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Enhanced bioactivity of Pure ZnO and ZnO-Ag nanocomposite Using Sol-Gel method for Self-Cleaning Application

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ABSTRACT

In the current study, Pure ZnO and Ag-ZnO nanocomposite are established using the sol-gel method with the influence of ammonia solution NH_4OH to incorporate Ag ions into ZnO and form Ag-ZnO nanocomposites. Then pure ZnO and Ag-ZnO nanocomposites were annealed at 450^{o}C for tow h in a muffle furaning using temperture controlling, and heat rate was set at temperature 100^{o}C per min. The structural and morphological properties of samples were characterized using XRD, FTIR, and FESEM with Energy dispersive X-rays (EDX). In addition, the antibacterial activity of pure ZnO and Ag-ZnO nanocomposite was evaluated against gram-positive and gram-negative organisms by plate count test. The results of the test revealed strong antibacterial behavior of nanocomposite against bacteria as compared to pure ZnO and improved efficiency of incorporation Ag ion on ZnO.

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INTRODUCTION

Different metal oxides like ZnO, TiO_2, and silicon dioxide (SiO_2) have attracted extensive attention due to their wide range of technological applications (García-Alamo *et al.*, 2013). As well as, inorganic antibacterial substance, like metal and a metal oxide, become more advantageous as compared to organic materials because of their stability (Salem *et al.*, 2015). Among semiconductor metal oxides, ZnO has found to be an interesting semiconductor because of wide band-gap and useful properties that can be grouped with different inorganic materials. Besides, ZnO nanoparticles show antibacterial activity due to increasing bacterial attachment on a biomedical surface with inherent features that shearing structures as Wurtzite (Farag *et al.*, 2010; Wang, 2004; Kaneva and Dushkin, 2011; Sharma, 2012; Bogutska *et al.*, 2013). Zinc oxide nanocomposites have attracted more interesting due to their optical, magnetic, and mechanical properties. They can also produce a new combination with new properties because of strong interacting between two various components (Shah *et al.*, 2013; Kołodziejczak-Radzimska and Jesionowski, 2014). ZnO nanocomposite have attracted large attention because of exceptional features and application in apparent electronic, Chemical Sensors, and biomedical applications using various methods like chemical precipitation, sol-gel method, chemical vapor deposition (CVD), Hydrothermal method; spray pyrolysis and vapor-liquid-solid method. In metal-oxide nanocomposite, various materials with various features can be combined in the same particles and performing multi-function (Vijayakumar *et al.*, 2013; Baruah *et al.*, 2012). However, it is a hard achievement Visible Light absorbing on a zinc oxide substance. Silver ions are most widely investigated because of their interesting candidates for varied bandgap substance and absorbed light. Silver ions reveal great absorbing in visible light without any extremely toxicity element (Chamjangali and Boroumand, 2013; Arakelova, 2010; Haider *et al.*, 2016a). There are various doping agents, such as (P, N, As, Li, and Ag). Among these, Ag is added as a doping agent. Because of the structure of silver ions and their surface, that becomes the main important as the size decreased, which makes them interesting substance and linkage matrices in the medical filed (Lupan *et al.*, 2010; Jamil *et al.*, 2014; Bordbar *et al.*, 2013; Bazant *et al.*, 2014). On the other hand, Ag-ZnO nanocomposite has pronounced interest since they show biological, photocatalytic activity, low toxicity, and exert a synergetic antibacterial effect. Besides, Ag nanomaterials exhibit some exceptional characteristics in chemical and biological sensors, that are related to localizing Surface Plasmon Resonance SPR, and metallic enhance fluorescence (Wang *et al.*, 2011). Briefly, Ag treatment inhabits DNA replication and Cell protein that result in inhibiting the growth of bacteria because of the existence of Ag in size of nanoparticles in the nanocomposite, which results in increasing surface area as well as enhancing antimicrobail activity of Ag-ZnO nanocomposite (Shao *et al.*, 2013).

In this paper, the Ag-ZnO nanocomposite was prepared using the cost-effective and simple Sol-Gel method without a need to vacuum equipment. Moreover, the influence of ammonia solution in incorporation Ag ions into the ZnO crystal structure was evaluated and followed by annealing to ensure the formation of Ag-ZnO nanocomposite. The structural and morphological of the nanocomposite were characterized using XRD, FTIR, and FESEM with Energy-dispersive X-Rays (EDX) to analyze the composition of the synthesized sample. Besides, the antibacterial activity of pure ZnO and Ag-ZnO nanocomposite samples were evaluated against gram-positive *(S. aureus*) and gram-negative (*E.coli*) microorganism using the plating method.

MATERIALS AND METHODS

Regents

Zinc acetate dehydrates ($Zn(CH_3CO_2)_2.2H_2O$,99.9%), silver nitrate ($AgNO_3$,99.9%), ammonia solution (NH_4OH) ,Sodium hydroxide (NaOH) and Polyvinylpyrrolidone (PVP,M.W= 4000) stabilizer agent.

Preparation Ag-ZnO nanocomposite

The synthesis of pure ZnO and Ag-ZnO nanocomposite had been performed by the sol-gel method. 2 grams of Zinc Acetate dehydrates is dissolved in 100 ml of deionize water (D.W), and 0.1 M NaOH was dissolved in 100 ml of D.W to form NaOH solution. Then NaOH solution was dropped step by step to zinc acetate until white color result and leave for 24h to obtain for aging and forming gel sample. For the same procedure, 2 grams of Zinc Acetate dehydrates is dissolved in 100 ml of deionize water (D.W) and 0.025-gram silver nitrate is dissolved in 100 ml of deionizing water with continuous stirred. Then, it was followed by a suitable amount of ammonia solution (0.001M) that was dropping step by step slowly until pH equal to 7 with more stirring of the mixture for 1h. With continuous stirring, about 0.5 %(w\v) of PVP was added in 100 ml of deionized water as a stabilizer agent. After one hour, the solution was left for 24h for aging and formed a glassy gel. The combination was filtered and washed twice with deionized water (DW) and dried at 100^{o}C for 24h. Row zinc oxide and Ag/ZnO nanocomposite were annealings at 450^{o}C for tow h in a Muffle Furnace constant temperature. The heating rate is set at 100^{o}C per min. All substances are used as received from the supplier without more purification.

Characterization

The preparation of pure ZnO and Ag-ZnO nanocomposite are characterized using X-Ray Diffraction (XRD, 6000-Shimadzu X-Ray Diffractometer, and wavelength λ=1.54178 Å) to determine the crystal structure and the average particle size using Scherrer formula (Salman *et al.*, 2017; Zheng *et al.*, 2008). FTIR analysis (Shimadze 8400S spectrophotometer) was used to characterize nanocomposite. Filed emission scanning microscopy (FESEM, Mira, 3-XMU, Day petronic-Iran) is using to determine morphological of samples and average particle size of Ag-ZnO samples with energy dispersive X-Ray (EDX) and analyses the composition of established samples.

Antibacterial Activity

Antibacterial activity was evaluated by using a viable count method test (ASTM E2149 suspension method). Two kinds of bacteria, gram-negative (*E.coli*) and gram-positive (*S.aureus*), were grown on nutrient agar at 37 C^o for 24h. Bacteria suspension was formed using normal saline (0.9%) to obtain 10^7-10^8 CFU/ml at 0.5 Mcfarland Standard, as it was reported previously (Dutta *et al.*, 2010; Haider *et al.*, 2016b). Then one ml of each bacteria suspended is added to 9 ml of normal saline that contain ZnO and ZnO-Ag in different concentration (1, 2, 5) PPM and incubation for 24h at 37 C^o in incubation shaker at 160 rpm, after that the mixture was serially diluted in normal saline and then 100 ml of diluted was cultured on Molar Hinton Agar and incubated at 37 C^o

for 24 h. The available bacteria colony was counting as in Equation (1), which represented the formula of bacterial survive rate (K) as following (Ahmed *et al.*, 2015),

No. of colonies (CFU/ml) = (No. of colony for each dilution)/ (dilution 10^3 factor)/sample Volume

$$Bacteria\ survival\ rate\ (K) = \frac{(A-B)}{A} \times 100\% \quad (1)$$

RESULTS AND DISCUSSION

The crystal structure and diffraction patterns of Ag-ZnO nanocomposite were recognized by X-Ray Diffraction in the range 20^o-80^o. From Figure 1 (b), a strong diffracted peak belongs to the Hexagonal Wurtzite texture of ZnO (Munshi *et al.*, 2018). These strongest peaks at values 2Θ: 31.9^o, 34.5^o, 36.3^o, 47.6^o, 56.7^o, 62.9^o, 66.4^o, 68^o, and 69.1^o were related to the planes: (100), (002), (101), (102), (110), (103), (200), (112) and (201), respectively and agreed with standard card (JCPDS No.36-1451). Besides, three weak peaks were obtained at 38^o, 44.9^o and 77^o were corresponded to (111),(200) and (311) indicating the presence of Ag ions (JCPDS No.04-0783) with their low content in ZnO lattice without other impurities in the structure (Hosseini *et al.*, 2015; Atarod *et al.*, 2016).

From XRD analysis, the diffraction peaks were the narrow and low-intensity peak of (100) and (002) as compared with pure ZnO in Figure 1 (a) because of the effect of Ag ions as a dopant in zinc oxide structures. From these results, most of the silver ions are located on the surfaces or interface of the ZnO structure, and there are some of silver ions peaks not detected in x-Ray Diffraction analysis because of the low content of metallic Ag and less dispersion. The calculated average particle size of Ag - ZnO product is from 25 nm to 50 nm in diameter, according to Debye-Scherer's Equation (2) , (Zheng *et al.*, 2008).

$$D = \frac{K\lambda}{\beta_{2\theta}\cos_{\theta}} \quad (2)$$

FT-IR Spectrum

From Figure 2 (a,b), FT-IR analysis of pure Ag-ZnO nanocomposite are recording in the range (400 - 4000) cm^{-1}. From this Figure, the important band at 418 cm^{-1} was related to a combination of Zn-O and Ag ions vibration and 486 cm^{-1} in pure ZnO. The bands at 3358 cm^{-1} and 1643 cm^{-1} corresponded to OH functional groups O-H, which revealed the presence of some water absorptions on Ag-ZnO nanocomposite. While the band at 2341 cm^{-1} was corresponded to the atmospheric CO_2 that appear on metallic. Stretch mode of C=O is recognized at 1516 cm^{-1} and 1570 cm^{-1} of pure ZnO and Ag-ZnO nanocomposite. In the state of Ag-ZnO nanocomposite, presence spectral bands at 1126 cm^{-1} and 885 cm^{-1} were due to the formation organic capping agent of Ag metals. The results of FTIR suggest, the presence some Ag ions in ZnO lattice at band 418 cm^{-1} and band 885 cm^{-1} as shown in Figure 2 (b) that reveal the combination between Zn-O vibration and Ag ions during the sol-gel method as compared with pure ZnO in Figure 2 (a) (Baranyai *et al.*, 2009).

Field Emission Scanning Electron Microscopy (FESEM) and EDX analysis

The morphological and composition studies of distributed Ag metallic in Ag-ZnO nanocomposite were determined FESEM and DEX to observe the composition of elements of formation Ag located on ZnO lattice as shown in Figure 3 (a,b,c). From the FESEM results in Figure 3 (b), the surface morphology was exhibited spherical grains of Ag-ZnO nanocomposite with the diameter range from 20-50nm as compared to pure ZnO in Figure 3 (a) that revealed particles with quasi-spherical nanoparticles, less uniformly shape and less size near from 20-30nm. These results reveal that surface morphology of Ag-ZnO nanocomposite was influenced by inserting Ag metals in ZnO lattice, which increased grain size of nanoparticles with good distribution.

Besides, Figure 3 (c) revealed the incorporation of Ag metallic with ZnO structure as characterized by EDX analysis that confirmed the presence of Zn, O and little amount of Ag attachment in ZnO lattice after continuous stirred and annealing time. These results may give initial evidences that the grain size after Ag deposition on ZnO causes less increase in the size of spherical nanoparticles with uniform distributed nanoparticles as well as reveals the role of ammonia solution (0.001M) in the synthesis between silver nitrate and zinc acetate and forms high crystalline nature and changes the morphology.

Antibacterial Activity of Pure ZnO and Ag-ZnO nanocomposite

The antibacterial activity of pure ZnO and Ag-ZnO nanocomposite suspensions were examined against gram-positive (*S.aureus*) and gram-negative (*E.coil*) bacteria using plating count test. In addition to, it determined the minimum inhibitory concentration and bactericidal concentration (MIC and MBC) assays of nanocomposites agents against various pathogens and achieved irreversible inhibition using a different concentration of pure ZnO and Ag-ZnO nanocomposite (1, 2, 5) ppm, respectively. As shown in Figure 4 (a), the results showed Ag-ZnO

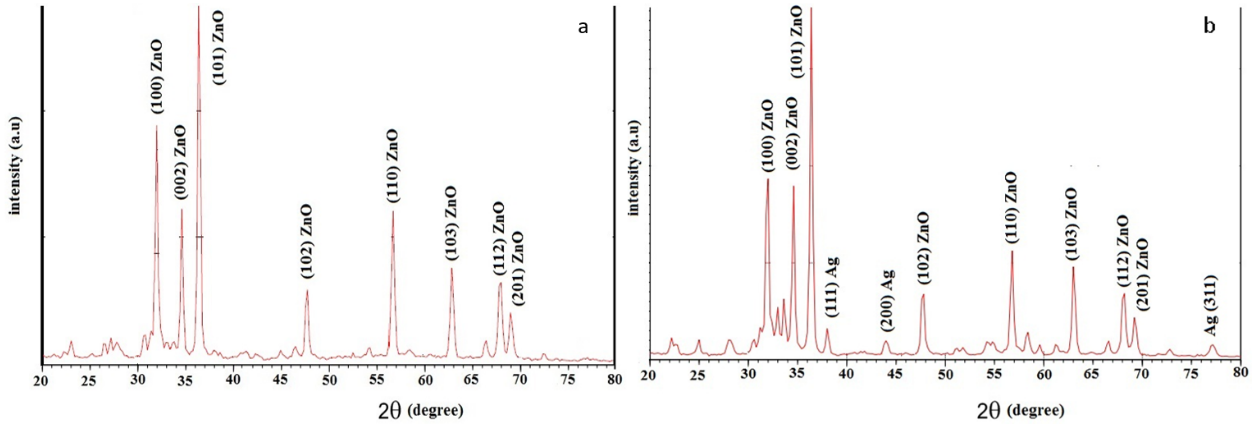

Figure 1: XRD analysis of a) Pure ZnO and b) Ag-ZnO nanocomposite result after annealing at 450°C for 2h

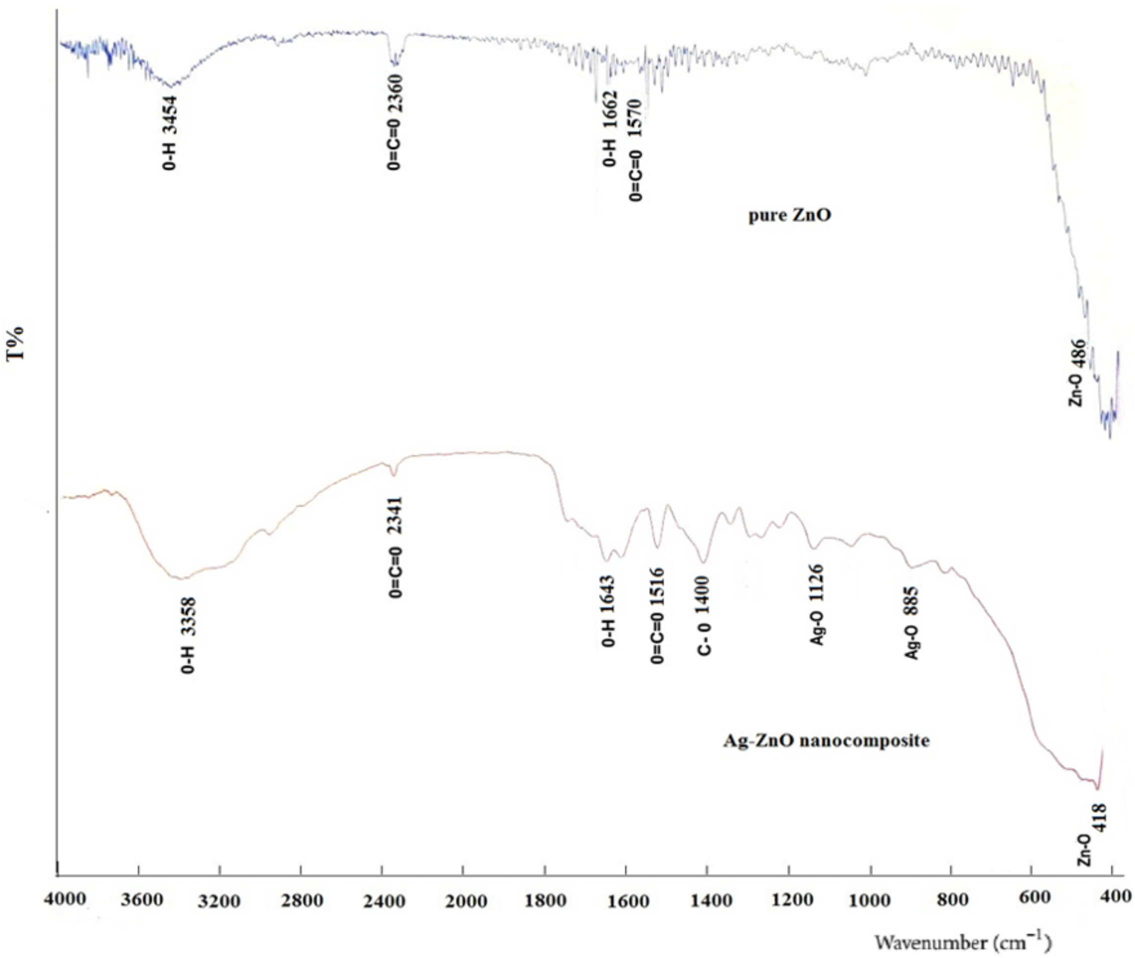

Figure 2: FTIR spectrum of a) pure ZnO and b) Ag - ZnO nanocomposite after annealing at450°C and 2h

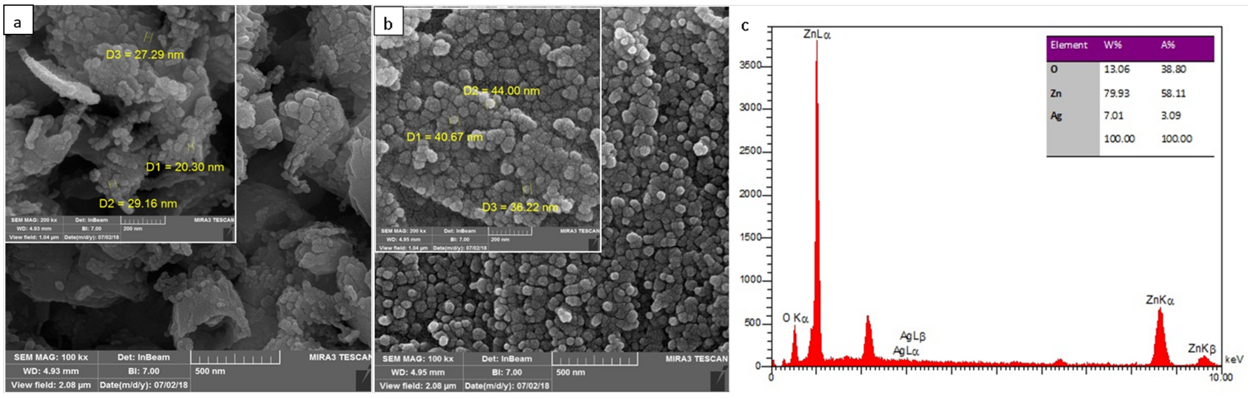

Figure 3: SEM images of a) ZnO and b) Ag-ZnO nanocomposites and c) EDX of Ag-ZnO nanocomposite product after annealing at 450°C for 2h

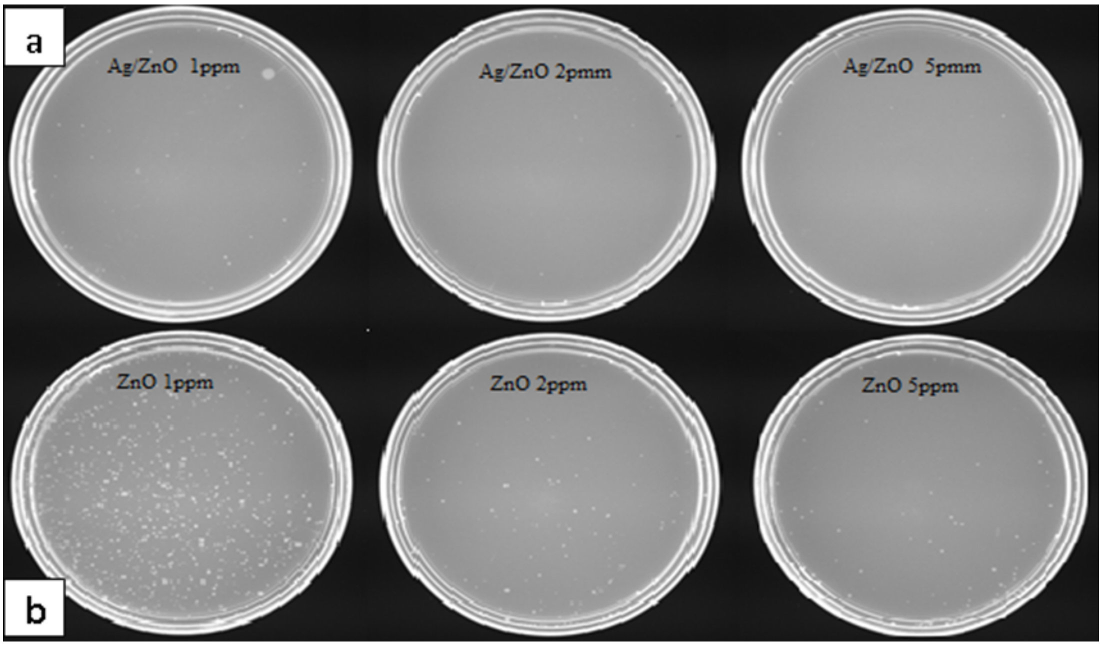

Figure 4: Viable cell number of a)Ag-ZnO nanocomposite and b) pure ZnO nanoparticles in different concentration against *E.coli* bacteria

nanocomposite can efficiently inhibit the growth of bacterial cells nearly (99%) when the concentration of Ag-ZnO nanocomposite is about 2 ppm, which indicated the lowest MIC of Ag-ZnO towards *E. coli* was 2 ppm. It was found that at the concentration 5ppm of Ag-ZnO nanocomposite, the percentage of inhabited cells reach more than (99%), suggesting that the MBC of nanocomposite against *E. coli* was between 2 and 5 ppm. While in the case of *S.aureus,* the percentage of antibacterial activity was nearly (80, 96, 98 %), respectively, at concentrations (1,2,5) ppm, as seen in Figure 5 a. In-state of using pure Zinc oxide with the same concentrations (1, 2, 5) ppm, the results indicated that the lower percentage of antibacterial activity was (20, 60, 80)% of *E.coli* and (10, 30, 60)% for *S.aureus*, respectively as shown in Figure 4 (b) and Figure 5 (b) which demonstrated the number of viable cell method using plating method.

Besides, (Figure 6) indicates the Antibacterial Activity of pure ZnO and Ag-ZnO nanocomposite, respectively, using a concentration of (1, 2, 5) ppm against the humane pathogen.

Since the results in this test suggest the effective of Ag-ZnO nanocomposite on both types of pathogens compared with pure ZnO Nanoparticles, and these results agree with previous studies, which performed the mechanism of bacteriostatic of Ag-ZnO nanocomposite by inhibited peptidoglycan formation through cells splitting. In addition to, the result was supported by the Hypothesis which the bacteria treatment develop to non-cultural due to inhibition of more cells splitting (Bogutska *et al.*, 2013).

The main action of Nanoparticles is performed by nanoparticle-bacteria interactions because of the

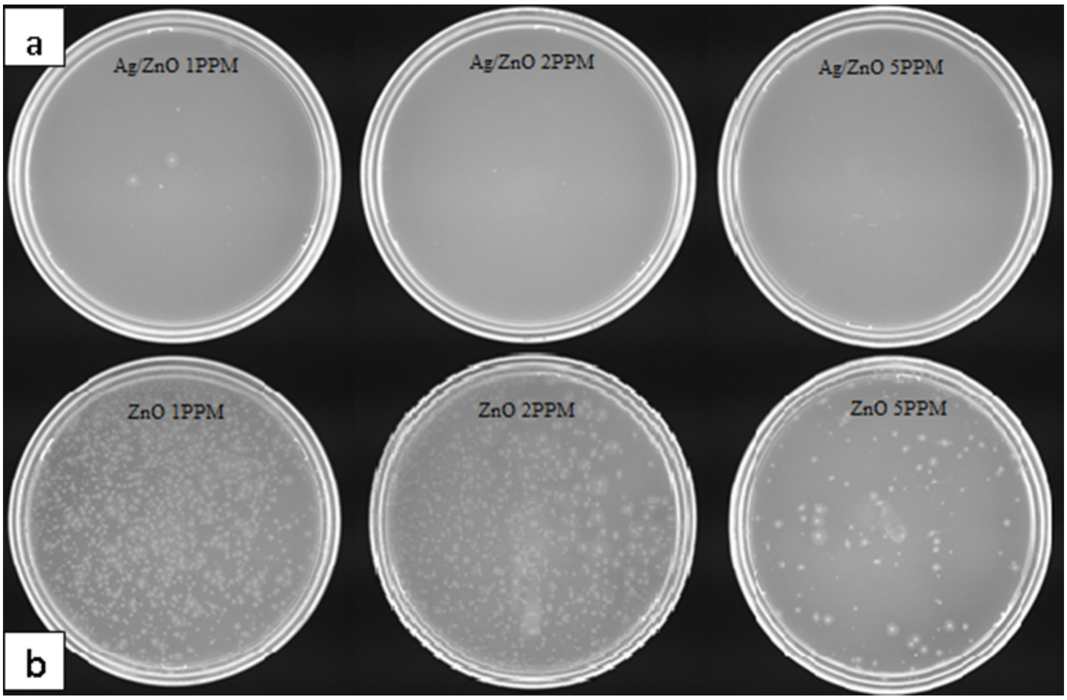

Figure 5: Viable cell number of a)Ag-ZnO nanocomposite and b) pure ZnO nanoparticles in different concentration against *S.aureus* bacteria

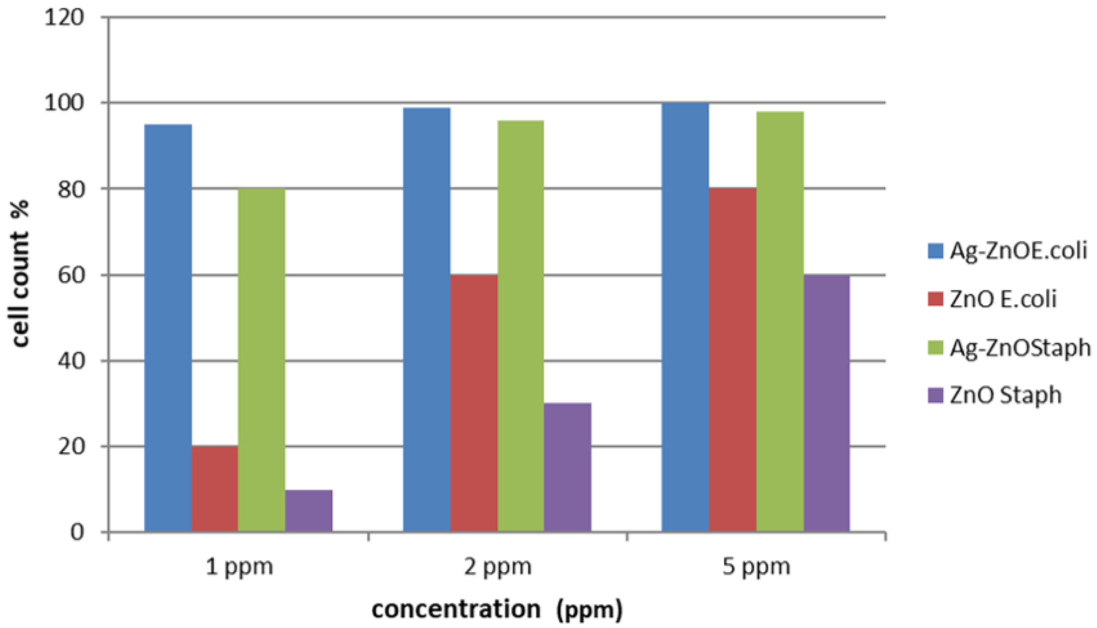

Figure 6: Antibacterial Activity of Ag-ZnO nanocomposite and pure ZnO nanoparticles using different concentration against *S.aureus* and *E.coli* microorganism

negative charge of the cell surface, which results in internalize of NPs into the bacteria membrane. The accumulation of pure ZnO and Ag-ZnO nanocomposite on the surface of bacterial cells either in the cytoplasm or in the periplasmic area leads to interrupted cell function and disorganized membranes.

From the results, it was suggested Ag-ZnO nanocomposites reveal successful antibacterial property as compared to pure ZnO. The mechanism of the biocidal action of pure ZnO and Ag-ZnO nanocomposite involves the disrupting and penetrating the membrane and oxidizing the cell component by production levels of reactive oxygen species (ROS) resulted in damage like hydroxyl radicals OH^- and superoxide (O^{2-}) that can toxic the cell as they damage cellular components such DNA and proteins since the physical contact between the cell membrane and nanocomposite can be resulted due to the electrostatic effect of attractive via prompting the interaction between the culture of bacteria and NPs due to the formation of active species from NPs that directly influence the cell membrane (Bamberger *et al.*, 1993; Feng *et al.*, 2000).

As a result, Ag-ZnO nanocomposite improved a good antibacterial agent as compared to pure ZnO. Besides, pure ZnO and Ag-ZnO nanocomposite had shown robust antibacterial activity against gram-negative *E.coli* as compared with gram-positive *S.aureus* bacteria because of a variable of bacteria membrane between *E. coli* and *S.aureus* microorganisms as well as increasing the surface – volume ratio of small particles size which results in high penetrate (Atarod *et al.*, 2016).

CONCLUSION

ZnO nanocomposite with deposition Ag metals have been prepared using the Sol-Gel method and revealed strong antibacterial activity against *E. coli* and *S.aureus* as compared to pure ZnO NPs. The results conclude the effect of the addition of a suitable amount of Ag metals on grain size, surface morphology, and crystal structure with hexagonal of Ag-ZnO nanocomposite as demonstrated by XRD analysis, FTIR spectrum and FESEM with (EDX). In addition, the resulted pure ZnO and Ag-ZnO nanocomposite have shown strong antibacterial activity against Gram-negative bacteria *E. coli* as compared with Gram-positive *S.aureus* because of the variable of the cell membrane of pathogens and the influence of deposition silver ion on the crystal structure of ZnO.

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