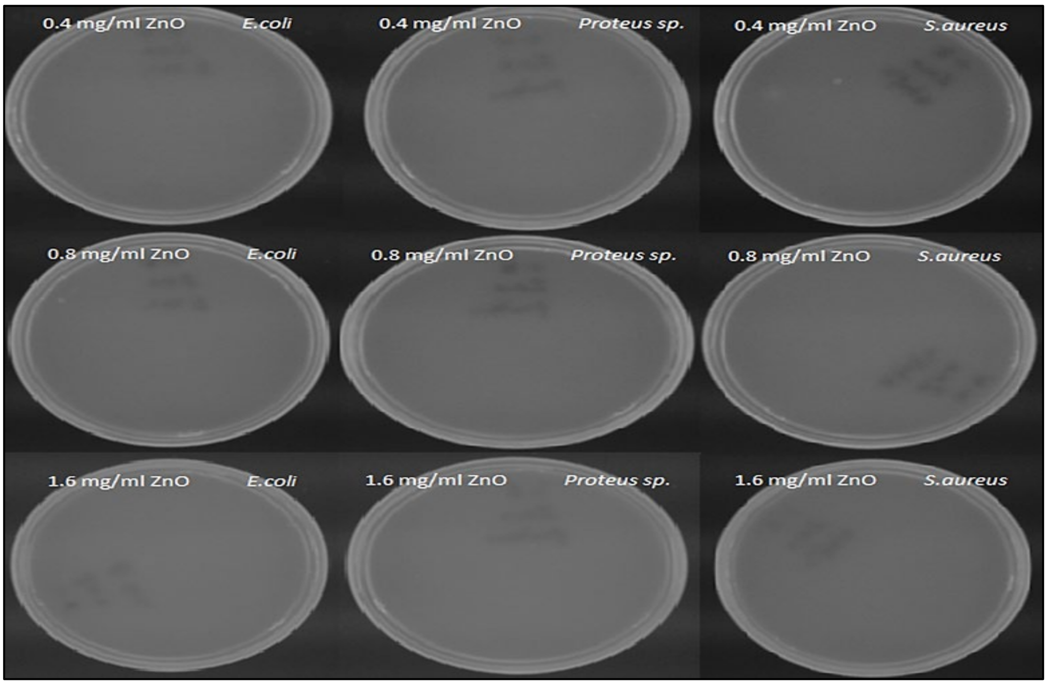


Figure 6: Photography effect of ZnO NanoParticles against Staph. Aureus, E.coli and Proteus

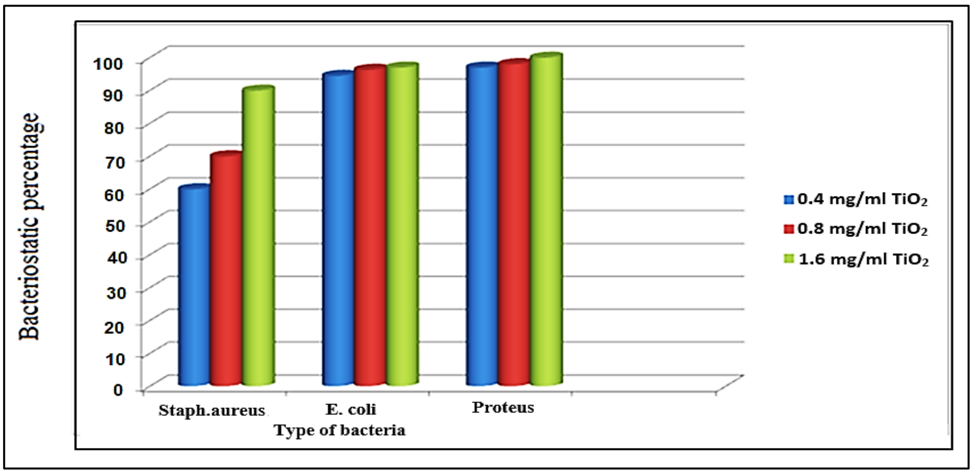


Figure 7: Bacteriostatic percentage of TiO_2 Nanoparticles against Staph. aureus , E.coli and Proteus

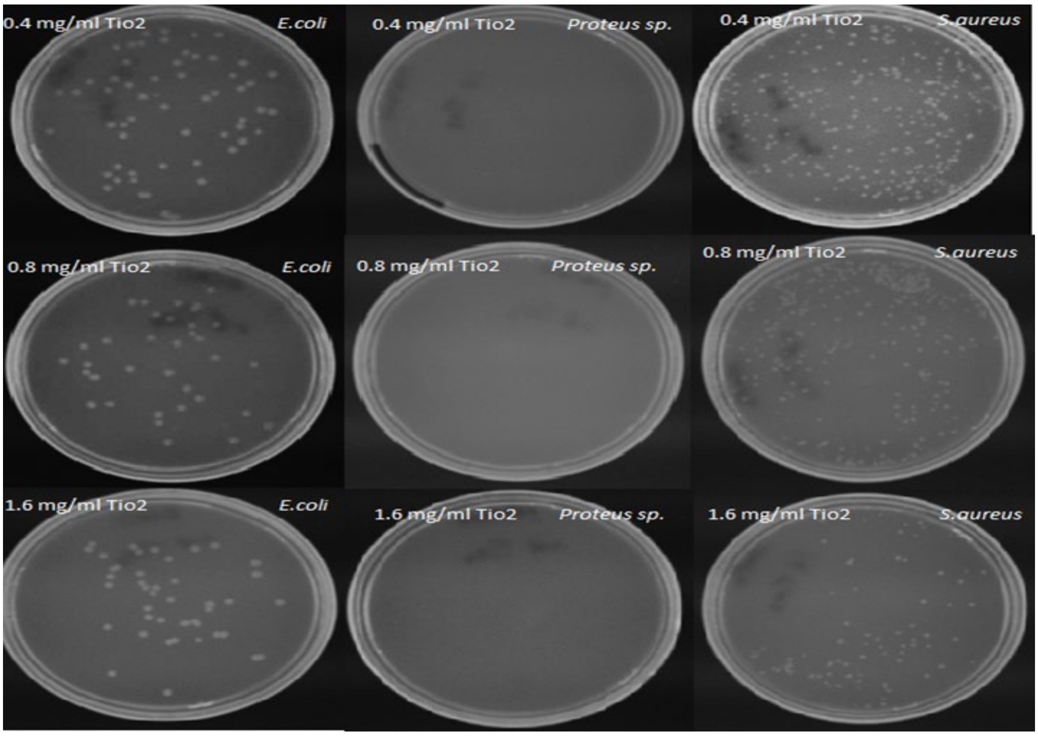


Figure 8: Photography effect of TiO_2 Nano Particles against Staph.aureus, E.coli and Proteus

Also, the results improved that bacteriostatic of both nanomaterials (ZnO & TiO_2) raised with concentration increasing. The distinction between Gram-negative and Gram-positive bacteria's antibacterial activity is determined by a variety of variables, including the following: Differing outer layer or cell wall architecture [28]. Gram-positive bacteria, like S. aureus, have thick cell walls that lack any periplasmic region and are mostly composed of peptidoglycans as well as other materials, such lipoteichoic acids (LTA). Gram-negative bacteria have relatively thin cell walls and periplasmic spaces between their inner and outer membranes [28]; (ii) the carotenoid pigments in S. aureus offer a better oxidant resistance [29] (iii) S. aureus can produce oxidative stress response gene products to combat oxidative stress conditions. In E. coli, comparable mechanisms are less effective [18]. Also, additional elements, including how the Nps associate with the cell membrane, the capacity of the bacterial cell to dissolve ZnO, and cell permeability, must be considered [28].

The unique characteristic of nanoparticles' ability to effortlessly pass the cell membrane of bacteria with hole sizes smaller than their own is thought to be the mechanism [30]. Additionally, the thiol (-SH) groups of the proteins found in the cell wall interact with the metal oxide nanoparticles' antibacterial activity, mostly visible on their surface. As a result of this interaction, cells become less permeable and lyse [31].

Cell death results from releasing genetic information, proteins, and minerals through damaged cell membranes. The protein leakage from bacterial pathogens was greater than that from fungal infections. These findings suggest that most of the cells exposed to nanoparticles were phantom cells that released intracellular content into the suspension of cells. The degree of harm to the cell membrane is determined by how long it is exposed to this stress on the cell wall. As a result, more lactate dehydrogenase enzymes were generated [32].

This study looked at the antibacterial effects of various TiO_2 concentrations. One of the most significant agents responsible for nosocomial infections, E.coli, is also resistant to broad-spectrum antibiotics. Antibiotic resistance in bacteria has significantly grown due to excessive antibiotic use [33].

The rates of infectious disease mortality and morbidity can be decreased by the introduction of innovative antibacterial medications. Numerous bacterial strains have been demonstrated to be significantly inhibited by nanomaterials. Studies suggest that because metal oxides have a positive charge and microorganisms have a negative charge, there is an electromagnetic attraction between them that results in oxidation and finally results in the death of the microorganisms [34]. Nanomaterials can also inactivate biological enzymes and DNA by collaborating with electron-donating groups like hydroxyls, indoles, thiols, polysaccharides, amino acids, and others. Bacterial cell walls develop pits, which increase permeability and cause cell death [35].

In the current work, we demonstrated that varying TiO_2 concentrations might stop an antibiotic-resistant strain of E.coli from growing. TiO_2 might be utilized as the appropriate disinfectant in hospital settings where resistant bacteria could spread readily and infect surgical wounds and burn victims. TiO_2 nanomaterials are used in the textile sector to create cotton textiles with an antibacterial effect [36]. To reduce the risk of infection in patients, creating sutures or wound bands from such cotton materials could be conceivable.

As was seen for E.coli in the current study, strong binding of nanoparticles to the outer membrane of E.coli inhibits active transport, dehydrogenase, and periplasmic enzyme activity, which in turn inhibits RNA, DNA, and protein synthesis, ultimately leading to cell lysis [37]. Such effective and quicker formulations may be useful in clinical settings where E.coli causes urinary tract infections (UTIs). Numerous bacterial strains have been demonstrated to be significantly inhibited by nanomaterials [38]. To determine the optimal concentration that can have the most efficient antibacterial ability against the E. coli culture, several nanoscale TiO_2 and CdO concentrations were investigated throughout the current investigation. Our results are consistent with earlier research on the antibacterial properties of nanomaterials [38–40]. Numerous studies have proposed potential pathways for nanomaterials' interaction with biological macromolecules. It is thought that metal oxides have a positive charge while microbes have a negative charge. The microbe and treated surface are attracted to one another in an "electromagnetic" manner. The moment the microorganism comes into touch, it oxidizes and promptly dies. The thiol groups (-SH) of the proteins on the bacterial cell surface are thought to interact with the ions produced by nanomaterials. These proteins protrude from the bacterial cell membrane, which allows nutrients to flow through the cell wall. Nanomaterials render proteins inactive, which reduces membrane permeability and ultimately causes cellular death [39] additionally, nanomaterials prevent the development of biofilms and bacterial adhesion. These proteins stick out from the bacterial cell membrane and allow nutrients to pass through the cell wall proteins rendered inactive by nanomaterials, which decreases membrane permeability and ultimately causes cellular death [39], nanomaterials prevent the development of biofilms and bacterial adhesion [40,41]. A widely sought goal is an antimicrobial modification to stop the growth of harmful germs. A compact bio-film matrix is created because microbial cell development and colonization can shield the underlying microorganisms from antibiotics and the host's defensive systems. Serious illness may ensue from microbial invasion [42,43]. These infections are also linked to material biofouling, food deterioration, and the development of food-borne illnesses [44]. As a result, Much emphasis is being paid to creating antimicrobial goods and surfaces for application in the biomedical, food, personal hygiene, and health industries. The metal-based nanoparticles' broad-spectrum biocidal activity against various bacteria, fungi, and viruses [43]. Nanomaterials are known to inactivate biological enzymes and DNA by coordinating with electron-donating groups such as thiols, carboxylates, amides, imidazole, indole, and hydroxyls. They puncture the bacterial cells' walls, increasing permeability and resulting in cell death [44].

4. Conclusion

Two distinct materials, ZnO and TiO_2, were successfully synthesized and characterized using diagnostic tools such as XRD, FTIR, and SEM. The analyses confirmed the successful preparation of ZnO and TiO_2 in accordance with their respective phases. The determined granular sizes were 18.6nm for ZnO and 12.6nm for TiO_2. The research demonstrates that both ZnO and TiO_2 are reliable materials with potential antibacterial applications. Notably, ZnO exhibited a superior antibacterial effect, displaying a more robust capability in eliminating Gram-positive and Gram-negative bacteria than TiO_2. This highlights the differential antimicrobial properties of the two materials, with ZnO emerging as a safer and more effective option for combatting bacterial infections. These findings contribute valuable insights into the synthesis, characterization, and antibacterial efficacy of ZnO and TiO_2 nanoparticles, emphasizing their potential applications in various fields, including medicine and healthcare.

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Data availability statement

The data supporting this study's findings are available on request from the corresponding author.

Conflicts of interest

The authors declare that there is no conflict of interest.

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