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Synthesis, Characterization and Biological Activity of Zinc Oxide Nanoparticles (ZnO NPs)

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ABSTRACT
The main target of this paper, synthesis ZnO Nanosize by rapid easy and Successful method as Nano technique, study the structural ,optical and antibacterial activity of ZnO NPs catalyst .The ZnO NPs had been synthesized by thermal-precipitation method. This method achieved in KOH (pH=8) as alkaline media was important condition in precipitation method, in 60-70°C to provide thermal factor. Then the precipitate filtrated, washed and dried in air room temperature for 48h.later the catalyst calcined in 300 and 500°C in electric furnace for 120 min. Part of precipitate don't treatment in furnace. The production was white nano powder characterized by SEM, EDX, and XRD.
The key aim of the current study is the investigation of antibacterial activity of ZnO NPs towards bacterial strains such as Gram-positive Staphylococcus aureus, Gram-negative Escherichia coli (E.coli) and Pseudomonas aeruginosa. This antibacterial activity was performed through the agar well diffusion method using different preparation temperatures of ZnO NPs, both with and without antibiotic (Oxytetracylin OTC). The resulting data showed that a relatively higher inhibiting zone was found in Gram-positive Staphylococcus aureus than for Escherichia coli and Pseudomonas aeruginosa, indicating that ZnO NPs do have antibacterial activity and could thereby improve the exact type of activity in antibiotics. It is therefore found that this study could be of use in formulating nano-drug conjugates that function as an antimicrobial agent within different aspects of research in medicine and pharmaceutics.

Key words: ZnO Nona size, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, precipitation method, physical properties, Parameters.

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INTRODUCTION
Modern material sciences mainly focus on nanotechnology, as it provides a wide variety of new applications ranging from food processing and agricultural producing to sufficient medical technicalities [1]. Among these applications are synthesizing, characterizing and exploring materials within the nano-meter span (1–100 nm), at which a definition of the characteristics and functionalities of living and anthropogenic systems is provided [2]. Whenever the nanoscale size of a material's structure exhibits novel and noticeably improving physio-chemical and biological characteristic, in addition to remarkable phenomena and functionality, it is considered to be pertinent [3]. In general, the nano-scale size can confer relatively wider surfaced areas to nano-particles (NP) than macro-sized ones [4], as the former are subjected to control or manipulation, showing remarkably differing characteristics from bulk materials [5] at the atom level (1–100 nm). Metal NPs like zinc oxide (ZnO), TiO_2, and silver are mainly distinguished by their compositional, crystalline, and morphological properties. The reduction to nano-scale size could affect the material properties in terms of chemistry, mechanics, electrics, structure, morphology and optics, eventually allowing NPs of interacting uniquely with bio cell molecules and facilitating the physical transmission of an NP inside inner cellular structures [6, 7]. Due to the semi-conductivity of ZnO having a wide band gap (3.3 eV), larger exciton binding energy (60 meV), and an n-type conduction, in addition to being environmental-friendly, this material is considered to be interesting to be applied in various cases such as solar cells, optical coatings, photo catalysts, electric devices, antibacterial coatings, active medium in UV semiconductor lasers, and in gas sensors [8- 10].
A number of approaches have been applied in synthesizing metal NPs, among which are sol-gel method, thermal decomposing, hydro-thermals, and microwave irradiating [11]. Yet, these techniques are found to be rather costly and time-consuming, in addition to their toxicity as adding chemical agents in the reducing process generates a large quantity of secondary waste materials [12].
Metal-oxide nano particles such as MgO, Cu_2O, CuO, ZnO, TiO_2, and WO_3 [13-15] have provided sufficient anti-microbial effects, presenting remarkable behavior and characteristics either for micro- or milli-metric particles [16]. It has been noted that ZnO NPs function as antimicrobial agents against pathogenic and spoilage microorganisms [17]. Applying zinc oxide nano particles as antimicrobials outweighs any other metallic nanoparticles [18].
Inducing oxidative stress as a result of forming oxygen species have led to the major antimicrobial mechanism of such particles. The accumulated zinc oxide NPs causes the membrane to disrupt so as to internalize the particles and release antimicrobial ions (Zn^{+2}) [19]. The process of synthesis determines the morphology of ZnO-NPs, as these could take the form of nano rods or -plates [20–21], nano spheres [22], nano boxes, hexagonal, tripods [23], tetra-pods [24], nano wires, -tubes, and -rings [25,26], or nano cages and –flowers [27,28]. A number of researches showed that a nano-particulate formulation could function as a sufficient bactericidal material [29-35].

MATERIALS AND METHODS
Materials
Zinc nitrate hexahydrate ($Zn(NO_3)_2.6H_2O$, 98%), and potassium hydroxide (KOH, 90%) were purchased from Sigma-Aldrich (Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa). These analytical grade materials have been utilized without a purifying process.

(a) Synthesis of ZnO nanoparticles

The direct thermal precipitation technique has been used in preparing zinc oxide NPs. Using deionized water, the preparation of KOH and zinc nitrate (0.4M and 0.2M, respectively) took place at room temperature, marginally adding the aqueous solution to the zinc nitrate while constantly stirring, followed by controlling the temperature at 60 ^{o}C for 120 min, forming a white precipitation. The resulting mixture is centrifuged at 500 rmp for 20 minutes, followed by a triple wash in deionized water and absolute alcohol. Kept at 300, 500^{o}C for 2 hours, the formation of zinc oxide is facilitated using a custom prepared tubular muffle furnace with no calcination.

(b) The antibacterial activity

The agar well diffusion technique has been used in producing the antibacterial activity of several zinc oxide nano particles. The measurement of these activities were compared to Gram-positive Staphylococcus aureus, Gram-negative E.coli and Pseudomonas aeruginosa bacteria. Each preparation of ZnO-NPs was inoculated in different wells on Mueller-Hinton agar plates seeded in advance by 100 µl of 24 h old bacterial inoculate. The zinc oxide samples underwent a 15-min sonication within distilled water before being inoculated. Incubation occurred at 37 °C for 24 hours so as to grow the bacteria. Antibacterial activity has been recorded through the measurement of the inhibition zone diameter (mm).

RESULTS & DISCUSS

Characterization of the Synthesis ZnO

(a) The SEM with EDX analysis

Figures (1, 2 and 3) display the Scanning Electron Microscope (SEM) photograph for zinc oxide, where zinc nitrate has been used in its preparation as the starting material at a temperature of 300, 500^{o}C. The morphological investigation of the prepared ZnO powder took place by means of scanning electron microscopy (SEM). As presented in Fig. 1, the estimation for the average size of ZnO particles would be about 23-33 nm. The SEM image illustrates the homogeneity and agglomeration of the powder.

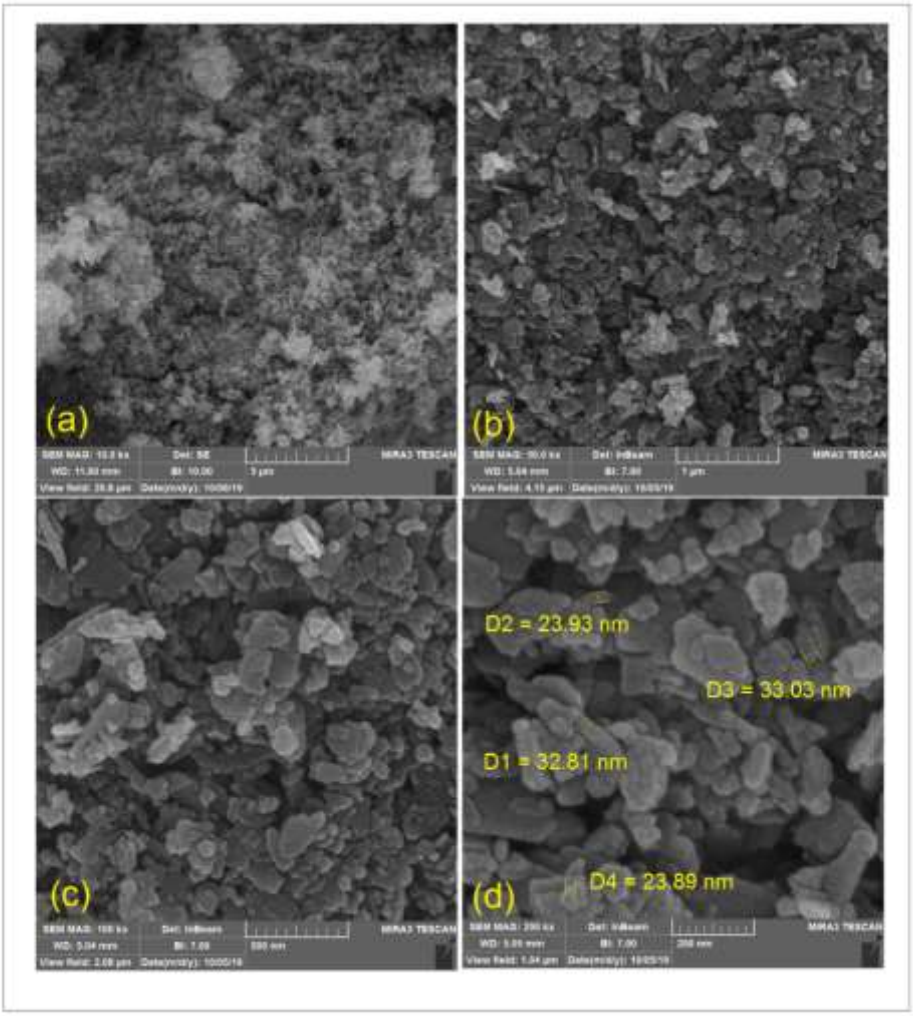

Fig .1: SEM of ZnO powder synthesized at 0^{o}C for different magnifications: (a) x 10 k, (b) x 50 k, (c) x 100 k, (d) x 200 k.

Figure 2 shows a microscopic photograph of the ZnO nanoparticles that have been obtained, through a variety of magnified illustrations. The nanomaterial examines the display particle sizes, ranging within a span of 17 < d <32 nm. The microstructure of nanocrystalline ZnO has a skeletal structure, as a result of the process of coagulation .

Figures 3 (a, b, and c) illustrate the formation of nanoparticles resulting from the decomposition, as they appear in form of faceted crystals (Fig. 3d). One of this material's characteristics is its relatively higher porosity.

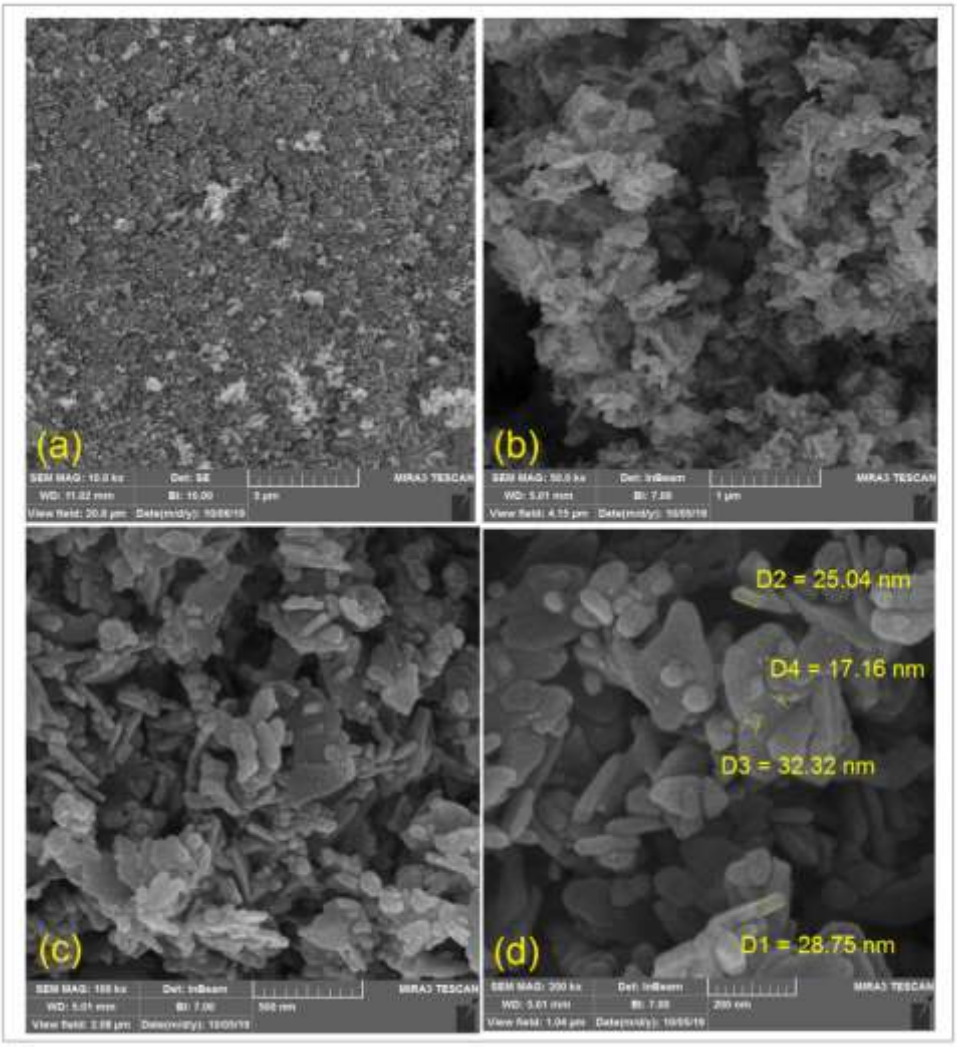

Fig .2: SEM of ZnO powder synthesized at 300°C for different magnifications: (a) x 10 k, (b) x 50 k, (c) x 100 k, (d) x 200 k.

Figure 3 displays the SEM images at a higher magnification, and demonstrates the formation of particles with a size of 14nm. It also provided a clearer idea about the particle separation, as the particles are seen to be separated smoothly, without being highly affected by agglomeration.

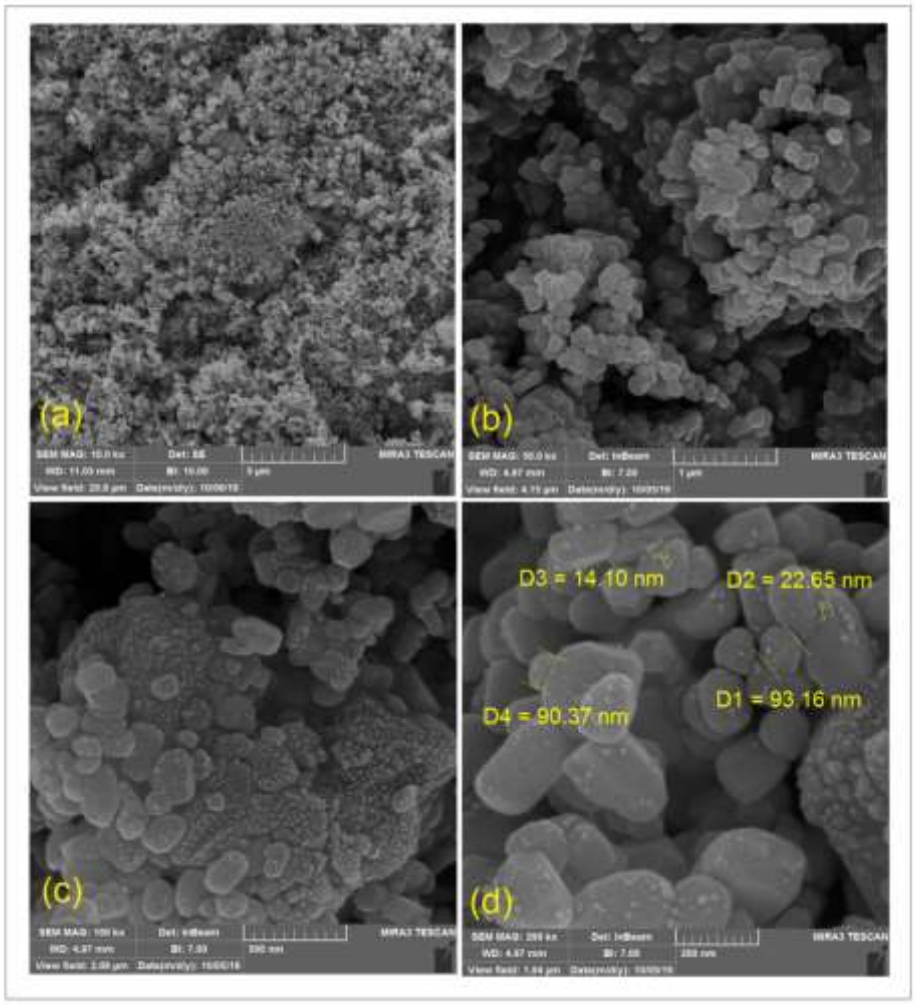

Fig .3: SEM of ZnO powder synthesized at 500°C for different magnifications: (a) x 10 k, (b) x 50 k, (c) x 100 k, (d) x 200 k.

Figure 4 represents the EDX spectrum of ZnO nanoparticles. EDX spectrum displays four peaks that could be identified as zinc and oxygen. Therefore, the conclusion can be drawn that pure ZnO nanoparticles could be prepared through the use of the precipitation method.

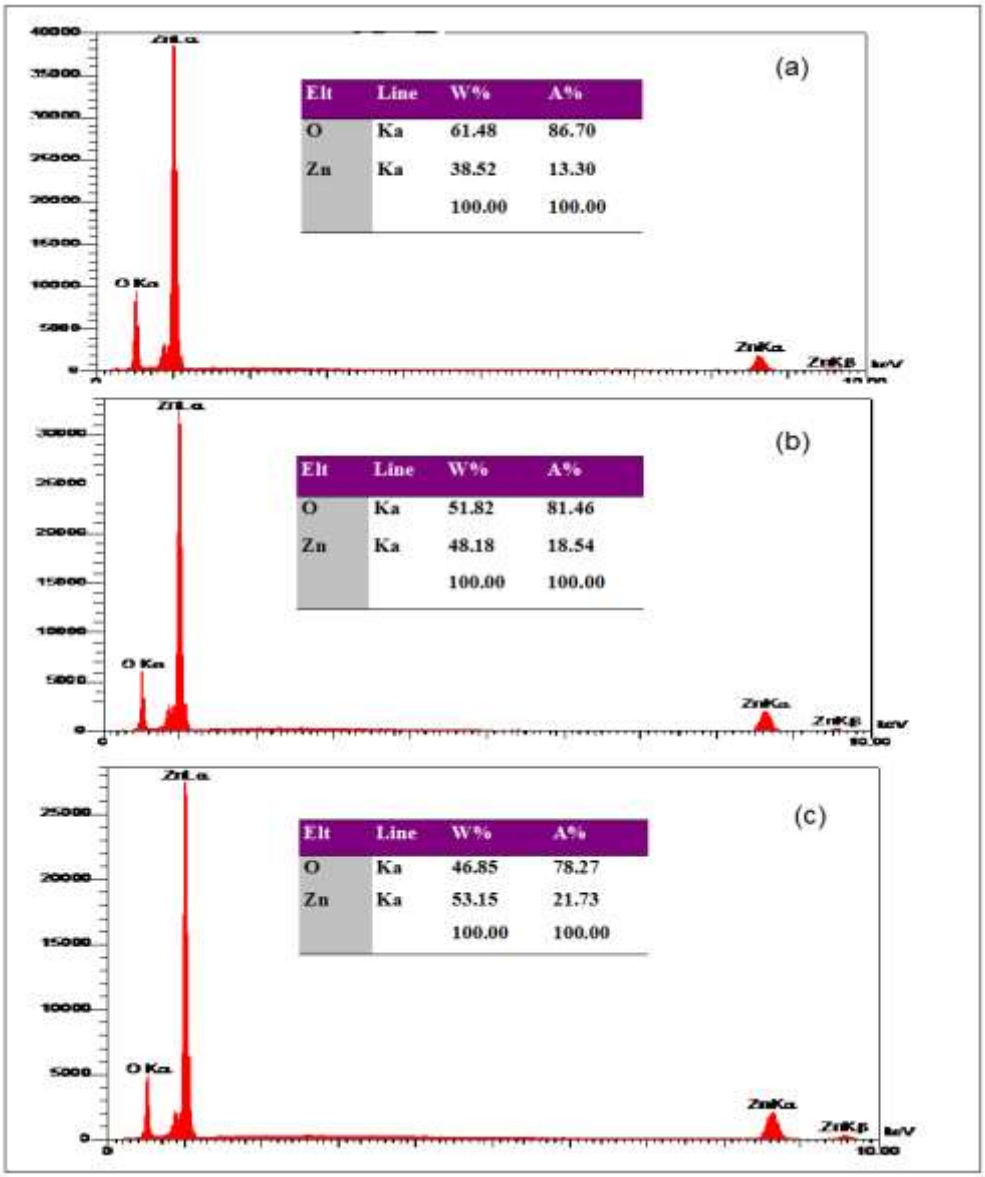

Fig .4: EDX of ZnO powder synthesized at (a)0°C ,(b)300°C ,and (c)500°C.

(b) The XRD analysis

Figure 5 presents the results of X-ray diffraction for the ZnO after synthesis, showing wide peaks at (31.9, 34.5, 36.3, 56.7, and 62.9) characteristic to the ZnO structure. The remarkable breadth of line for such diffracting peaks indicates that the material falls within the nanometer span.

Figure 5 demonstrates the possibility of obtaining ZnO NPs through decomposing the zinc compound thermally. With the use of Scherrer's equation, the diameter of the crystallite domain (D) was taken through the XRD peaks:

$D = \lambda k / \beta \cos\theta$

where λ represents the wavelength of the incident X-ray beam (1.54 A° for the Cu Kα), θ stands for the Bragg's diffraction angle, and β represents how wide the X-ray pattern line is at half peak-height in radians. The average sizes of the particles of ZnO measured were about 34.06 nm, 28.1 nm, 59.5 nm at temperatures of 0°C, 300°C, and 500°C, respectively.

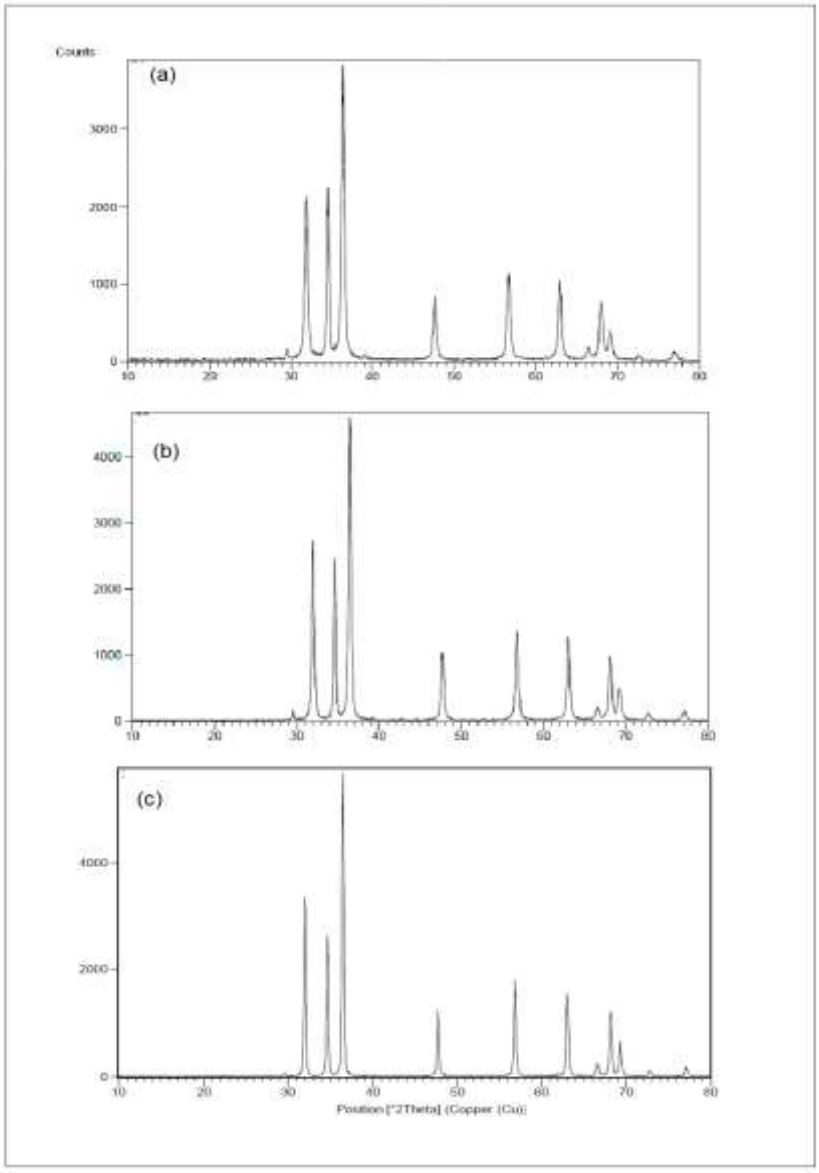

Fig .5: XRD of ZnO powder synthesized at (a)0°C , (b)300°C , and (c)500°C.

The Catalyst Applied on Bacterial

The resulting data in Table (1) indicate that each zinc oxide NP shoed antibacterial activity towards the Gram-positive *Staphylococcus aureus,* while relatively low antibacterial activity was observed for Gram-negative *E. coli and P. aeruginosa* bacteria. The higher inhibiting zone was created through the combination of antibiotic and zinc oxide NP effects in *Staphylococcus aureus,* having a comparatively larger inhibition zine than *E. coli* or *P. aeruginosa*. Such activities are the result of amines and carboxyl groups located on the cell surfaces, as well as the fact that larger affinity of zinc oxide ions occurs toward these groups [36]. The agar diffusion test as a qualitative test was done for observing and predicting the zinc oxide NP antibacterial behavior. Among the advantages of this method are the fact that it is simple and of lower cost, as well as that it can examine a large amount of bacteria and antimicrobial agents [37].

The results illustrated the greater resistance of gram-negative bacteria towards NPs, unlike gram-positive bacteria. This difference in performance can be traced back to the dissimilarities found in the structure and composition of their cell membranes [36]. The outer membrane of gram-negative bacteria, such as *Escherichia coli* and *P. aeruginosa* have a predominant composition of a fierce lipopolysaccharide layer (LPS), which are found to be a permanent barrier against nanoparticles, thereby decreasing how sensitive Gram-negative bacteria are [38].

A typical characteristic of any metal nanoparticle is begin able of reducing or removing microorganisms through two essential mechanisms: (a) the free metal ion toxicity that arises from dissolving metals from the surface of nanoparticles [38], and (b) releasing reactive oxygen species (ROS) and zinc ions. The generated ROS species, such as hydrogen peroxide (H_2O_2), OH-(hydroxyl radicals), O_2^{-2} (peroxide) and the zinc ions from ZnO NPs, attach to the

negative surface of cell membranes, causing the inner cellular material to leak after the cells are disrupted, thus eventually resulting in the cell's death [39].

A number of researches have pointed out that the anti-microbial activeness of zinc oxide nanoparticles O-NPs antimicrobial activity is remarkably influences by the morphology of the particles. Such shape-dependency could be clarified through the percentage of active facets on the nano-particles, thereby motivating NP researches so as to obtain the selected nano-structured zinc oxide in an antibacterial test. Other influential factors were the particle size and concentration. Previous studies have shown that a negative correlation exists between the nanoparticle size and the toxicity level affecting the microorganisms; the smaller the size, the larger its effect. A small nanoparticle has a comparatively wider interfacial area, penetrating bacterial membranes without difficulty, which increases their antibacterial effectiveness [37]. Nano particles cause a significant disturbance to how permeable the cell wall and membrane are, resulting in a further influence on biomolecules like DNA and protein, as it prevents certain processes including DNA replication and protein synthesis [40].

Table 1: Diameter of zone of inhibition by ZnO NP against Staphylococcus aureus, E.coli and Pseudomonas aeruginosa

sample	Zone of inhibition in diameter (in mm)		
	Staphylococcus aureus	*E.coli*	*Pseudomonas aeruginosa*
d	1.5	1.5	1
Z1	2	1.25	1
D+z1	2.5	2	1
d	1.5	1.25	1
Z2	1.5	-	1
D+z2	2	-	1
d	1.5	1.5	1
Z3	1.75	1.5	-
D+z3	2.25	2	-

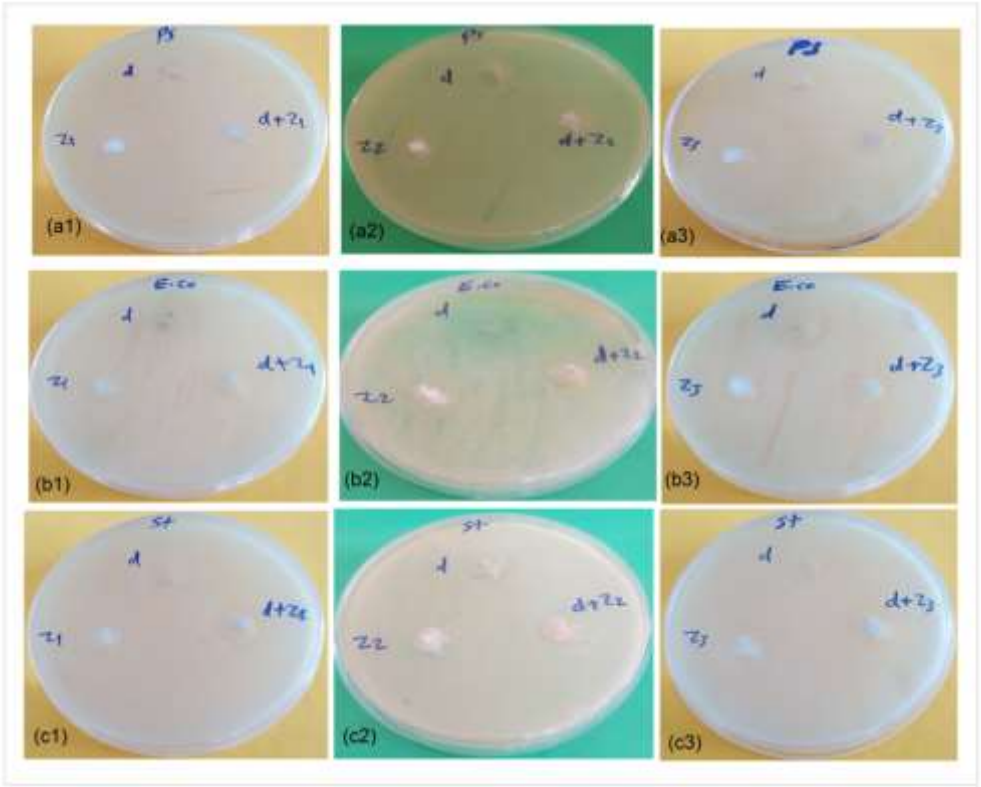

Fig .6: Antibacterial activity of ZnO NPs against (a) *Pseudomonas aeruginosa*, (b) *Escherichia coli,* (c) *Staphylococcus aureus*, (1) ZnO synthesized at 0°C , (2) ZnO synthesized at 300°C , and (3) ZnO synthesized at 500°C.

CONCLUSION

The ZnO NPs has been prepared in an easy and fast way at different temperatures, studying their physical properties which are biologically tested. The prepared nanoparticles were characterized through SEM, XRD, and EDX. The ZnO NPs synthesized at 300°C have the best properties, having an average particles size of about 28.1 nm. The antibacterial activity of nanoparticles depended on the sort of nanoparticle and the species of microorganism (whether gram positive or negative).

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