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Role Enhancement of ZnO nanoparticles and ZnO/Ag composite for Medical Applications

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Abstract

ZnO nanoparticles assisted with ethanol and 2propanol as capping agent investigated the medical activities of ZnO nanoparticles and ZnO/Ag composite. ZnO nanoparticles were prepared using zinc acetate and silver nitrate as a source of zinc and silver. ZnO/Ag composite also prepared and studied the medical activities. XRD pattern indicates that the structure of ZnO was hexagonal wurtzite with average size 5 nm according to Scherre's formula. The optical band gap of ZnO nanoparticle showed very sensitive for using different capping agent as shown in UV-VIS spectra and also showed blue shift in wavelength corresponding to capping agent. Finally, the antibacterial properties of ZnO and ZnO/Ag composite against Gram-negative and Gram-positive bacteria have been demonstrated using well diffusion method and indentify their antibacterial effects compared with organic antibacterial agents.

Keywords: ZnO nanoparticles, ZnO/Ag composite, antibacterial.

1. Introduction

Zinc oxide (ZnO) with an energy gap of 3.37 eV at room temperature has attracted much attention for their optical and electronic properties such as catalytic, electrical, optoelectronic, and photochemical properties [Brida et al. 2002; Ashour et al. 2006]. Also due to high catalytic with large surface area activity, ZnO nanostructures have a wide apply as a catalytic reaction process [Chen & Tang 2007]. According to the morphology of zinc oxide nanostructures, it shows different physical and chemical properties with various synthesis methods. It is also that the physical and chemical properties of synthesized zinc oxide are to be investigated in terms of its morphology [Kumar1 et al. 2013].

Silver nanoparticles find use in many fields, and the major applications include their use as catalysts, as optical sensors of zetomole (10−21) concentration, in textile engineering, in electronics, in optics, and most importantly in the medical field as a bactericidal and as a therapeutic agent [Prabhu & Poulose 2012]. Nanocomposites materials with different properties can be grouped in same particle to perform multiple technological functions. However, they can also show new properties and functionalities due to the strong interaction between the two different functional components [Fie et al. 2010]. ZnO /Ag composites have attracted large attention, not only because ZnO is one of the most important wide-band gap semiconductors and has various applications, including use in sensors, electronics, solar cells and photo electronics, but also because silver nanomaterials display some unique features in chemical and biological sensing, which are based on surface-enhanced Raman scattering (SERS), localized surface plasmon resonance (SPR), and metal-enhanced fluorescence [Yu et al. 2009]. In addition, silver modification is found to be effective for the fabrication of p-type ZnO, as the naturally occurring ZnO displays n-type conductivity due to its native defects such as zinc interstitials and oxygen vacancies [Shah et al. 2013].

Most researchers generally considered studying the medical activities of pure ZnO nanoparticles which is limited to affect of the concentration of zinc, effect of pH, and method of preparation. In the present work, ZnO/Ag nanocomposite were prepared using two different of zinc acitate concentration and silver nitrate and were exploited on the medical application. In recent years, inorganic antibacterial agents like (ZnONPs and AgNPs) have attracted the attention of researchers because of their thermal resistance and the persistence of their antibacterial effects compared with organic antibacterial agents [Korai 1999]. A more recent study showed that nano ZnO has an enhanced bactericidal power vs. bulk ZnO against Gram-negative [Voicu et al. 2013]. Currently, the antibacterial properties of AgNPs against Gram-negative and Gram-positive bacteria have been widely demonstrated [Ahearn et al. 1995; Jeon et al. 2003; Zhao et al. 2001; Sondi & Sondi 2004]. The objective of

the present work was to investigate the antimicrobial activities of the ZnO NPs and AgNPs against models of Gram-negative like Escherichia coli (E.coli) and Pseudomonas aeruginosa (P. aeruginosa) and models of Gram-positive bacterium like Staphylococcus aureus (S. aureus). Then models of bacteria was expanded to other types like Lactobacillus and Staphylococcus aureus as Gram-positive bacteria and Pseudomonas aeruginosa, E. coli and citrobactraas as Gram-negative bacteria and tested antibacterial assay of ZnO/Ag composite against them to investigate the advantage of such ZnO/AgNPs composite.

2. *Experimental procedure*

2.1 Synthesis of the ZnO nanoparticles

The ZnO nanoparticles were synthesized by precipitation fromsolution using Zn(CH3CO2)2 and NaOH. The overall reaction for the synthesis of ZnO nanoparticles from Zn(II) acetate can be written as

$$Zn(CH3CO2)2 + 2NaOH \rightarrow ZnO + 2Na(CH3CO2)2 + H2O.$$

For a typical preparation, 0.02 M of zinc acetate dehydrate was prepared by adding Zn(CH3CO2)2·2H2O to the solvent water, ethanol and 2-propanol in a covered flask under vigorous stirring at 50 ◦C. A 0.02 M NaOH solution was prepared by adding sodium hydroxide to the solvent (ethanol and 2-propanol) in a covered flask under vigorous stirring at 60oC. After that cooling the final solution to room temperature and 0.02 M of Zn(CH3CO2)2·2H2O was mixed with it and labeled to ZE and ZP respectively.

2.2 Synthesis of the Ag nanoparticles.

0.8 ml of 0.01 M Ag(NO3)2 was added to the 15 ml of 0.0005 M polyvinyl pyrrolidone (PVP).Then 0.8 ml (0.01 M) of sodium borohydride (NaBH4) was added to result.

2.3 Synthesis of the Ag@ZnO nanocomposites.

0.5 ml of the Ag nanoparticles were added to the 2 ml for each sample of ZnO nanoparticles with vigorous stirrer for 2 h.

2.4 Test for antibacterial activity of AgNPs and ZnONPs

Another improvement about the formation of metal nanoparticles is determined by anti-pathogenic activity of reduced silver nanoparticles (AgNPs) using agar well diffusion method. Media and glassware used were sterilized in an autoclave at 121°C for 15 min. three types of bacteria like Escherichia coli (E.coli) and Pseudomonas aeruginosa(P. aeruginosa) which are Gram-negative and Staphylococcus aureus (S. aureus) which is Gram-positive were used for antibacterial study. Bacterial suspensions were prepared by growing a single colony overnight in nutrient broth and by adjusting the turbidity to 0.5 McFarland standards. Each strain was swabbed uniformly into the individual plates using sterile cotton swabs. Wells of 5mm diameter were made on Muller Hinton agar (MHA) plates using gel puncher and 100 µl of each AgNPs (0.1mM) and ZnO NPs separately were added to the prepared wells with a diameter of 5 mm and control well was filled with distilled water. These plates were incubated at 37°C for 24 h in a bacteriological incubator and the zone of inhibition (ZOI) was measured by subtracting the well diameter from the total inhibition zone diameter.

2.5 The antimicrobial activity of ZnO and ZnO/Ag composite

The antimicrobial activity of ZnONPs and ZnO/Ag composite was tested using well diffusion method against other types of Gram-negative bacteria like E. coli, P. aeruginosa and citrobactraas and Lactobacillus and S. aureus as Gram-positive bacteria. These microorganisms are standardized and supplied from (biology lab, Biotechnology branch/ Department of Applied Science, University of Technology /Baghdad) for sterility testing of medicinal products and are among the microorganisms used in public health tests. Microbial cultures were developed on solid media, Mueller-Hinton agar (MHA) recommended for bacterial strains.

3. Results

The XRD result as shown in Fig. 1 reveals that all peaks belonging to hexagonal wurtzite ZnO phase which coincide with JCPD card no. 79-0207.The precursor has been completely composed and no excess peaks detected in XRD result. The most intensive peak 101 for ZnO phase at 36.2° was analyzed together with the 110 diffraction line at 56.5° to obtain information about the crystallite size of ZnO particle using Scherer equation. Due to the crystal symmetry the ZnO NP is the thermodynamically stable crystallographic phase. The width of the peaks in case of ZnO NP has increased due to the quantum size effect. The average particle size was estimated to be 42 nm using Scherer equation.

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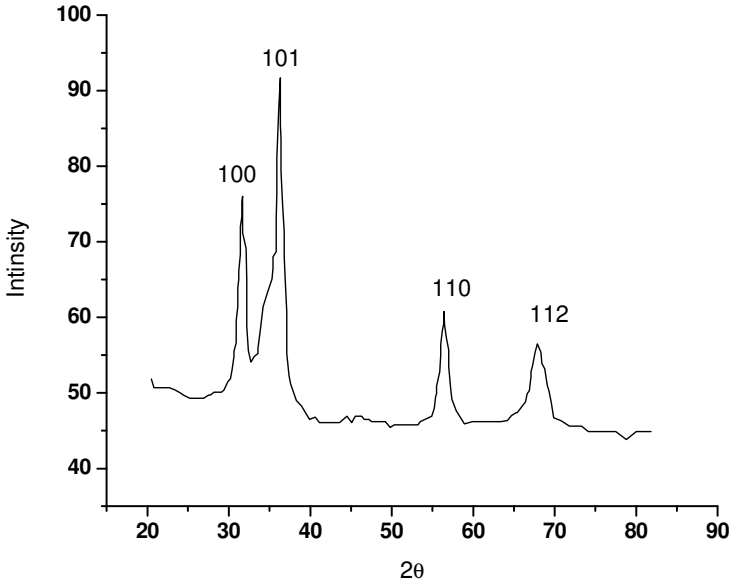

Fig. 1 XRD for ZnO nanoparticles prepared using capping agent.

The UV-Vis absorption spectra of the Ag NP were shown in Fig 2. Absorption spectra of Ag nanoparticles formed in the reaction media has absorbance maxima at 421 nm. A remarkable broadening peak at 350 nm to 480 nm indicates that the particles are polydispersed.

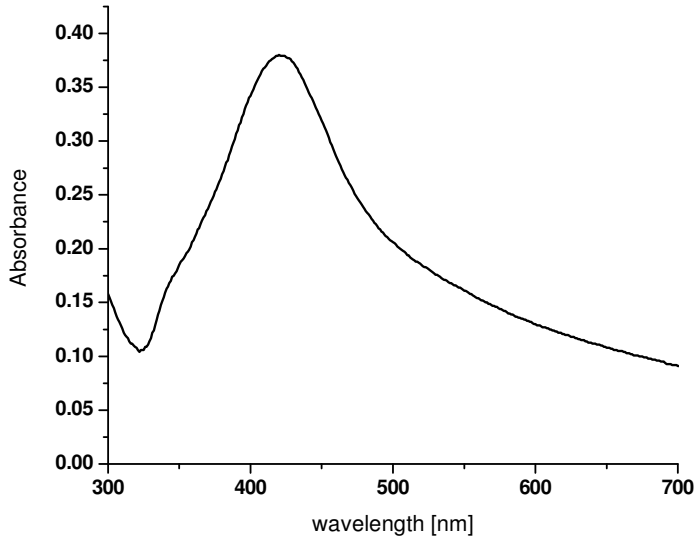

Fig. 2 UV-Vis spectra for Ag nanoparticles.

The UV-Vis spectra of ZnO NP prepared using different capping agent (ethanol and 2-propanol) was shown in Fig 3. The absorption peak of the prepared nano ZnO was found at around 360 nm.

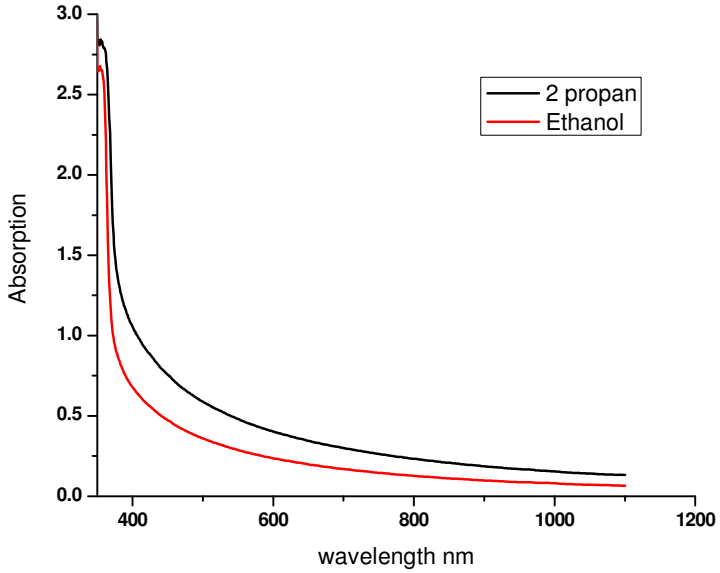

Fig. 3 UV-Vis spectra for ZnO nanoparticles prepared using different capping agent.

The UV-Vis spectra of ZnO/Ag composite prepared using different capping agent (ethanol and 2-propanol) was shown in Fig 4. The absorption peak of the prepared composite was found at around 360 nm.

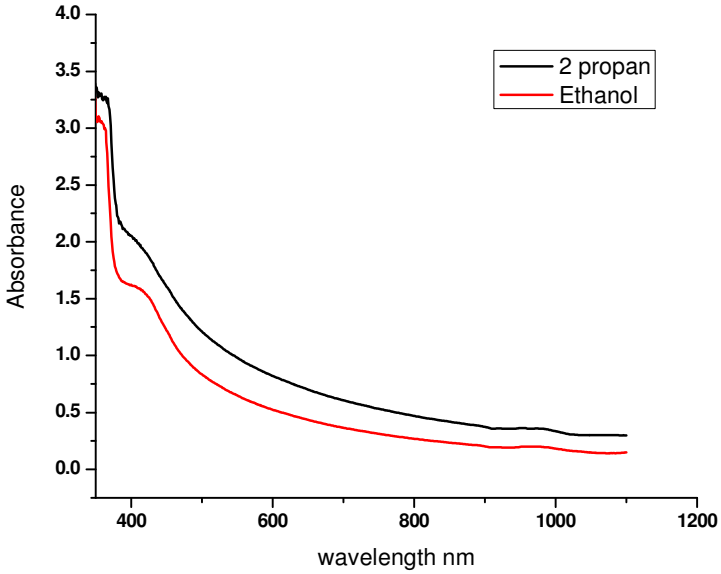

Fig. 4 UV-Vis spectra for ZnO/Ag composite prepared using different capping agent.

Fig 5 shows typical energy gap calculation for ZnO nanoparticles and ZnO/Ag composite with different capping agent (ethanol and 2-propanol). The extrapolated linear portion of the curve to absorption equal to zero which plot of $(\alpha h\upsilon)2$ versus $h\upsilon$ is made to determine direct band gap. It can be seen also there is a blue shift from 3.3eV using ethanol to 3.376 eV using 2-propanol for ZnO nanoparticles. The similar observation of blue shifting in ZnO/Ag composite as shown in Fig 6 when using 2-propanol instead of ethanol as a capping agent.

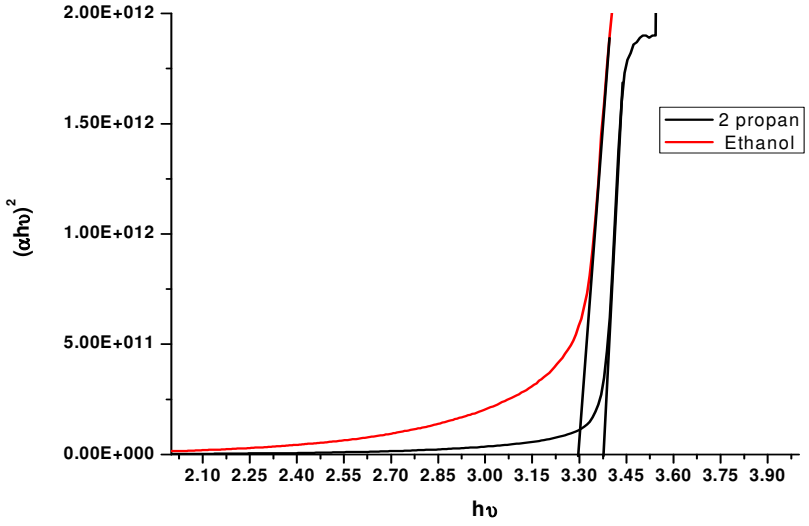

Fig. 5 Energy gap for ZnO nanoparticles prepared using different capping agent.

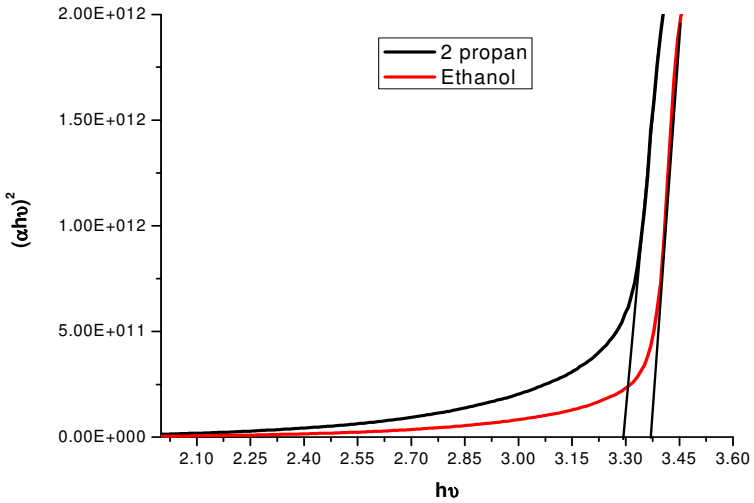

Fig. 6 Energy gap for ZnO/Ag composite prepared using different capping agent.

Antibacterial activity studies using Well diffusion method

The modified well diffusion method was used to evaluate the antimicrobial activity of AgNPs and ZnONPs that prepared in different method to compare between their antimicrobial activity like ZE and ZP as shown in Fig (7a,b,c) against coli (E.coli),(P. aeruginosa) and (S. aureus) which represented the antimicrobial activity of AgNPs and ZnO prepared by different methods against coli (E.coli),(P. aeruginosa) and (S. aureus),respectively.

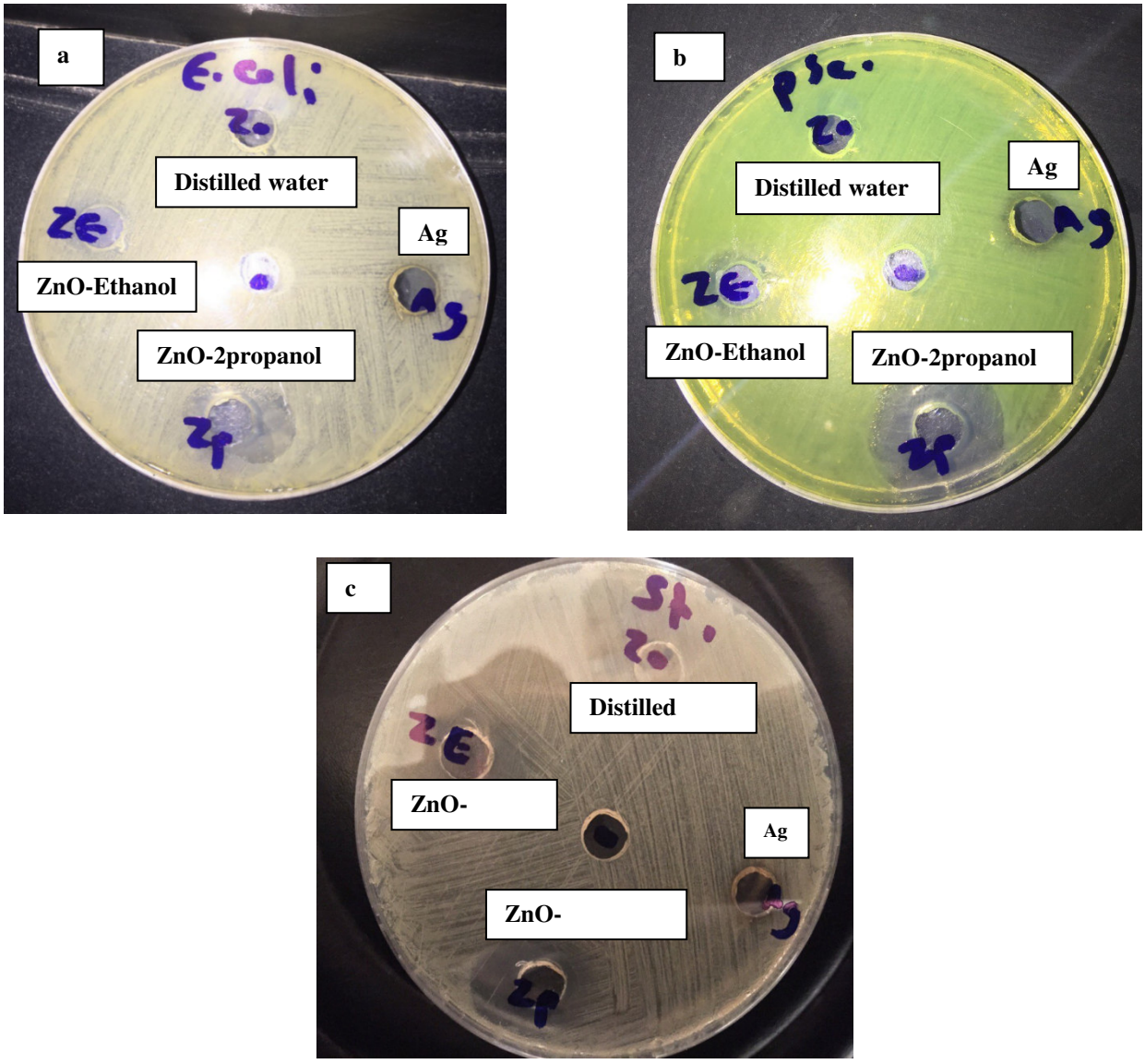

Fig.7: Images of antibacterial activities of well diffusion method using distilled water, Ag nanoparticles 0.1mM, ZnO prepared Ethanol and ZnO prepared 2 propanl against a) E.coli, b) P. aeruginosa and S. aureus bacteria.

This method was performed in (MHA) Petri dish. In Fig (7a) a small inhibition zone of AgNPs (0.1mM) about 0.9mm of against E.coli as compared with ZnO-P (0.01mM) and ZnO-E (0.01mM) which have large inhibition zone about 2.1mm and 1.3mm, respectively . While there is no inhibition zone forming with ZnO-W. In the case of P. aeruginosa it's found that inhibition zone of AgNPs and ZnO-W are small as compared with both ZnO-P and ZnO-E which about 2.5mm and 1.5mm as shown in Fig 7b. While in case of S. aureus bacteria, the clear zone of Ag NPs is smaller as compared with both ZnO-P and ZnO-E which about 2 mm and 2.4mm as shown in Table 1 and as shown in Fig 7c. Since it's found that there a high resistance for gram negative and positive cell against Ag NPs as compared with the others types of nanoparticles due to the increasing the aggregation state of AgNPs and there is a little resistance for gram negative and positive cell when using ZnO-P (0.02mM) as compared with the others types of nanoparticles due to the little the aggregation state of ZnO-P which leads to penetrate the membrane of cell and kill the bacteria as shown in Table 2.

Table 1: Inhibition Zone of Antibacterial Test for ZnO (0.01 M from zinc acetate) and Ag nanoparticles

Bioactive agent		Inhibition Zone (Diameter, mm)		
		E.coli	S. aureus	P. aeruginosa
Ag NPs	0.1mM	0.9mm	1mm	1.1mm
ZP	0.01M	2.1mm	2mm	2.5
ZE	0.01M	1.3mm	2.4mm	1.5mm
DW	0.01M	-----	------	------

Table 2: Inhibition Zone of Antibacterial Test for ZnO (0.02 M from zinc acetate) and Ag nanoparticles

Bioactive agent		Inhibition Zone (Diameter, mm)		
		E.coli	S. aureus	P. aeruginosa
ZnO-P	0.02M	2.1mm	2mm	2.5
Ag NPs	0.1mM	0.9mm	1mm	1.1mm
ZnO-E	0.02M	1.3mm	2.4mm	1.5mm
ZnO-W	0.02M	-----	------	------

In the case of well diffusion method, the experiments were conducted by measuring inhibition zone diameters for each of Gram-negative and Gram-positive bacteria as shown in Table 2. The different concentration of the composite leading to an inhibitory action on the microorganisms' growth was also determined. Since, it's found that the presence of an inhibition zone clearly indicated the antibacterial effect of composite material which represented by ZnO/Ag composite in different concentrations as compared with ZnO NPs.
The diffusion well method were tested other types of Gram-positive and Gram-negative bacteria showed in Fig 8. In Fig 8, clear Inhibition zone values of the different concentrations of ZnO/Ag composite, tested against Lactobacillus and Staphylococcus aureus as Gram-positive bacteria and Pseudomonas aeruginosa, E. coli and citrobactraas Gram-negative bacteria as shown in Table 3.

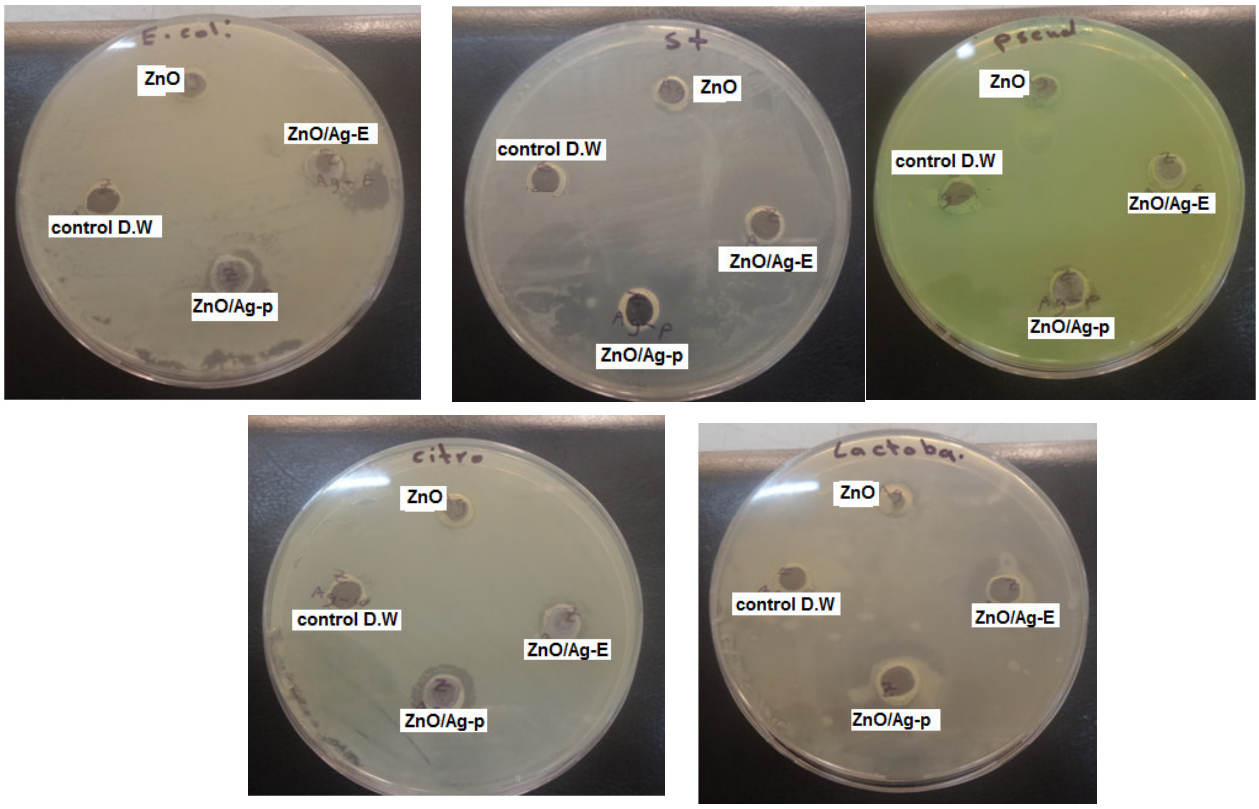

Fig.8: Images of antibacterial activities of well diffusion method using distilled water, Ag nanoparticles 0.1mM, ZnO prepared Ethanol and ZnO prepared 2-propanl against a) E.coli, b) P. aeruginosa, S. aureus, citrobactra, Lactobacillus bacteria.

Since, the results in Tables 3 showed a clear inhibition zone and synergistic effect of ZnO/Ag composite material prepared by different chemical methods for most of the studied Gram-positive and negative isolates. While there is no Inhibition zone values were obtained for the ZnO NPs prepared by different methods as shown in the Fig 8.

Table 3: Inhibition Zone of Antibacterial Test for ZnO/Ag composite

Bioactive agent		Inhibition Zone (Diameter, mm)				
		E.coli	S. aureus	P. aeruginosa	citrobactra	Lactobacillus
ZnO-E/Ag	0.2M	1.1cm	2.5cm	1cm	1.3cm	1.6
ZnO-P/Ag	0.2M	1.8cm	2.7cm	1.1cm	1.5cm	2cm
ZnO-W/Ag	0.2M	-----	------	------	-----	------

It has already been proved that composite material are more active than alone ZnONPs in inhibiting the bacteria growth; the macro composite material ZnO/Ag composite suspension clearly has much higher activity than the ZnONPs suspension. Furthermore, the result showed that the differences in the susceptibility of bacteria to the composite material could be related to differences in cell wall structure, cell physiology, metabolism or degree of contact.

4. Conclusion

ZnO NPs and ZnO/Ag composites were prepared using different capping agent (Ethanol and 2-propanol). The XRD result confirm that the wurtzite-phase of ZnO NPs. The UV-VIS absorption spectra showed blue shift when used 2-propanol while no blue shift with using ethanol in wavelength corresponding to bulk. Large band gap energy and highly blue shifted absorption edge confirm that the prepared ZnO NPs and ZnO/Ag composite exhibit quantum confinement effect. We have demonstrated the antibacterial activity of addition of ZnO NPs as compared with other metal oxide. Besides, the result showed the higher antibacterial activity and synergistic effect between ZnO NPs and Ag NPs in forming ZnO/Ag composite for different bacterial isolate.

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