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COMPARISON OF PREPARATION OF ZNO NANOPARTICLES BY CHEMICAL AND AQUEOUS HYDROLYSIS AND EVALUATION OF THEIR ANTIBACTERIAL ACTIVITY

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Abstract

In this work, ZnO nanoparticles are prepare by two different methods (chemical and hydrolysis) and characterize of by UV-Visible spectroscopy, FTIR spectrum, (XRD) analysis, scanning electron microscope SEM analysis and Atomic force microscope (AFM). The resulted showed that ZnO NPs have antibacterial activity against bacteria *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Bacillus subtilis*.

Key words: ZnO nanoparticles, *SEM, XRD, AFM.*

Introduction

Zinc oxide nanoparticles are semiconductor, having unique physical and chemical properties, it has been used in biomedical applications as bio-imaging, drug delivery and antibacterial activity, et. This activity related to size similarity with bio-molecules, their abundant functionality on large surface areas and their quantum size effects (Xiong, 2013; Çakir, 2012). Recently the medical field has towards nano-materials to decease complications and increase the efficiency of chemotherapy agents (Ahmed, 2016). For preparation of zinc oxide nanoparticles by several methods either chemical or physical methods, such as sol-gel process, nanolithography, chemical vapour deposition, physical vapour deposition (PVD), spray conversion processing and precipitation method (Masaki Raghad, D.H., 2017). Zinc oxide nanoparticles have antimicrobial properties therefore focus of industrial applications in biocides coating in water treatment, paints and cosmetic products (Ravishankar Rai, 2011). The field of Zinc oxide nanoparticles was a very important field of biology because of its distinguishing antimicrobial activity that opened new frontiers for biological science (Allahverdiyev, 2011). Especially in its nanoparticle form, it has a strong toxicity towards a wide range of microorganisms including bacteria, fungi (Huang, 2008; He, L., 2011). This research, zinc oxide nanoparticles were prepared *via* two different method (std and chemical methods) and characterized by UV-VIS spectroscopic, XRD, SEM and EDX and FTIR, activity of these nanoparticles were test against bacteria.

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Material and Methods

Preparation of ZnO nanoparticles by chemical methods

100 ml of Zinc nitrate (1M of $Zn(NO_3)_{2)}$ and Sodium hydroxide (1 M of NaOH) were dissolved in ethanol and mixed then added to 100 µl of HCl with stirrer at 60ºC for 1 hours and leaved to cold, centrifugation and washed four times then dried at 50°C/1day.

Preparation of ZnO nanoparticles by chemical methods

1M of zinc oxide is prepared and added to100 µl of HCl with stirrer at 60ºC for 2 hours until it is completely dissolved and transform into a transparent color. ZnO nanoparticles were characterized by UV-Vis spectrophotometer to determine absorption spectra, scanning electron microscope (SEM) which utilized for visual the morphology of zinc oxide nanoparticles, furthermore to Atomic force microscope AFM, (FTIR) analysis was used to investigate the functional groups of zinc oxide nanoparticles were prepared *via* two method. The crystalline structure of zinc oxide nanoparticles was determined by X- ray diffraction (XRD). Different

bacteria using well diffusion agar methods. To prepare bacterial suspension take a loop full of each test bacterial isolate suspended with sterile normal saline, the density of colloidal was adjusted with 0.5 McFarland standards. Take 100 µl of suspension and inoculated to mueller hinton agar plates then spread by L- shape, then made 4 well on the surface on the inoculated mueller hinton agar plate by cork borer, after that added concentration (0.01, 0.02, 0.04 and 0.06 mg/ml) was added to each well and the plates were incubated at 37°C /1day. Finally the zone of inhibition was measured after 1 day incubation.

Results and Discussion

Characterization of the synthesized zinc oxide nanoparticles

The fig. 1,2 shows the UV-Vis-NIR spectra of ZnO nanoparticle were prepared in chemical and hydrolysis methods.

FTIR spectroscopy showed that photochemical analysis of powder of ZnO nanoparticles (chemical and hydrolysis methods), it shows prominent bands of absorbance at peaks XRD pattern of zinc oxide nanoparticles showed distinct diffraction planes of 31.7°, 34.4°, 38.2°, 46.6° and 56.6° at 2θ values indexed to

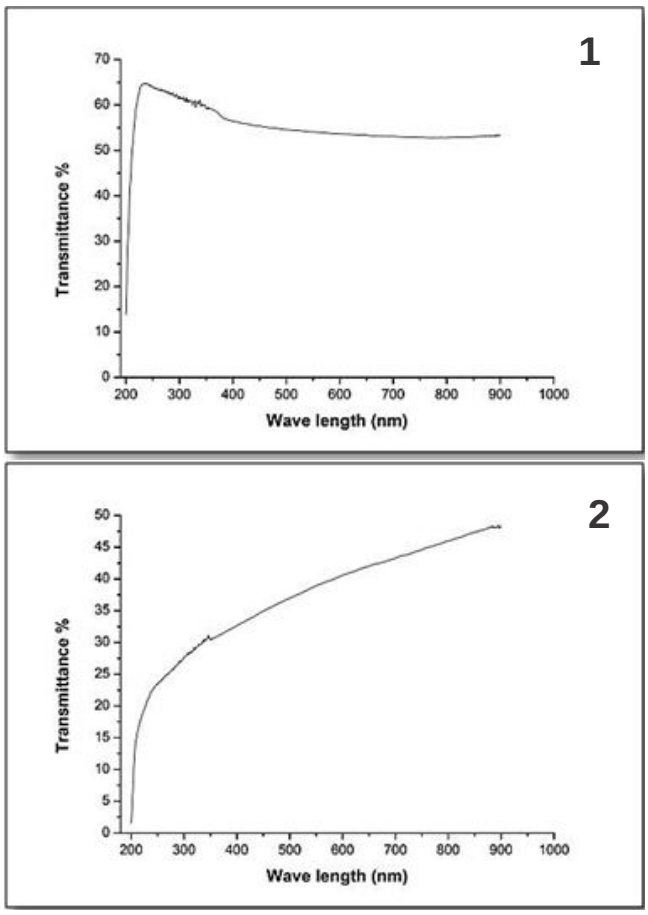

Fig. 1: UV-Vis-NIR ZnO nanoparticles (1) chemical method, (2) aqueous hydrolysis.

(100), (002), (101), (102) and (110) planes. It shows in fig. 4 and in aqueous hydrolysis showed distinct diffraction planes of 31.7°, 34.6°, 36.18°, 47.42°, 56.56°, 62.76° and 66.14° at 2θ values indexed to (100), (002), (101), (102), (110), (103) and (200) planes. It shows in fig. 5. XRD is

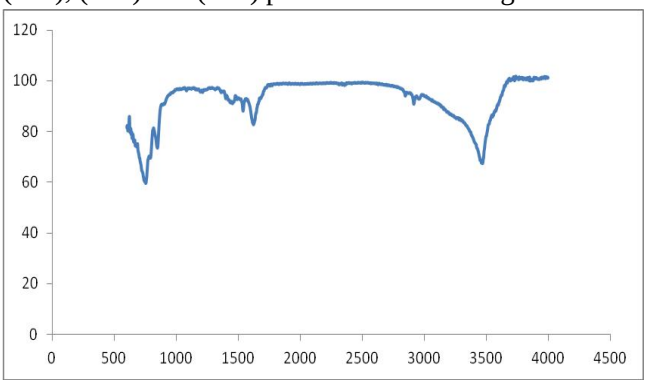

Fig. 2: FTIR analysis of ZnO nanoparticles chemically.

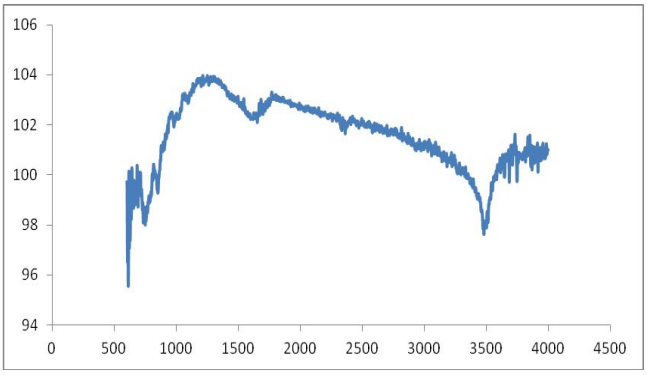

Fig. 3: FTIR analysis of ZnO NPs hydrolysis.

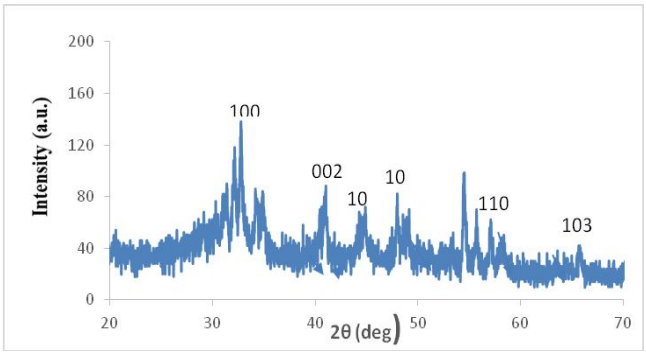

Fig. 4: XRD of ZnO nanoparticles chemical.

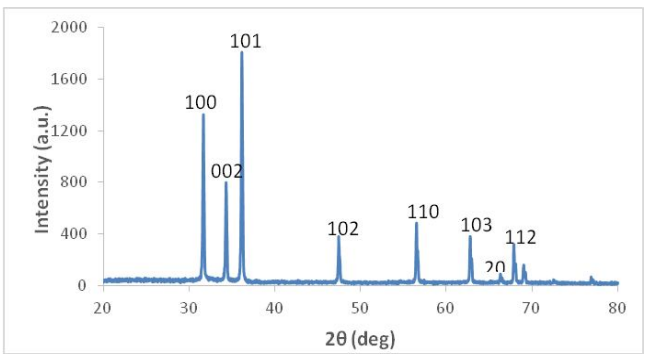

Fig. 5: XRD of ZnO nanoparticles hydrolysis.

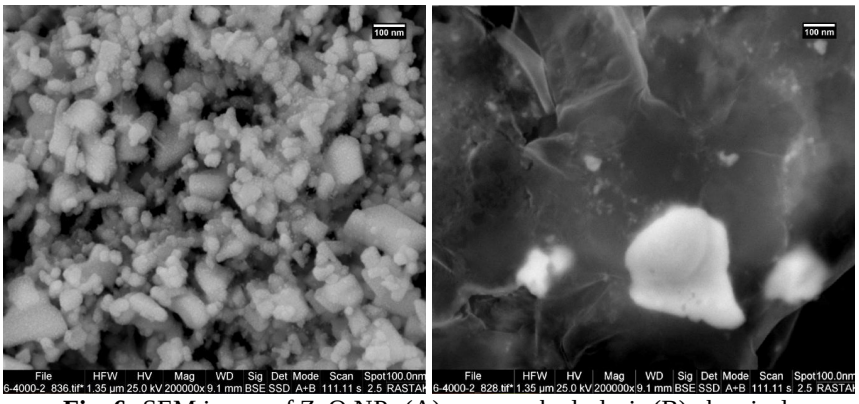

Fig. 6: SEM image of ZnO NPs (A) aqueous hydrolysis (B) chemical.

used to calculate the chemical composition and the crystalline nature of material (Ravishankar Rai, 2011).

It shows in fig. 6A and 6B the Scanning electron microscopic (SEM) images of zinc oxide nanoparticles obtained by aqueous hydrolysis, chemical method, SEM images confirmed the surface morphology of zinc oxide nanoparticles at different methods.

The topography of the surface was studied using an atomic force microscope.

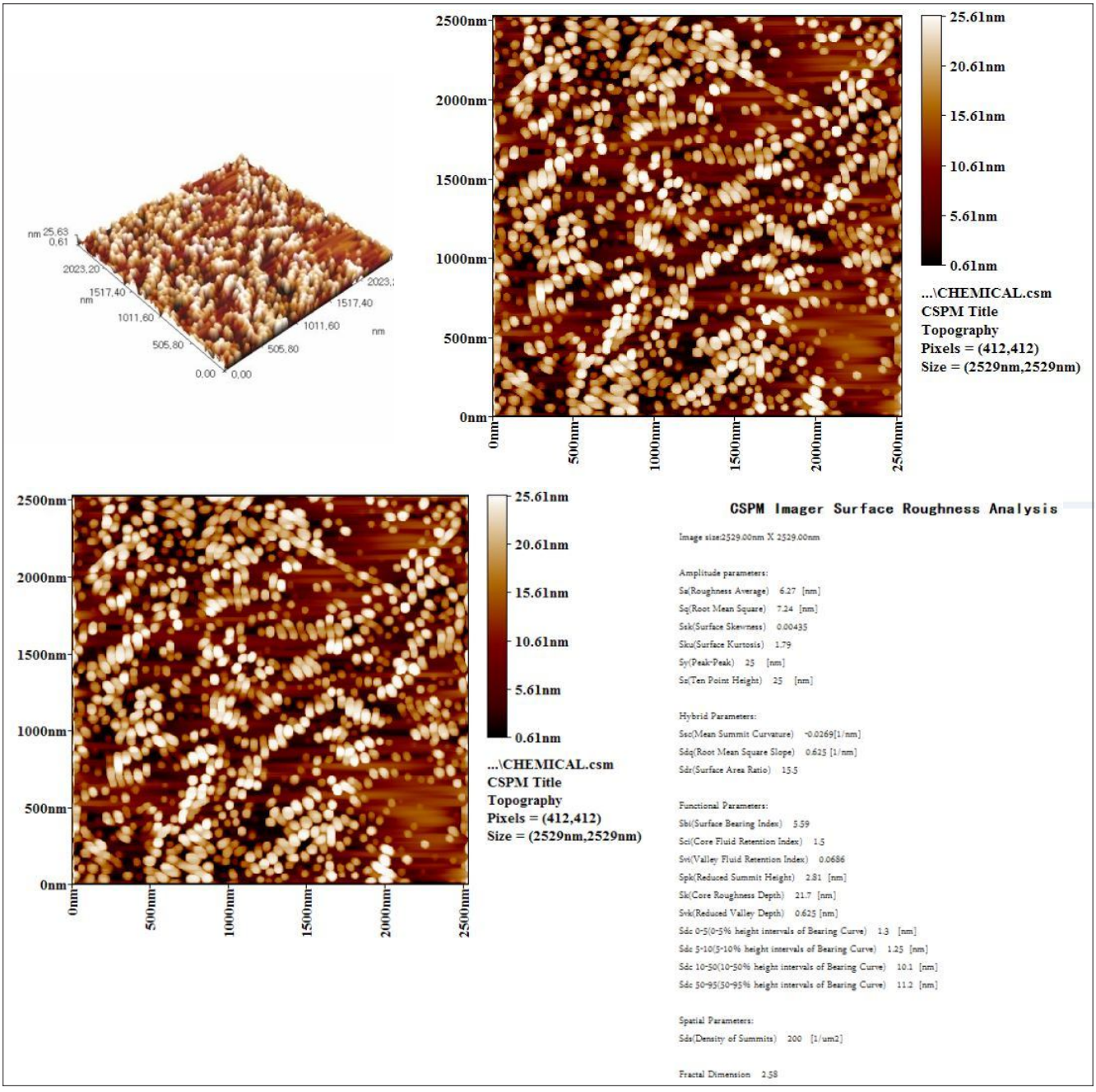

Fig. 7: Image of AFM (Chemical)

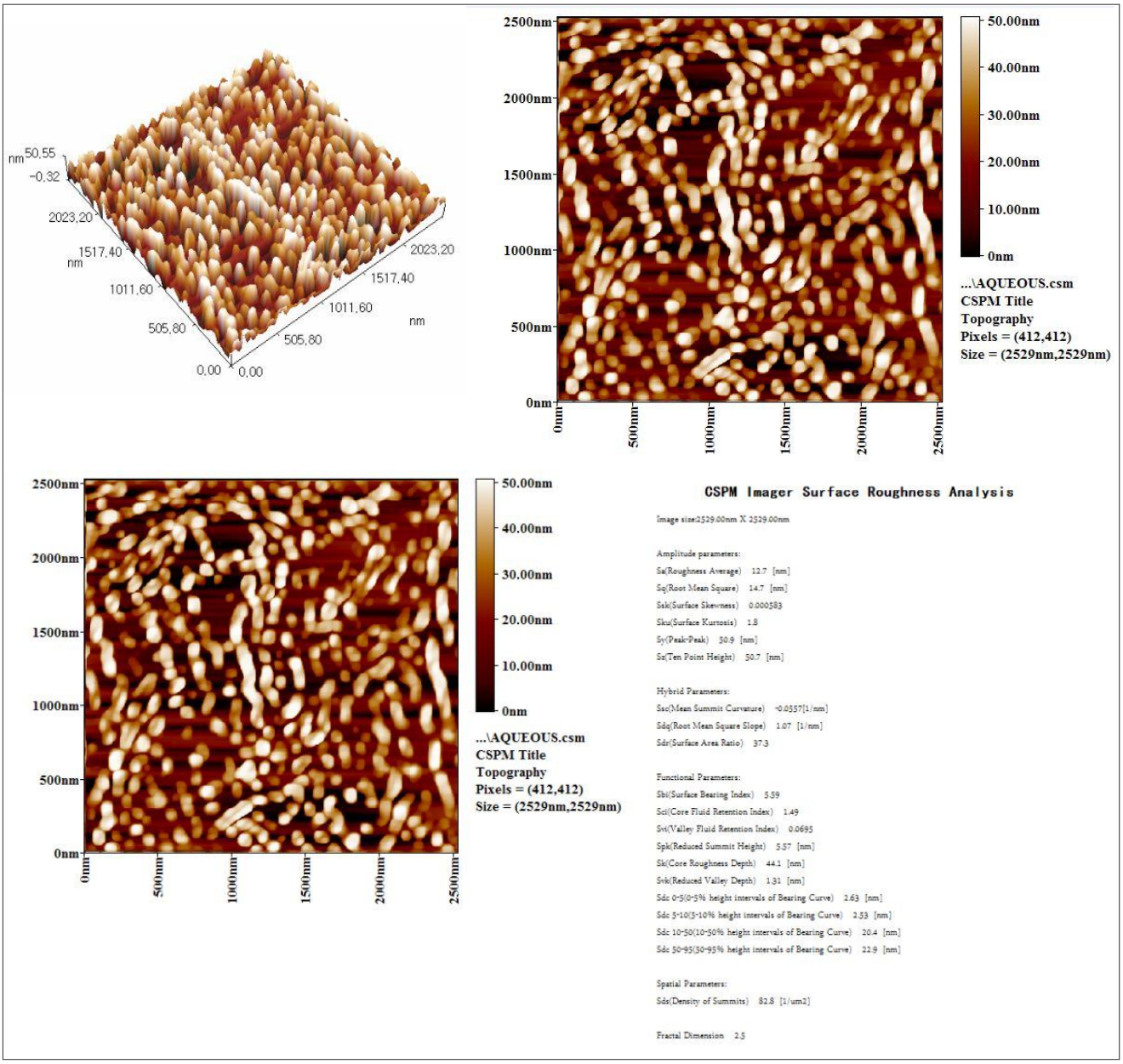

Fig. 8: Image of AFM (Hydrolysis)

The fig. 7,8 showed two dimensional image of ZnO NPs showing in clusters of spherical shapes. The fig. 7,8 showed three-dimensional image of ZnO nanoparticles revealed a population of homogeneous particles with a regular surface fig. The average diameter of ZnO nanoparticles in chemical method is 58.87 nm while in aqueous hydrolysis is 75.96 nm.The topography of the surface like roughness average value and root mean square, surface skewedness, surface kurtosis, the value peak-peak in chemical method were greater than that prepared in aqueous hydrolysis, this indicates a good uniform crystallites of ZnO nanoparticles prepared in chemical method.

Table 1: Value of surface roughness analysis.

Parameters	Chemical	Aqueous Hydrolysis
Avg. Diameter	58.87	75.96
Roughness Average	6.27	12.7
Root mean Square	7.24	14.7

The antibacterial activity of ZnO NPs were evaluated bacteria including *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* using

Table 2: Antibacterial activity of ZnO NPs by chemical.

Bacterial isolate	Concentration of Zn NPs (mg/ml)			
	0.01	0.02	0.04	0.06
	Inhibition zone rate (mm)			
E. coli	-	-	-	-
S. aureus	-	-	24	24
Klebsiella pneumoniae	-	-	-	-
B. subtilis	-	-	-	14

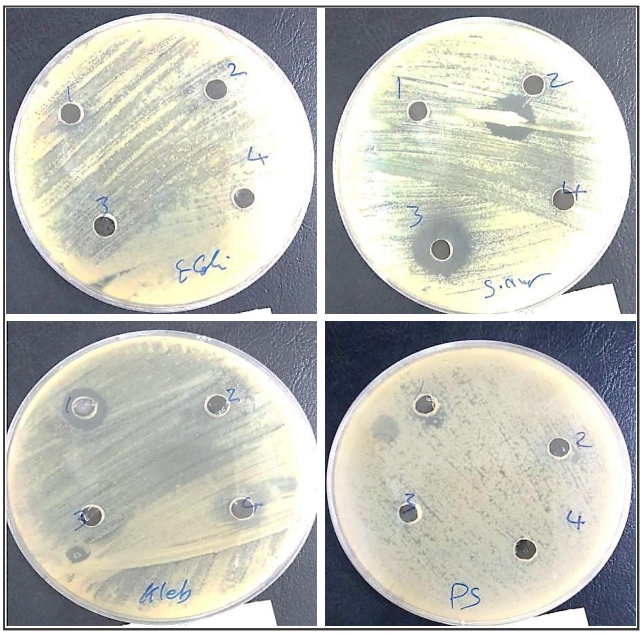

Fig. 9: Image of inhibition zone of bacteria by aqueous hydrolysis.

Fig. 10: Image of inhibition zone of bacteria by chemical.

Table 3: Antibacterial activity of ZnO NPs by hydrolysis.

Bacterial isolate	Concentration of Zn NPs (mg/ml)			
	0.01	0.02	0.04	0.06
	Inhibition zone rate (mm)			
E. coli	-	-	-	-
S. aureus	-	-	16	-
Klebsiella pneumoniae	-	-	-	12
B. subtilis	-	-	-	-

agar well diffusion method. The results showed clearly that ZnO nanoparticles which had efficient against these isolates of bacteria as shown in table 1,2. Several previous studies for antibacterial activity of ZnO nanoparticles, that suggested many mechanisms for antibacterial activity, to contact of ZnO nanoparticles with cell walls that result the destruct the bacterial cell integrity, separately with Zn^{+2} ions and ROS formation (Sirelkhatim, 2015; Siddiqi, 2018).

Conclusion

This research was aimed to study zinc oxide nanoparticles by different method, the results showed that zinc oxide nanoparticls had antibacterial effect against *S. aureus* and *B. subtilis* in chemical methods while in aqueous hydrolysis had antibacterial effect against *S. aureus* and *Klebsiella pneumoniae*.

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