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STUDY ON THE ACTIVITY OF ZnO-SnO₂ NANOCOMPOSITE AGAINST BACTERIA AND FUNGI

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Abstract: ZnO-SnO₂ nanocomposite was prepared by the sol-gel method. The as-prepared nanocomposite was characterized by X-ray diffraction (XRD), scanning electron micrograph (SEM-EDX), FTIR and UV-Visible spectrometer analysis techniques. The average particle size of nanocomposite was calculated from the XRD study. The average particle size of the prepared nanocomposite was 22 nm. According to the UV-Visible spectrum, the band gap value of 5.06 eV was obtained for the ZnO-SnO₂ nanocomposite. From the analysis techniques it was found that the metal oxides of ZnO-SnO₂ mainly consist of ZnO and SnO₂ metal oxides. The antibacterial and antifungal activities of the ZnO-SnO₂ nanocomposite were studied against *Staphylococcus aureus* (ATCC25923), *Listeria monocytogenes* (ATCC 11994) (Gram-positive), *Salmonella typhi* (ATCC14028), *Escherichia coli* (ATCC 25922) (Gram-negative), *Candida albicans* (ATCC10231), and *Aspergillus niger* (ATCC 16404) (fungi) by two methods through the turbidity method or reading optical density and inhibition zone, which were carried out in the absence of irradiation. We observed an effective antibacterial and antifungal activity of the ZnO-SnO₂ nanocomposite against bacteria and fungi.

Keywords: ZnO-SnO₂ nanocomposite, bacteria, fungi, sol-gel method

Introduction

In the past centuries *penicillin* was extracted from *Penicillium*, a fungal class, and used as antibiotic (Grabley and Thiericke, 1999). The development of science leads to the synthetic organic drugs which are becoming common practice to treat bacterial diseases (Mascaretti, 2003; Adam, 2001). The increase of using drugs developed resistance in bacteria which is a cause of a decrease in the efficiency of drugs and the antibiotics become less efficient (Diallo and Savage, 2005). Therefore, this type of

problem motivates scientists to synthesize drugs having high efficiency and more effectiveness on multisystem of bacteria, causing the bacteria finding difficulty in developing resistance against them. Organic antibiotics are found less stable (shorter shelf life) than inorganic antibiotics at elevated temperature or pressure. For this reason various metal oxide nanoparticles such as ZnO and SnO₂ have been considered as antibacterial and good inhibitor of different bacterial strains due to their selective toxicity to biological systems (Zhang et al., 2007; Hajipour et al., 2012). Therefore, the inorganic metal oxide nanoparticles are used as antimicrobial agents because they have several advantages such as great effect on resistant strains of microbial pathogens, less toxicity and heat resistance (Nagarajan and Rajagopalan, 2008). The activities of nanoparticles are directly dependent on the bacterial strain i.e., Gram-positive and Gram-negative because they have differences in their cell wall. Electrostatic interactions are directly responsible for the attachment of nanoparticles to bacteria. These interactions changes the integrity of cell membranes of bacteria and toxic free radicals is released, which induce oxidative stress on bacteria (Hajipour et al., 2012). Recently, the antibacterial properties of the metal oxide nanocomposites of ZnO-SnO₂ have been attracting significant attention due to their stability under high temperature and pressures, and various potential applications in catalysis (Limthongkul et al., 2001), photocatalytic (Tiekun et al., 2014), sensors (Sin et al., 2014), and possessing excellent electrical properties as new transparent materials, where the much higher conductivity than ZnO and SnO₂ (Kurz et al., 2006), at same time the ZnO-SnO₂ nanocomposite have high surface area which allow for better interaction with bacteria and exhibiting great antibacterial activity regarding to biomedicine surface and coating onto cotton glass substance. Furthermore, there is a growing awareness of the use of antibacterial fabrics in the form of medical, protective garments (Issa et al., 2013) and textile fabric (Sojka et al., 2008).

The removal of bacteria from water is an extremely important process for drinking and sanitation systems, especially against concerns on growing outbreaks of water borne diseases (Shannon et al., 2009). In the United States, only between 2003 and 2005, there were four reported water borne disease outbreaks attributed to pathogens in drinking water affecting 282 people (US Environmental Protection Agency, 2006). Conventional methods for disinfection of water are dependent on chemical agents, that are ineffective against the *cyst-forming protozoa* such as *Giardia* and *Cryptosporidium* and also these methods often produce harmful by-products. Nanotechnology is considered as a new generation of technology that can have a great impact on economies through new consumer products, manufacturing methods and materials used (Albrecht et al., 2006). This technology can lead to cost effective and high performance water treatment systems (Diallo, 2005). By using nanotechnology, the implementation of oligodynamic nanoparticles for water disinfection is being explored. Oligodynamic nanoparticles based disinfection includes the use of metal oxides such as silver oxide, gold oxide, zinc oxide, tin oxide and copper oxide due to their antimicrobial properties. Besides their oligodynamic nature, they also possess

catalytic properties (Rodriguez et al., 2007). Among these transition metal oxides, the ZnO-SnO₂ nanocomposite attracts much interest due to the low cost of ZnO and SnO₂ nanoparticles. Additionally, in the ZnO-SnO₂ nanocomposite, the valence band of ZnO is positioned between the valence band and the conduction band of SnO₂ and the conduction band of ZnO is positioned above the valence band and the conduction band of SnO₂ (Gratzel, 2001). Recently, Nasrin et al. (2011) reported the synthesis of nano-sized coupled oxides ZnO-SnO₂ thin films in a different molar ratio of ZnO and SnO₂. They were prepared using sol-gel dip coating method and taking their bacterial activity against *E.coli*. As far as we know, there is no report on activity of ZnO-SnO₂ nanocomposite against such bacteria as *Salmonella typhi*, *Staphylococcus aureus*, *Listeria monocytogenes*, and fungi *candida albicans* and *Aspergillus niger*.

The aim of this research is synthesis of nano-sized ZnO-SnO₂ in a molar ratio 1:1 by the sol-gel method and study their antibacterial and antifungal activities against *Salmonella typhi* (ATCC14028), *Escherichia coli* (ATCC 25922) (Gram-negative), *Staphylococcus aureus* (ATCC25923), *Listeria monocytogenes* (ATCC 11994) (Gram-positive), as well as *candida albicans* (ATCC10231) and *Aspergillus niger* (ATCC 16404) (fungi) as a model by two methods.

Experimental

Synthesis of ZnO-SnO₂ nanocomposite

All of the chemical reagents used in the experiments were analytic grade without further purification and treatment. To synthesize ZnO-SnO₂ nanocomposite, 0.22 M zinc chloride (ZnCl₂) aqueous solution and 0.22 M tin(IV) chloride pentahydrate (SnCl₄·5H₂O) aqueous solution were prepared in distilled water, each solution stirred separately until formed clear solutions. The two solutions were mixed together at room temperature to prepare 100 cm³ solution having the Zn:Sn ratio of 1:1 and the mixed solution placed on the plate of magnetic stirrer at a speed of 350 rpm for 24 hours. The pH of the reaction volume brought to 12 by adding drop-wise of diluted ammonia while the solution was continuously stirred. Finally, the precipitate was separated from the solution and washed with distilled water three times to remove any soluble products and dried at 100 °C in an oven. The schematic steps of the synthesis of ZnO-SnO₂ nanocomposite of using the sol - gel method is shown in Figure 1.

Antibacterial and Antifungal performance

The prepared nanocomposite sample was employed for the inactivation of Gram-negative *Salmonella typhi* (ATCC14028), *Escherichia coli* (ATCC 25922), and Gram-positive *Staphylococcus aureus* (ATCC25923), *Listeria monocytogenes* (ATCC 11994) and also employed for the inactivation of fungi *candida albicans* (ATCC10231) and *Aspergillus niger* (ATCC 16404). The antibacterial and antifungal activities of the synthesized nanocomposite were evaluated by two methods.

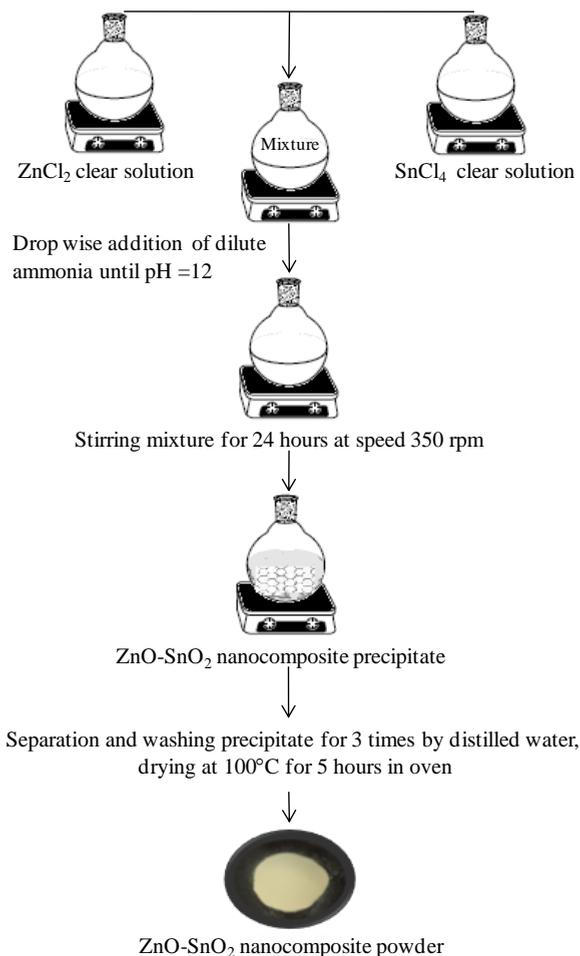


Fig. 1. Schematic procedure for synthesis of ZnO-SnO₂ nanocomposite

The first was turbidity method. A few colonies from the overnight culture of nutrient agar or MacConky agar were transferred into 5 cm³ of nutrient broth medium turbidity of suspension which was adjusted to (0.45–0.5) through the optical density reading at 600 nm by spectrophotometer. The minimum bactericidal concentration (MBC) of nanocomposite powder was determined through the standard curve which is the relation between absorbance reading and the number of bacteria, and the following dilution was prepared for each extract (1, 2, 3, 4, 5, 6, 7, 8 and 9) mg/cm³ of the stock solution which is prepared by dissolving 10 mg of nanocomposite powder in 1 cm³ of 70% ethanol. The second method was the inhibition zone: nutrient agar was prepared and sterilized by autoclave and then poured about 20 cm³ of agar into Petri plate,

solidified and inoculated with the bacterial isolates that were obtained from prepared control. The inoculums were uniformly spread using a sterile cotton swab on a sterile Petri dish nutrient agar. The stock solution of the nanocomposite prepared by dissolving 10 mg and 20 mg of nanocomposite in 1 cm³ 70% ethanol. Each Petri plate has 6 wells (4 mm diameter holes cut in the agar gel) and 6·10⁻²cm³ of the stock solution was added to the test wells and 6·10⁻²cm³ of antimicrobial at a minimum inhibition concentration (MIC) by using pure extra HPLC powder of *Ampicillin* and *Rifampicin* were added into two adjusted wells. The mixed of 3·10⁻²cm³ antimicrobial with 3·10⁻²cm³ nanocomposite were added into two wells and also one of the wells used for control 6·10⁻²cm³ of 70% of ethanol was used. The systems were incubated for 24 hours at 36 ± 1°C under aerobic conditions and inhibition zones of the microbial growth was measured in mm.

Results and discussions

Characterization of ZnO–SnO₂ nanocomposite

The crystal phase composition of ZnO–SnO₂ nanocomposite was determined by the XRD analysis (XRD, Rigaku Mini with Cu K α radiation, $\lambda = 0.1541$ nm, Koya University, Kurdistan-Iraq). The diffractograms were recorded in range of 10–80°. Figure 2 displays XRD patterns of synthesized ZnO–SnO₂ nanocomposite. The existence of strong and sharp diffraction peaks located at 31.19°, 34.17°, 39.91°, 46.04 and 53.80° corresponding to (100), (101), (200), (102) and (110) planes, respectively. All the peaks can be readily indexed to crystalline size of ZnO–SnO₂ nanocomposite (standard data of JCPDS file number of 89-0511) with hexagonal phase (*space group* P63mc, and $a=b= 0.32490$ nm and $c= 0.52052$ nm) and (JCPDS file number of 77-0452 and *space group* P42mm, with a lattice parameter of $a=b= 0.47552$ nm and $c= 0.31992$ nm). It indicates the formation of ZnO–SnO₂ nanocomposite with no impurities such as SnO, ZnSnO₃ and Zn₂SnO₄. The average particle size of ZnO–SnO₂ nanocomposite was calculated with the Debye-Scherrer formula,

$$D = \frac{K\lambda}{\beta \cos\theta} \quad (1)$$

where D is the average particle size, K shape factor, λ X-ray wavelength (0.1541 nm), θ diffraction angle of X-ray and β the full width at half maximum in radians. The average particle size calculated by using the above formula was round 22 nm.

The formation and purity of the ZnO–SnO₂ nanocomposite powders were also confirmed by FTIR spectroscopy, which operated in the range of 400–4000 nm. Figure 3 shows the FTIR spectra of non calcined nanocomposite powders. The data reveal that the significant absorption peaks at 456.26 cm⁻¹ is assigned to the Zn–O–Sn bonding of the ZnO–SnO₂ nanocomposite. The broad absorption peak observed at 3413.83 cm⁻¹ reveals to the stretching band of H–O–H caused by absorbing water

molecules, and the absorption peak at 1384.64 cm⁻¹ is assigned to the N-H bonding. The FI-IR analysis clearly confirmed the presence of metal oxides of ZnO-SnO₂ in the prepared sample.

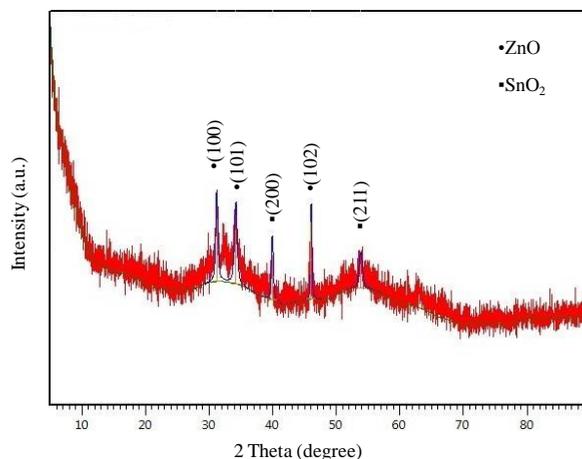


Fig. 2. XRD Pattern of ZnO-SnO₂ nanocomposite at room temperature

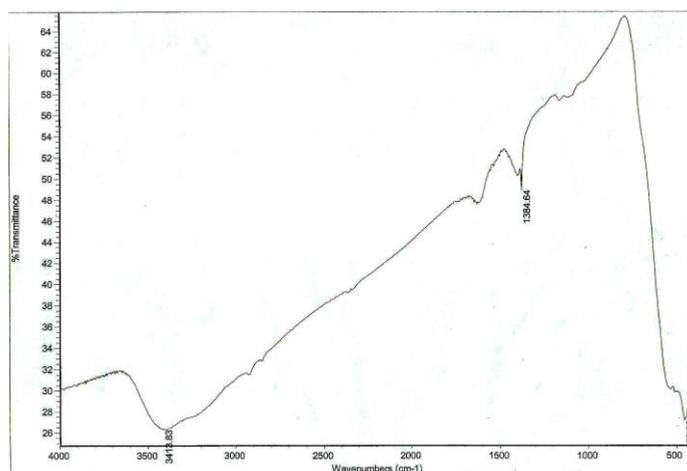


Fig. 3. FT-IR Spectrum of ZnO-SnO₂ nanocomposite

The UV-visible spectroscopy measurement was carried out by using a double-beam spectrophotometer Cary 500 scans and operated in the range of 200–450 nm at a resolution of 2.0 nm. The photo-absorption ability of the ZnO-SnO₂ nanocomposite was detected by the UV-Visible spectrum as shown in Figure 4. The ZnO-SnO₂ nanocomposite showed strong absorption at the wavelength of 245 nm. The band gap energy (E_g) of the nanocomposite calculated by the formula: $E_g = 1.240/\lambda_g$, where λ_g is the wavelength. The wavelength of the absorption edge of the prepared nanocomposite

sample was 245 nm. Thus, the band gap energy estimated from the absorption edge was about 5.06 eV. This result indicates that the nanocomposite suspension has a high ability to absorb ultra-violet light.

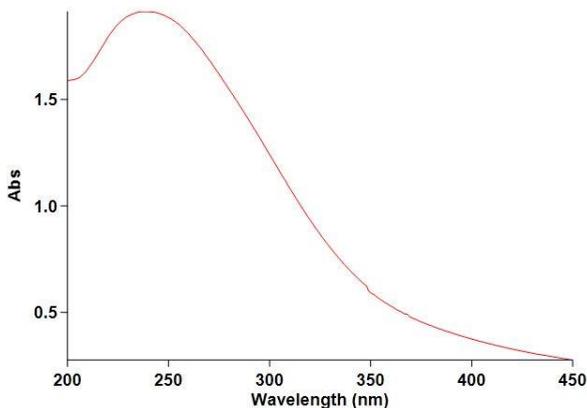


Fig. 4. UV-Visible spectra of ZnO-SnO₂ nanocomposite

The SEM image of synthesized nanocomposite showed that the irregular shape of nanocomposite powder due to agglomeration which occurred during the synthesis process as shown in Figure 5. The EDX spectrum of the prepared nanocomposite is shown in Figure 6. It shows the presence of Zn, Sn, O, N, Si and C elements in preparing nanocomposite. The silicon and carbon were also detected.

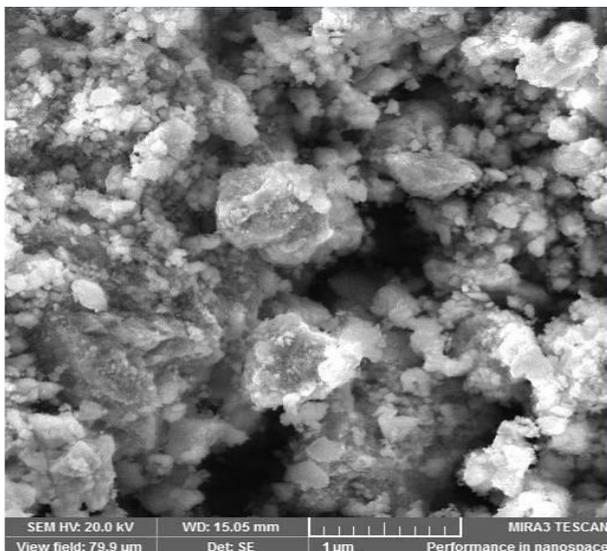


Fig. 5. SEM image of ZnO-SnO₂ nanocomposite

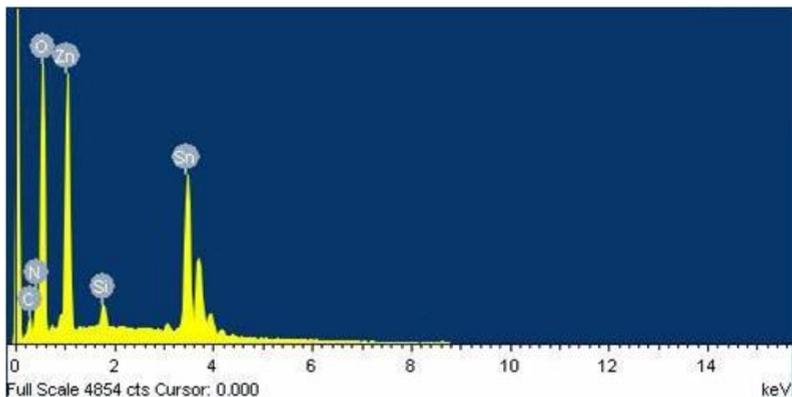


Fig. 6. EDX image of ZnO-SnO₂ nanocomposite

Bactericidal and fungicidal activity

The antibacterial and antifungal activities of prepared nanocomposite powder were determined by examining the inhibition of bacterial growth of *Staph aureus*, *Listeria monocytogenes* (Gram-positive) and *E. Coli*, *Salmonella typhi* (Gram-negative) and fungi *Candida albicans* and *Aspergillus niger*. We observed significant effects of nanocomposite powder at 22 nm. Various concentrations of ZnO-SnO₂ nanocomposite powders were examined for inhibition studies to determine the minimal inhibitory concentration. The effect of varying concentrations of nanocomposite powders on the percentage of bacterial growth is shown in Figures 7 and 8.

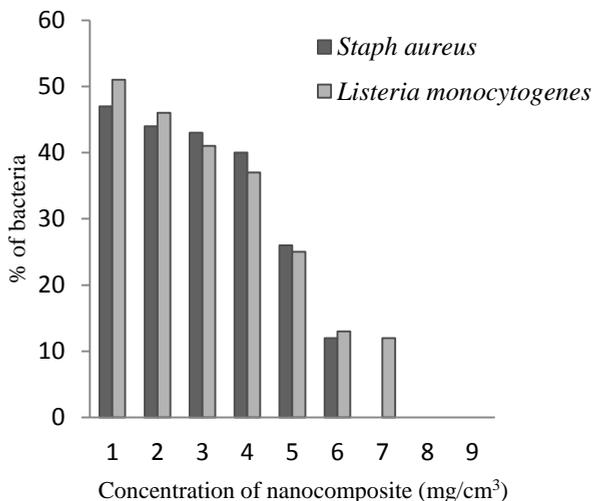


Fig. 7. Relative percentage growth of Gram positive bacteria at different concentration of nanocomposite

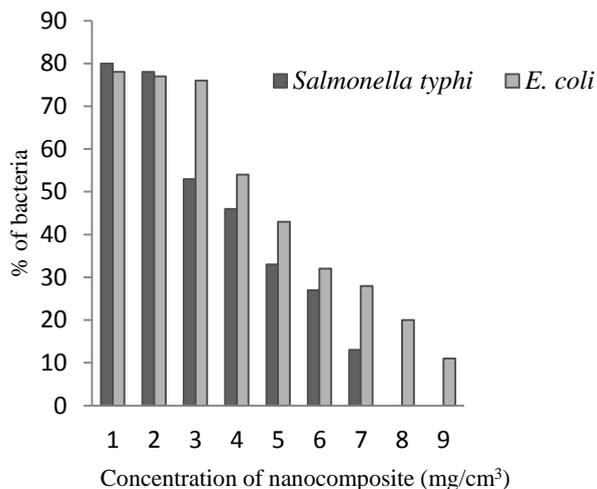


Fig. 8. Relative percentage growth of Gram-negative bacteria at different concentration of nanocomposite

We observed that the nanocomposite powder was more effective against *Staph aureus* rather than *Listeria monocytogenes*. At higher concentrations ($7 > \text{mg/cm}^3$), the growth of bacteria was completely inhibited (100%) in *Staph aureus* and inhibited (90%) in *Listeria monocytogenes*. We also observed that the nanocomposite powder was more effective against *Salmonella typhi* rather than *E. coli*. At higher concentrations ($8 > \text{mg/cm}^3$), the growth of bacteria was completely inhibited (100%) in *Salmonella typhi* and inhibited (80%) in *E. coli*, and the antifungal activity of nanocomposite powder was observed more effective against *Aspergillus niger* rather than *Candida albicans*. At higher concentrations ($7 > \text{mg/cm}^3$), the growth of fungi was completely inhibited (100%) in *Aspergillus niger* and inhibited (95%) in *Candida albicans* as shown in Figure 9.

The inhibition zones were observed and recorded in Table 1. It shows that the nanocomposite is more active against Gram-positive bacteria and fungi than Gram-negative bacteria. The nanocomposite powder is more active against *Aspergillus niger* and *Listeria monocytogenes* than *Candida albicans* and *Staph aureus* at higher concentrations (10 mg/cm^3). It also have been observed that the nanocomposite powder is more effective against *Salmonella typhi* rather than *E. coli* at higher concentrations (20 mg/cm^3). However, the antifungal *actinozol* shows resistance for both isolates with and without mixing with nanocomposite. Also, *Ampicillin* and *Rifampicin* used as antibiotics against Gram-positive bacteria of *Staphylococcus aureus* and *Listeria monocytogenes*, both of them showed susceptibility and good activity or synergistic activity with and without mixing with nanocomposite. Also, the *Ampicillin* and *Rifampicin* were used as antibiotics against Gram-negative bacteria of *Salmonella typhi* and *Escherichia coli*. The *Salmonella typhi* shows susceptibility to

both antibiotics with and without mixing with nanocomposite, while *E. coli* shows less susceptibility to both antibiotics and when exposed to mix of *Ampicillin* with nanocomposite. It shows resistance due to the mixed structure of *Ampicillin* with nanocomposite which has an antagonistic activity.

Table 1. Inhibition zone of nanocomposite and antibiotics against bacteria and fungi

Isolates	Diameter of zone (mm)						
	Nanocomposite	<i>Actinozol</i>	<i>Ampicillin</i>	<i>Rifampicin</i>	Nanocomposit + <i>Actinozol</i>	Nanocomposit + <i>Ampicillin</i>	Nanocomposit + <i>Rifampicin</i>
Fungi							
<i>A. niger</i>	17	Resistance	-	-	Resistance	-	-
<i>C. albicans</i>	12	Resistance	-	-	Resistance	-	-
G+ve Bacteria							
<i>S. aureus</i>	10	-	20	15		14	11
<i>L.monocytogenes</i>	14	-	20	16		20	16
G-ve Bacteria							
<i>E. coli</i>	10	-	8	10	-	Resistance	9
<i>S. typhi</i>	15	-	20	15	-	20	13

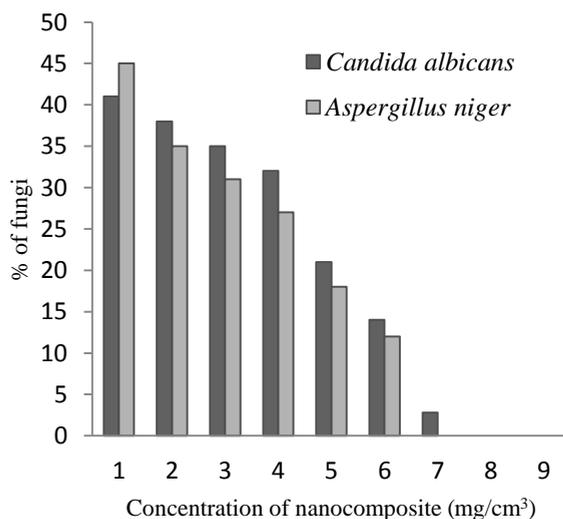


Fig. 9. Relative percentage growth of fungi at different concentration of nanocomposite

Comparing the activity of nanocomposite powders against Gram-positive and Gram-negative bacteria shows that the ZnO-SnO₂ nanocomposite powders were more active against the Gram-positive than Gram-negative. This may be because the Gram-positive bacteria are encased in a plasma membrane covered with a thick wall of *peptidoglycan*, while Gram-negative bacteria are encased in a triple layer, the

outermost layer being a *lipopolysaccharide*. For this reason the Gram-negative bacteria may be more resistant to chemical agents than Gram-positive bacteria which clearly appears in the case of ZnO-SnO₂ nanocomposite as shown in Figure 10.

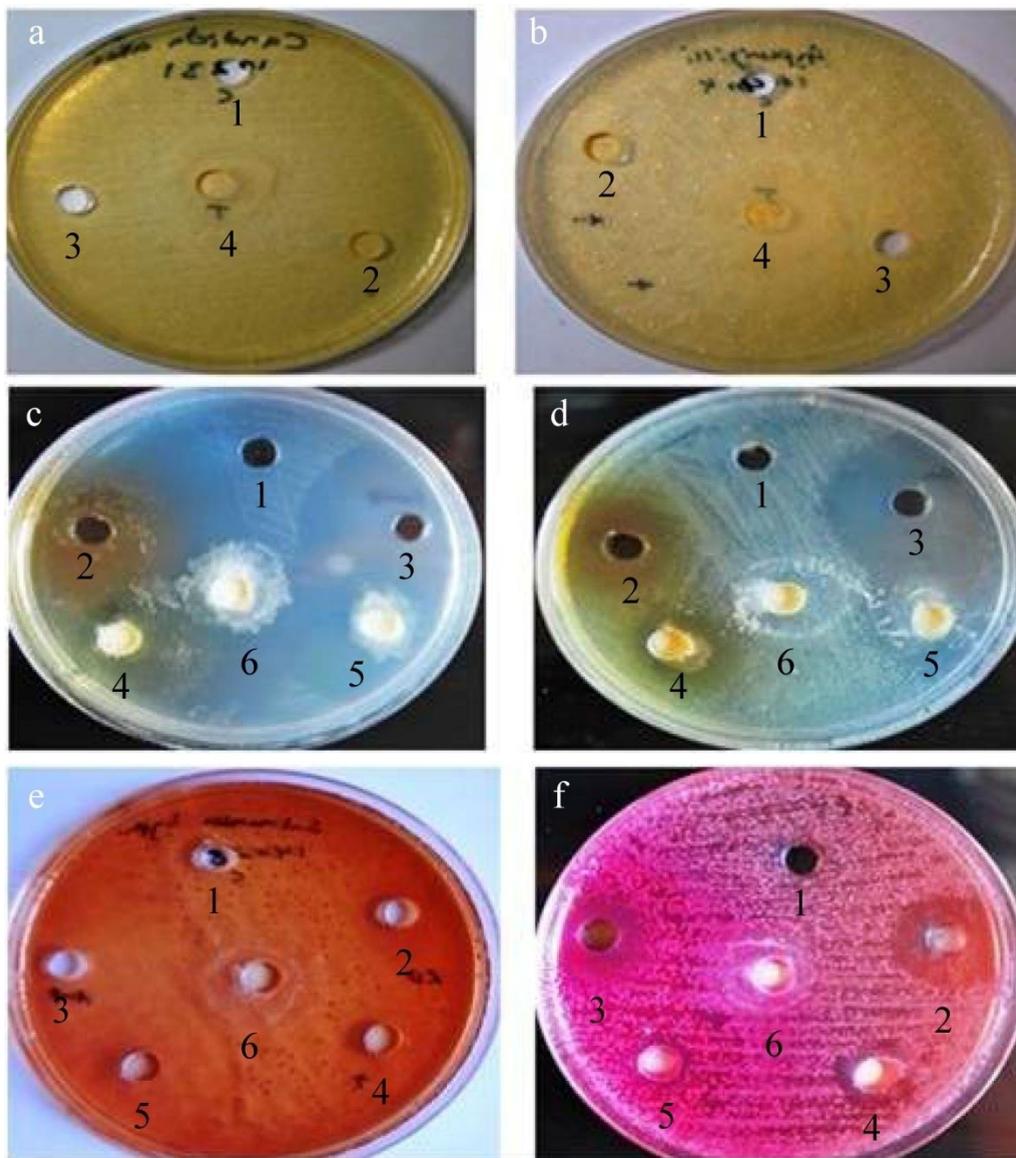


Fig. 10. Inhibition zone of nanocomposite and antibiotics against bacteria and fungi: a) *Candida albicans* b) *Aspergillus niger*, 1= Control, 2=Actinozol+nanocomposite, 3= Actinozol, 4= Test (nanocomposite), c) *Staphylococcus aureus* d) *Listeria monocytogenes*, e) *Salmonella typhi* and f) *Escherichia coli*, 1= Control, 2= Rifampicin, 3= Ampicillin, 4 =Rifampicin+ nanocomposite, 5= Ampicillin+ nanocomposite, 6=Test (nanocomposite)

Conclusions

The nanocomposite of ZnO-SnO₂ have been successfully synthesized through the sol-gel method. The synthesized nanocomposite and their characterizations were investigated by the XRD and reveals that the nanocomposite average particle size was 22 nm. The SEM image reveals that the nanocomposite have irregular shape due to agglomeration and presence of Zn, Sn, O, N, C and Si elements in the prepared sample, revealed by EDX, FT-IR spectral analysis, and shows that the characteristic peaks of Zn-O-Sn are stretching. The UV-Visible analysis reveals that the strong absorption peak for the prepared nanocomposite at 245nm have the band gap of 5.06 eV.

The different concentration of ZnO-SnO₂ nanocomposite was applied as an antibacterial and antifungal against Gram-positive of *Staph aureus*, *Listeria monocytogenes*, Gram-negative of *E.coli*, *Salmonella typhias* well as against fungi of *Candida albicans* and *Aspergillus niger* by two methods. They showed that the ZnO-SnO₂ nanocomposite powder was more active against the Gram-positive than Gram-negative and also active against fungi. Through the turbidity method it was observed that both bacteria and fungi were inhibited (80-100%) at nanocomposite concentration of $7 > \text{mg/cm}^3$. Through the inhibition zones, which was carried out in the absence of irradiation, clear zones were developed for bacteria, fungi and antibiotics. The fungi showed resistance against *Actinozo* land mixed *Actinozol* with nanocomposite while *E. coli* against mixed *Ampicillin* with nanocomposite.

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