



## International Journal of Molecular and Clinical Microbiology



### Synthesis & Antibacterial Effect of Metallic Oxide Nanoparticle against of *Escherichia coli* Isolated of Tabriz Aviculture

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#### ARTICLE INFO

##### Article history:

Received 4 February 2016

Accepted 6 March 2016

Available online 1 June 2016

##### Keywords:

Antibacterial, Ag/ZnO

Nanoparticle, *Escherichia coli*,

Tabriz Aviculture

#### ABSTRACT

*Escherichia coli* being one of the common microbial flora of gastrointestinal and respiratory tracts of poultry but may become pathogenic to both. Antibiotic treatment is considered the most important issue that promotes the emergence, selection and deploying of antibiotic-resistant microorganisms in veterinary medicine. With the increase of resistant to antibiotics, many researchers have tried to develop new. So, antibacterial nanoparticles have attracted great interest. The aim of this research is comparison antibacterial activity of Ag/ZnO with Zinc Oxide and Silver nanoparticles, against *E. coli* Isolated of Tabriz Aviculture. Nanoparticles are synthesis via wet method and avouch with oxalate decomposition in high temperature (500°C). FT-IR, XRD, SEM and TEM were used for determination of characterization of samples, respectively. Also the nanoparticles were digested and break down by ICP-AES for defining the presence of residual chemical element in the nanoparticles. Bacterial sensitivity to nanoparticles, after sonication, was commonly tested using by disc diffusion test and agar dilution test, also with determination of minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC). The particles size were less of 12 nm, approximately. This study shows that the Ag/ZnO nanoparticle has great antimicrobial agent against *E. coli* and just mixture of ZnO and Ag nanoparticles, give increase their bactericidal effect.

#### 1. Introduction

At the recent time, multi drug resistant strains of *E. coli* are presents in both human and animal isolates (Amara, Ziani, & Bouzoubaa, 1995) and multiple drug resistant, nonpathogenic *E. coli* found in the intestine is probably an important reservoir of resistance genes (Österblad et al., 2000) and momentarily drug-resistant *E. coli* of animal origin may colonize the human intestine (Marshall, Petrowski, & Levy, 1990). Acquired multi drug resistance to antimicrobial agents make an extensive trouble in case of the

management of intra and extra intestinal infections caused by *E. coli*, which are a major source of illness, death, and increased healthcare costs (Gupta, Hooton, & Stamm, 2001) and cause a variety of lesions in immune-compromised hosts moreover in poultry. Among the diseases some are often severe and sometimes lethal infections such as meningitis infection, endocarditis, urinary tract infection, septicemia, epidemic diarrhea of adults and children (Daini, Ogbolu, & Ogunledun, 2004) and Yolk Sac Infection, omphalitis, cellulitis, swollen head

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syndrome, Coli granuloma, and Colibacillus's (Gross, 1994). Antibacterial properties of nano metal oxide have been discovered as new generation of antimicrobial agent and researchers have introduced the use of silver and zinc ions as superior disinfectants for from hospitals infectious microorganisms (Lin, Vidic, Stout, & Victor, 1996; Sondi & Salopek-Sondi, 2004; Yu-sen, Vidic, Stout, McCartney, & Victor, 1998). Although, they have believed that residual these metal ions may adversely affect human health (Blanc, Carrara, Zanetti, & Francioli, 2005), but scientists' experiments demonstrated selectivity in the toxic nature of ZnO nanoparticle to different bacterial systems and human T lymphocytes. They suggested that ZnO nanoparticle may potentially useful as nanomedicine based antimicrobial agents (Sondi & Salopek-Sondi, 2004). We know that the bactericidal effect of metal nanoparticles have been ascribed to their size, photo-catalytic activity, which allows them to interact with microbial membranes and is due to the release of metal ions in solution (Morones et al., 2005). While various hypotheses have been proposed to explain the mechanism of antimicrobial activity of silver nanoparticle, it is widely believed that silver nanoparticle are incorporated in the cell membrane, which causes leakage of intracellular substances and eventually causes cell death (Cho, Park, Osaka, & Park, 2005; Thirumurugan, Shaheedha, & Dhanaraju, 2009). Some of the silver nanoparticle also interpenetrate into the cells. It is also reported that bactericidal efficiency is affected by the type of microorganism. In investigate with gram negative, *E.coli*, and gram positive, *S.aureus*, Kim reported greater biocidal efficiency of silver nanoparticle for *E. coli* and attributed it to difference in cell wall structure between gram negative and gram positive microorganisms (Kim et al., 2007). Also, Jayesh suggested that combination of metal oxide nanoparticles may give rise to more complete bactericidal effect against bacterial population (Jafari, Ghane, & Arastoo, 2011; Jafari, Ghane, Sarabi, & Siyavoshifar, 2011). This study has been undertaken to isolate and characterize *E.coli* strains from aviculture of Tabriz for assessing their susceptibility patterns to some nanoparticle antimicrobials.

## 2. Materials and Methods

### 2.1. Sampling from excrement swab

Sampling for 4 months, from 15 April to 15 July 2015 was conducted. After the autopsy, samples were prepared from liver of chicken with sterile swabs from different aviculture of Tabriz and were transferred via BHI broth to the microbiology laboratory.

### 2.2. Bacteriological analysis

A loop sample from Lung swab were spread on the *Eosine-Methylene Blue* (EMB) agar medium (MERCK, Germany). All samples were incubated for 24 hours at 37°C into EMB for successful isolation of typical colonies. Identification was done base of biochemical tests included gram staining, tests for Oxidase, Methyl red, *Voges-Proskauer* reactions, Indole, Catalase, Urea hydrolysis, Citrate, Gelatin hydrolysis tests, also Lactose fermentation, Nitrate reduction, casein hydrolysis and sugar fermentation.

### 2.3. Synthesis of ZnO nanoparticle

Zinc acetate (MERCK, Germany) was added to ethanol (MERCK, Germany) in a two neck flask giving a 0.3 M white solution. The temperature was elevated to 50 °C and after 30 min of continuous stirring oxalic acid (MERCK, Germany) was rapidly added to the solution. The molar ratio Zn:OA was 1. The system was kept at 50 °C under reflux for 2 h and a white precipitate was obtained; then the acetic acid and some of the ethanol were released moisture and the arising viscous gel was dried at 80 °C overnight. The dried Zinc oxalate was ground and calcined at 550 °C for 2 h.

### 2.4. Synthesis of Ag monometallic nanoparticle

Silver nitrate (MERCK, Germany) was added to ethanol (MERCK, Germany) in a two neck flask giving a 0.3 M gray solution. The temperature was elevated to 50 °C and after 30 min of continuous stirring oxalic acid (MERCK, Germany) was rapidly added to the solution. The molar ratio Ag:OA was 1. The system was kept at 50 °C under reflux for 2 h and a white precipitate was obtained; then the acetic acid and some of the ethanol were released moisture and

the arising viscous gel was dried at 80 °C overnight. The dried silver oxalate was ground and calcined at 550 °C for 2 h.

### 2.5. Synthesis of Ag/ZnONanoparticle

Zinc chloride (MERCK, Germany) and silver nitrate (MERCK, Germany) were added to ethanol (MERCK, Germany) in a two neck flask giving a 0.3 M gray solution. The temperature was raised to 50 °C and after 30 min of continuous stirring, oxalic acid (MERCK, Germany) was rapidly added to the solution. The molar ratio Zn/Ag:OA was 1. The system was kept at 50°C under reflux for 2 h and a gray precipitate was obtained; then the resulting viscous gel was dried at 80 °C overnight. The dried Ag/ZnO oxalate was ground and calcined at 550 °C for 2 h.

### 2.6. Characterization

Experiences of dependent on the crystallinity of the nanoparticles were carried out using a X-ray diffractometer set (XRD, Bruker D8-Advance Diffractometer using Cu K $\alpha$  radiation). Also the nanoparticles were digested and analyzed by inductively coupled plasma-atomic emission spectroscopy (ICP-AES, LIBERTY-RL, Varian Australia Co.) for determining the presence of residual chemical element in the nanoparticles. Fourier transform infrared (FT-IR) spectra were recorded on a Bruker spectrophotometer in KBr pellets. Surface morphology of product was characterized by using a scanning electronic microscopy (SEM, Cam Scan MV2300) with an accelerating voltage of 30 KV and TEM.

### 2.7. Disk diffusion test

Bacterial sensitivity to antibiotics is commonly inspected by a disk diffusion test, employing antibiotic impregnated disk (Case & Johnson, 1984). A 10 ml suspension of each nanoparticle (approximately, 16384  $\mu\text{gml}^{-1}$ ) was prepared into the Muller Hinton Broth medium (MERCK, Germany) and then suspension of nanoparticles was sonicated at room temperature and frequency of 28 KHz, during at the 10 minute, subsequently filtered through a membrane filter (0.2  $\mu\text{m}$ , 15 mm diameter ShimieRasanTeb). The nanoparticles laden filter

paper were dried in an oven for 1 h and small disks of uniform size (6 mm diameter) containing 16384  $\mu\text{gml}^{-1}$  nanoparticles were punched out and stored in a desiccator at room temperature. A content of  $1.5 \times 10^8$  CFU  $\text{ml}^{-1}$  of *E. coli* bacteria were cultured completely on the surface of a Muller Hinton agar plate (MERCK, Germany) before placing the disks on the plate. The plate was incubated at the 35 OC for 24 h, after which the average diameter of the inhibition zone enclosing the discs was measured with a ruler with up to 1 mm resolution. Subsequently, the tests were reported on three replicates.

### 2.8. Agar Dilution test

A 16384  $\mu\text{gml}^{-1}$  suspension of each nanoparticle was prepared into the Muller Hinton Broth medium, approximately, and then each nanoparticle was sonicated at room temperature and frequency of 28 KHz, during at the 10 minute. The bacterial inoculum ( $1.5 \times 10^8$  CFU $\text{ml}^{-1}$ ) was cultured completely on the surface of a Muller Hinton agar plate before excavating the cavity on the plate. Then 100  $\mu\text{g.ml}^{-1}$  from suspension of nanoparticles were filled into the cavities. The plate was incubated at 35°C for 24h, after which the average diameter of the inhibition zone enclosing the cavities was measured with a ruler with up to 1 mm resolution. The examination was also replicated, and so the results were compared together. Subsequently, the tests were reported for each nanoparticle and with *E. coli* strain on three replicates.

### 2.9. Determination of the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The lowest concentration of material that inhibits the growth of an organism was defined as the minimum inhibitory concentration (Sondi & Salopek-Sondi, 2004) (MIC). From the serial dilution method, were employed for determining the MIC of the nanoparticles (Daini et al., 2004). The test tubes were filled with 1ml of the liquid Muller Hinton broth medium. Into each of the test tubes number 1 and 2, one ml solution containing 16384  $\mu\text{g.ml}^{-1}$  of nanoparticles that had been sonicated at room temperature and frequency of 28 KHz, during at the 10 minute,

already was added and mixed thoroughly with the culture medium. The concentration of nanoparticles in each test tube become  $8192 \mu\text{g}\cdot\text{ml}^{-1}$ . Then, 1 ml of the content of test tube number 2 was added to test tube number 3 and mixed completely. This process was performed serially to test tube number 16. Consequence, 1 ml content of test tube number 16 was discarded. In order to have equal amounts of material in all the test tubes, 0.9 ml of test tube number 1 was discarded. Finally, 0.1 ml of microbial suspensions (*E.coli*) containing  $1.5 \times 10^8 \text{ CFU ml}^{-1}$  microorganism, were added to test tubes number 2 to 17, and the test tubes were incubated at  $35^\circ\text{C}$  for 24 h. Then, the microbial growth was studied by turbidimetric measurement, using a spectrophotometer (Nano-volume-spectrophotometer, Scandrop 250, Analytikjena Co.). The experiments also included a positive control (test tube containing nanoparticle and Muller Hinton broth medium, devoid of inoculum) and a negative control (test tube containing inoculum and Muller Hinton broth medium, devoid of nanoparticle). The negative controls indicated the microbial growth profile in the absence of nanoparticle (Jafari, Ghane, Sarabi, et al., 2011; Kawashita et al., 2000; Yang et al., 2006). All the experiments were carried out in triplicate. The minimum bactericidal concentration (MBC), i.e., The lowest concentration of nanoparticles that kills 99.9% of the bacteria was also determined from the batch culture studies. For growth inhibitory concentration (MIC) the presence of viable microorganisms was tested and the lowest concentration causing bactericidal effect was reported as MBC as suggested by Avadi (Avadi et al., 2004). Two experiments for bactericidal effect, a loopful from each test tube (Especially, negative & positive test tubes) was inoculated on Muller Hinton agar and incubated at  $35^\circ\text{C}$  for 24 h. The nanoparticle concentration illustrating the bactericidal effect was picked out based on absence of colonies on the agar plate. The release of  $\text{Ag}^+$  and  $\text{Zn}^{2+}$  ions from the nanoparticles into DI water and Muller Hinton broth medium were deliberated by suspending 10 mg of nanoparticles in 100 ml DI water/medium and sonicating with ultrasonic set (PARSONIC 7500s, Pars Nahand ENGG. Co. IRAN) for 10 min. The suspension was kept in a rotary shaker (Gyrotwister 3-Dshaker, labnet Co. USA) under the same conditions as in the above

studies and residual  $\text{Ag}^+$  and  $\text{Zn}^{2+}$  concentration in the aqueous phase was defined by ICP-AES after 24 h.

### 2.10. Antibiotic Sensitivity Test

To determine the sensitivity of standard strain of *E.coli* to common antibiotics, Disc Diffusion AgarTest with Ceftriaxone ( $30\mu\text{g}$ ), Gentamicin ( $10\mu\text{g}$ ), Ciprofloxacin ( $5\mu\text{g}$ ) and Cefazolin ( $30\mu\text{g}$ ) antibiotic disks on Mueller Hinton agar medium was done. After 24 hours of incubation, the diameter of growth inhibition around each disc was measured and compared. The disc, which has the maximum diameter of the inhibitory zone; it is more effective to destroy bacteria.

## 3. Results

### 3.1. Bacteriological analysis

*Escherichia coli* isolated from poultry and poultry environment, was subjected to various morphological and biochemical tests used for identification. The distribution pattern and the biochemical tests for identification of *E. coli* isolated from poultry sources is summarized in Table 1.

### 3.2. The FT-IR spectra analysis

Fig. 1 shows FT-IR spectra of (a) Ag, (b) ZnO and (c) Ag/ZnO. The supplement of oxalic acid to the ethanol solution of Ag caution was caused to the precipitation of a gray solid of silver oxalate as shown by FT-IR spectrum in Fig. 1a. The broadband at  $3427.33 \text{ cm}^{-1}$  was allocated to both the  $\nu_s(\text{O-H})$  and  $\nu_{as}(\text{O-H})$  of hydration water. The extreme band at  $1634.68 \text{ cm}^{-1}$  was allocated to asymmetric and water tensional tremble  $\delta(\text{H-O-H})$ . The shoulder at  $1428.89 \text{ cm}^{-1}$  is present in the spectrum evidence of (N-O) tremble and the closely spaced bands at  $875.31 \text{ cm}^{-1}$  and  $577.35 \text{ cm}^{-1}$  are present in the spectrum evidence of (O-C-O) tensional tremble and (M-O) tremble, respectively. Fig. 1b depended to ZnO FT-IR spectrum. The broadband at  $3445.05 \text{ cm}^{-1}$  was allocated to both the  $\nu_s(\text{O-H})$  and  $\nu_{as}(\text{O-H})$  of hydration water. The extreme band at  $1629.57 \text{ cm}^{-1}$  was allocated to asymmetric and water tensional tremble  $\delta(\text{H-O-H})$ . The shoulder at  $1428.89 \text{ cm}^{-1}$  is present in the spectrum evidence of (N-O) tremble and the

closely spaced bands at  $876.14\text{ cm}^{-1}$  and  $551.12\text{ cm}^{-1}$  are present in the spectrum evidence of (O-C-O) tensional tremble and (Zn-O) tensional tremble respectively. Also, Fig. 1c conclude Ag/ZnO FT-IR spectrum. The broadband at  $3426.47\text{ cm}^{-1}$  was allocated to both the  $\nu_s(\text{O-H})$  and  $\nu_{as}(\text{O-H})$  of hydration water. The extreme band at  $1628.14\text{ cm}^{-1}$  was allocated to asymmetric and water tensional tremble  $\delta(\text{H-O-H})$ . The shoulder at  $1458.22\text{ cm}^{-1}$  is present in the spectrum evidence of (N-O) tremble and the closely spaced bands  $625.36\text{ cm}^{-1}$  are present in the spectrum evidence of (Ag/ZnO) tensional tremble respectively.

### 3.2. The XRD spectra analysis

The XRD pattern of Ag, ZnO and Ag/ZnO nanoparticles (Fig. 2 a, b and c) were compared and interpreted with standard data of International Centre for Diffraction Data (ICDD). The average crystallite sizes (C.S) of the nanocrystal was calculated using the Debye-Scherrer Equation from the major diffraction peaks ( $C.S = K \cdot \lambda / \beta \cdot \cos \theta$ ). Where  $\lambda$  is the wavelength of the incident X-ray,  $\beta$  is the full width at half maximum (FWHM) of the diffraction peak in radians and  $\theta$  is the Bragg angles of the main planes. The average crystallite size of the Ag, ZnO and Ag/ZnO were 8.66 nm, 24.75 nm and 12.15 nm, respectively.

### 3.3. The ICP-AES spectra analysis

By ICP-AES analysis, we succeed to estimation of residual ions, after digestion of nanoparticles by sonication. They indicated ions levels of 120 ppm of silver and  $\leq 1$  ppm of zinc oxide, in the silver/Zinc Oxide nanoparticles, respectively.

### 3.4. The TEM and SEM images analysis

TEM images of silver nanoparticles were taken (Fig. 3) and approved that the metal particles were in the nano range, approximately. However, SEM images (Fig. 4) of nanoparticles were shown that silver; zinc oxide and silver/zinc oxide metal particles were exactly in the shape of spherical and clustered.

### 3.5. Antibiotic Sensitivity Test

After 24 hours of incubation, the diameter of growth inhibition around each disc was measured. In fact, the disc, which has the

maximum diameter of the inhibitory zone; it is more effective to destroy bacteria. The results in depended of the antibacterial effects of currency antibiotic against *E.coli* via the disc diffusion test show that the *E. coli* had most sensitivity against of Ciprofloxacin ( $5\mu\text{g}$ ) with  $\geq 30$  mm inhibition zone.

### 3.6. The antibacterial activity of Ag/ZnO analysis

The antibacterial activity of silver, zinc Oxide and silver/zinc oxide nanoparticles were compared for *E.coli* using the diameter of inhibition zones in disk diffusion test and Agar dilution test. In fact, the diameter of inhibition zone (DIZ) reflects the dimension of impressionability of the bacteria. We knew, the strain susceptible to disinfectants demonstrates larger DIZ, while resistant strain exhibit smaller DIZ. The disks with silver and Zinc oxide nanoparticles were compared to the silver/zinc oxide nanoparticles for *E.coli* strain. The DIZ for zinc oxide and silver/zinc oxide nanoparticles impregnated disks were almost greater than that studied with the silver nanoparticles impregnated disks for *E.coli* strain. Correspondingly, for *E.coli*, the silver and silver/zinc oxide nanoparticles impregnated disks were found to be more effective compared to zinc oxide nanoparticles impregnated disks, however the difference in the DIZ was merely 10–15% percent. Since DIZ was measured on agar plates using a ruler with 1 mm resolution, the possibility of measurement errors exists. The results in depended of the antibacterial effects of nanoparticles against different of bacterial via the disc diffusion test, the agar dilution test the MIC and the MBC are summarized in Table 2. A greater lag phase and lower maximum absorbance (at 600 nm) were observed as the concentration of nanoparticles increased.

A Similar observation was reported by Sondi (Sondi & Salopek-Sondi, 2004). We investigate silver, Zinc oxide and silver/zinc oxide nanoparticles against *E.coli*. The bactericidal effect of nanoparticles is dependent on the concentration of nanoparticles and the initial bacterial concentration. In this study, the initial bacterial concentration was constant at  $1.5 \times 10^8$  CFU  $\text{ml}^{-1}$  regardless of nanoparticles concentration and microbial strain. The MIC observed for zinc oxide nanoparticle was  $64\mu\text{gml}^{-1}$  for *E.coli*. Surprised, antibacterial

effect of the silver nanoparticle was weak for *E.coli*. The MIC observed for silver nanoparticle was  $2048\mu\text{gml}^{-1}$ . In contrast with the nanoparticles that picked out for this study, the most antibacterial effect was seen to silver/zinc oxide nanoparticle. Interestingly, the *E.coli* was most sensitivity against of silver/zinc oxide nanoparticle. In fact, our research shows that silver/zinc oxide nanoparticle has got antibacterial effects against *E.coli*. The MBC observed in this study for silver/zinc oxide nanoparticle was  $128\mu\text{g.ml}^{-1}$ .

#### 4. Discussion

Studies of Ruparelia indicated that in aqua medium, no systematic change in the size of nanoparticles observed after 24 h(Ruparelia, Chatterjee, Duttagupta, & Mukherji, 2008). In the current study, after synthesis of nanoparticles of metal oxides Ag, ZnO and combined nanoparticles of Ag/ZnO, their antibacterial effects compared. Though, studies of several authors in recent years, confirmed the antibacterial effects of Ag nanoparticle (Marshall et al., 1990; Williams, Ehrman, & Holoman, 2006). In the current study, disc diffusion and agar dilution methods used for determining the antibacterial effects of nanoparticles. Ruparelia and Jafari, et al. performed extensive experiments in the determination of microbial sensitivity of various bacteria to silver and copper nanoparticles, using Disc Diffusion Method(Jafari, Ghane, & Arastoo, 2011; Ruparelia et al., 2008). Regarding that the diameter of inhibition zone (DIZ), reflects the sensitivity of the organism, strains of sensitive, show larger DIZ and the resistant strains show smaller DIZ. The Results of the disc diffusion with Ag nanoparticle, by Thirumurugan against strains of pathogens *E.coli*, *salmonella typhi*, *Bacillus subtilis*, *Staphylococcus aureus*, indicated higher sensitivity to silver nanoparticle which is in contrast with the results of our study (Thirumurugan et al., 2009). Cho studies the MIC of Ag nanoparticle against *Pseudomonas aeruginosa* in which its growth in a concentration of  $7.5\mu\text{gml}^{-1}$  completely inhibited (Cho et al., 2005). Following their research project, Cho reported the MIC rate of silver nanoparticle for *Staphylococcus aureus* as  $12.6\mu\text{g.ml}^{-1}$ , but the MIC for these bacteria affected

by silver nanoparticle reported  $2048\mu\text{gml}^{-1}$ . The *E.coli*, used in the current study, showed the least and the most sensitivity to silver and Ag/ZnO nanoparticle, respectively. Actually, the least degree of MIC in *E.coli* were related to combined nanoparticles of silver and zinc with concentration of  $128\mu\text{g.ml}^{-1}$ . i.e, notwithstanding those nanoparticles had the most growth inhibitory effect in *E.coli*. Lock concluded that the most the dimension of silver nanoparticle, the better would be the MIC and their antibacterial effect would decrease(Lok et al., 2006). The aim was to indicate that the sensitivity of different strains of one bacteria to silver nanoparticle shows meaningful difference. Kim studied the gram negative bacteria *E.coli* and gram positive *Staphylococcus aureus*, also reported that the antibacterial silver nanoparticle mostly affects the *E.coli*, which is due to the difference between cell wall of gram negative & positive microorganisms(Kim et al., 2007). Rate of MIC obtained by Ping Li (Li, Li, Wu, Wu, & Li, 2005) for silver nanoparticle and against *Staphylococcus aureus* were  $0.625\mu\text{gml}^{-1}$ . Limited studies performed on the antibacterial properties of ZnO. Reddy were amongst few authors worked on the toxicity of the ZnO nanoparticle in gram negative & positive bacteria (Reddy et al., 2007). They found nanoparticle is able to completely inhibit the growth of *E.coli*. Up to now, no complete and comprehensive study reported in the field of combining antibacterial nanoparticles and the comparison of their antibacterial properties for *E.coli*. Amongst few studies, Yang combined silver nanoparticle with Zn to improve the antibacterial activity of Zn nanoparticle and investigate the antibacterial effect of Zinc oxide and silver nanoparticles and also comparing them with Ag/ZnO nanoparticle (Yang et al., 2006). They obtained interesting results. According to the findings of Kawashita and Pak-soo silver significantly increased antimicrobial activity(Kawashita et al., 2000; Park & Jang, 2003). Actually, Yang believed that the photocatalytic ability of ZnO nanoparticle plus silver nanoparticle improves and also increases its oxidation and reduction abilities, while suppressing bacteria growth (Yang et al., 2006). However, silver ions, eventually, release during sterilization and kill bacteria due to their high antibacterial activation. They theorized that silver ion release following bacteria death and

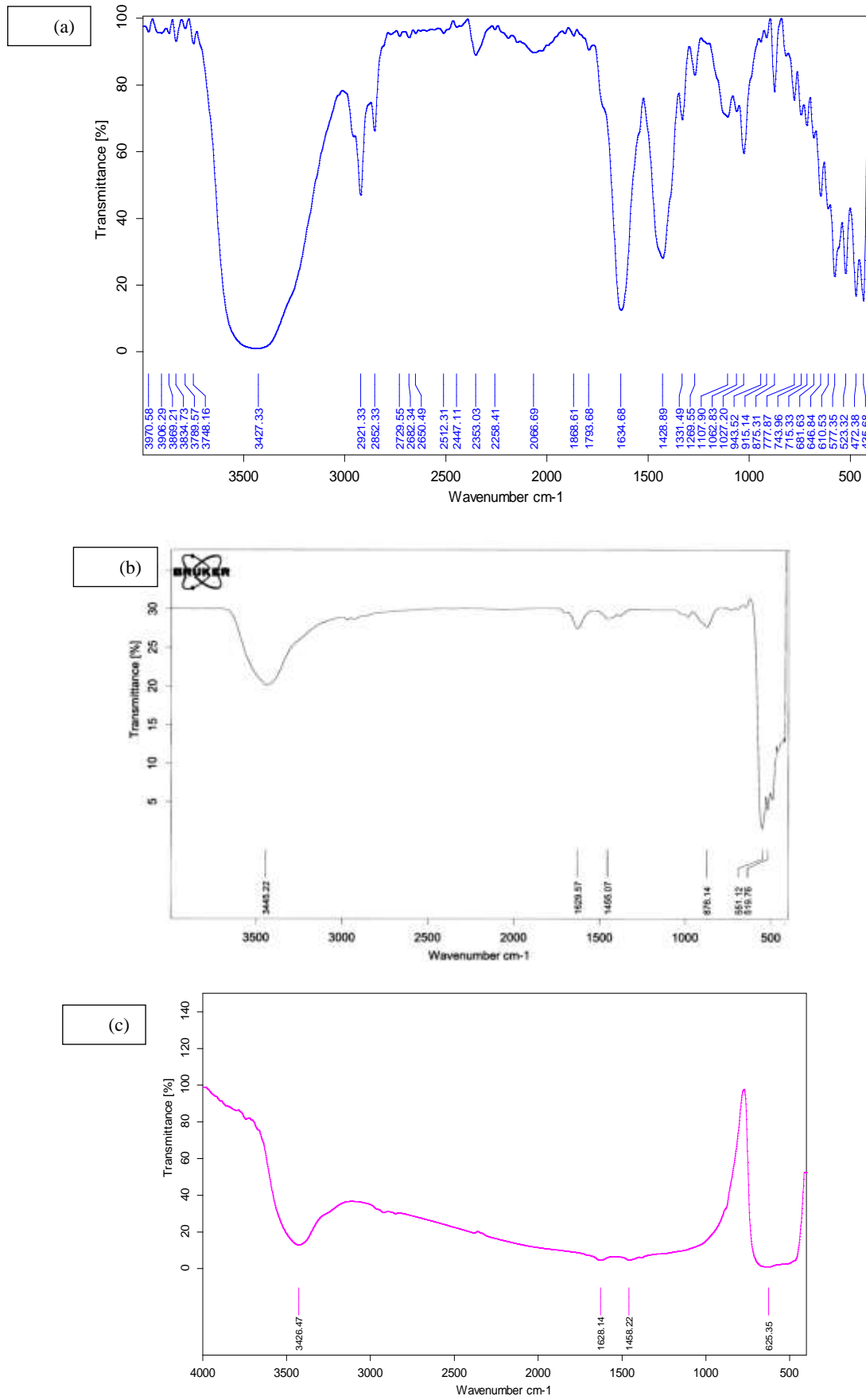


Figure 1. FT-IR pattern of (a) Ag, (b) ZnO and (c) Ag/ZnO nanoparticles

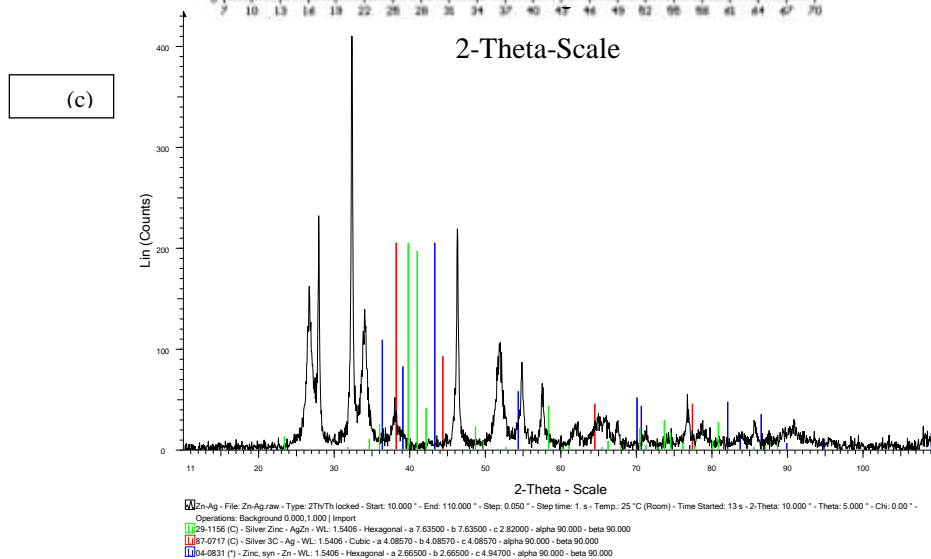
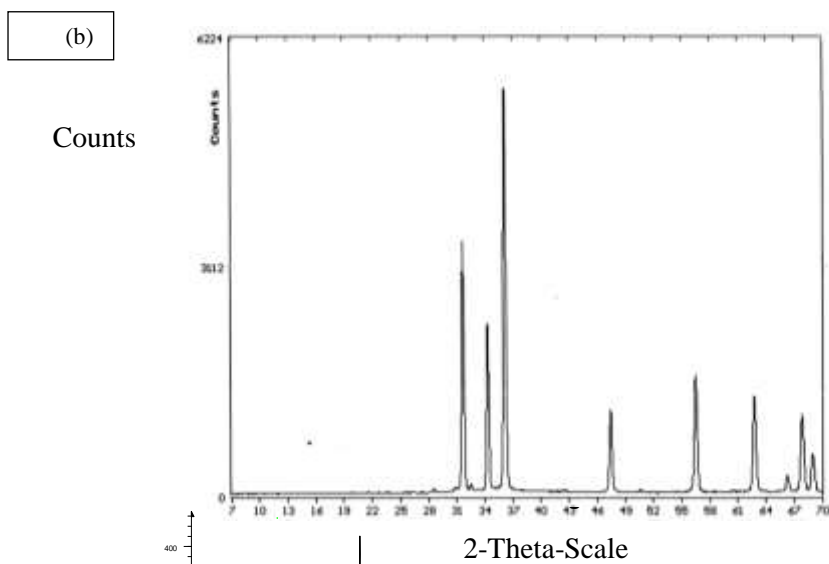
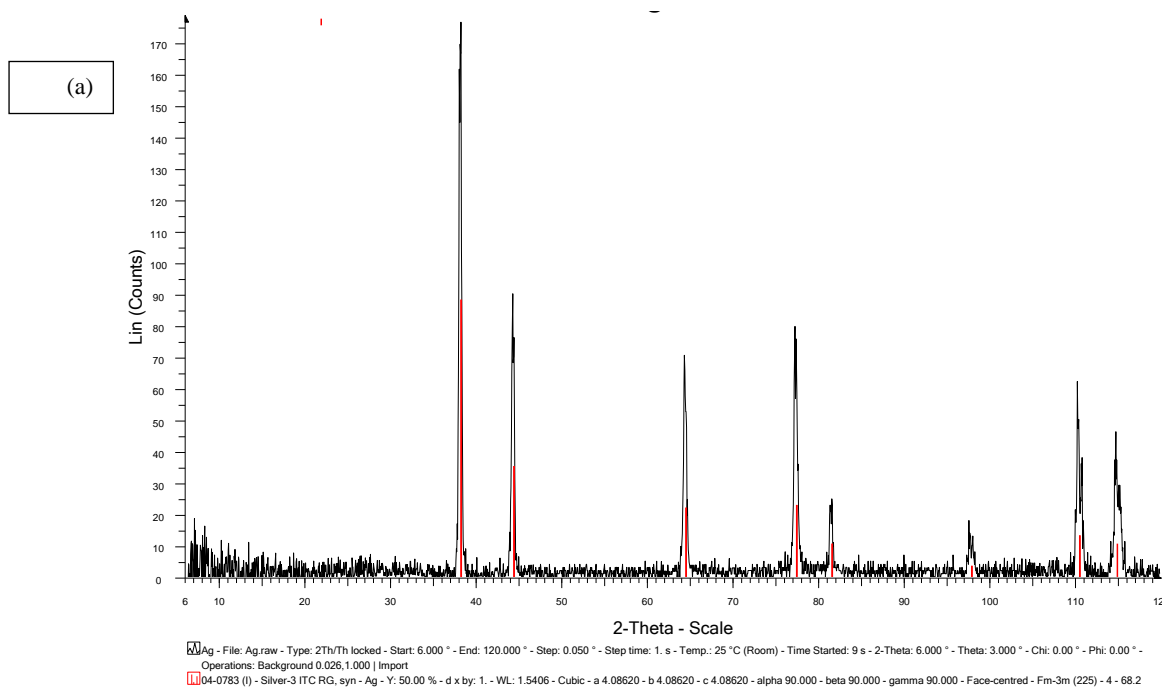
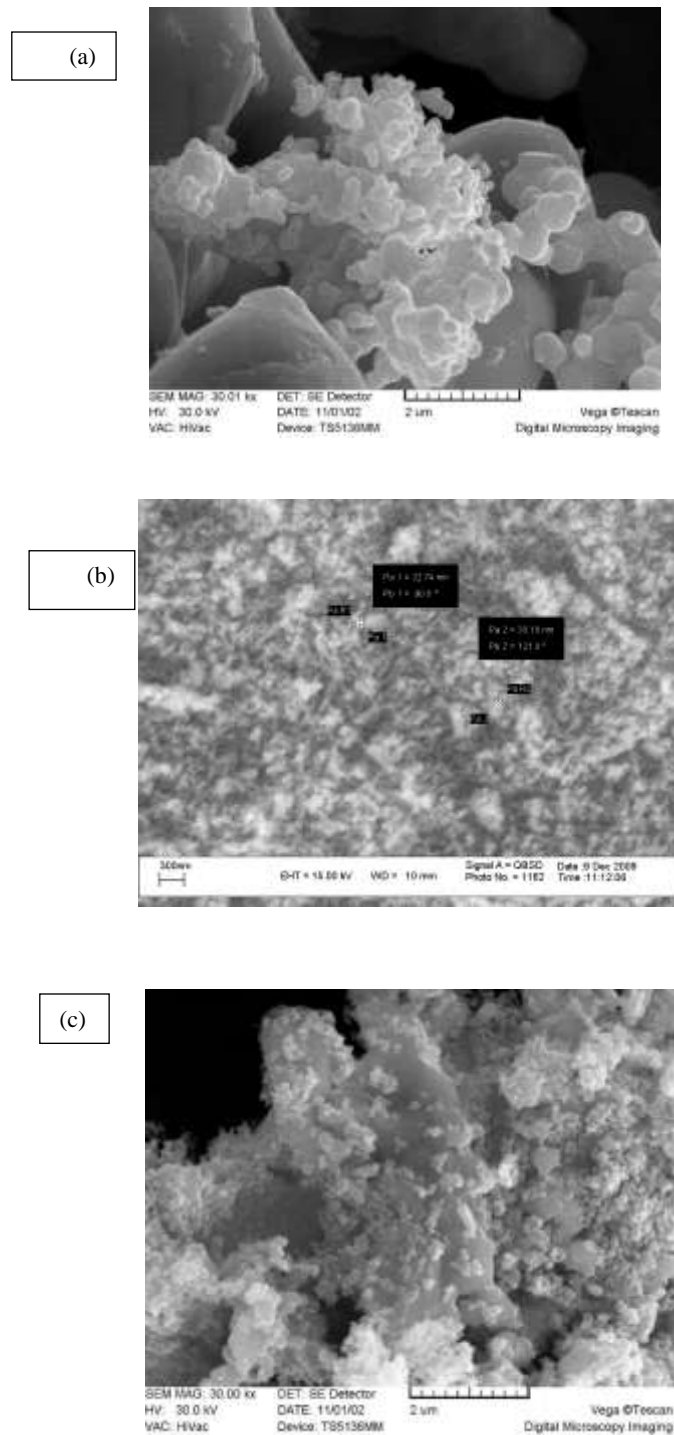


Figure 2. XRD pattern of (a) Ag (b) ZnO and (c) Ag/ZnO nanoparticles





**Figure 4.** SEM images of (a) Ag, (b) ZnO and (c) Ag/ZnO nanoparticles

colloid with other bacteria and repeat their sterilization behavior. It was also mentioned that silver covered on the surface of Zn nanoparticle has the ability to involve the electrons produced through photo-catalytic reactions of Zn nanoparticle which increases electron isolation and makes gaps in the cell membrane, so increase its antimicrobial activity. Regarding studies of these authors,

antibacterial property of silver and zinc oxide nanoparticles improves with their combination. In fact, our study confirmed that the gram negative strains of bacteria had most sensitive to silver/zinc oxide nanoparticle. Further, our study approved that the Ag/ZnO nanoparticle has a great antimicrobial agent against *E.coli* and just combination of zinc oxide and silver nanoparticles, give increase their bactericidal effect.

## Acknowledgements

We are indebted to the research Vice Chancellor of Islamic Azad University Ardebil Branch, Iran University of Medical Sciences, for supporting this research. Also, we gratefully acknowledge to University of Industrial Sahand, Tabriz, Department of Nanotechnology, for the XRD and SEM analysis. The authors would like to acknowledge to Iranian Mineral Processing Research Center (IMPRC) for ICP-AES analysis. We gratefully acknowledge the Faculty of veterinarian Science of Islamic Azad University, Tabriz Branch, Department of Pathobiology for preparation and isolation of bacteria and Executive Director of Iran-Nanotechnology Organization (Govt. of Iran). The anonymous reviewers are acknowledged for providing valuable comments and insights for improving the manuscript.

## Refereces

- Amara, A., Ziani, Z., & Bouzoubaa, K. 1995. Antibioresistance of *Escherichia coli* strains isolated in Morocco from chickens with colibacillosis. *Veterinary Microbiology*. 43(4); 325-330. doi: [http://dx.doi.org/10.1016/0378-1135\(94\)00101-2](http://dx.doi.org/10.1016/0378-1135(94)00101-2)
- Avadi, M., Sadeghi, A., Tahzibi, A., Bayati, K., Pouladzadeh, M., Zohuriaan-Mehr, M., & Rafiee-Tehrani, M. 2004. Diethylmethyl chitosan as an antimicrobial agent: Synthesis, characterization and antibacterial effects. *European Polymer Journal*, 40(7); 1355-1361.
- Blanc, D., Carrara, P., Zanetti, G., & Francioli, P. 2005. Water disinfection with ozone, copper and silver ions, and temperature increase to control *Legionella*: seven years of experience in a university teaching hospital. *Journal of Hospital Infection*, 60(1); 69-72.
- Case, C. L., & Johnson, T. R. 1984. *Laboratory experiments in microbiology*: Benjamin /Cummings Publishing Company.
- Cho, K.H., Park, J.E., Osaka, T., & Park, S.G. 2005. The study of antimicrobial activity and preservative effects of nanosilver ingredient. *Electrochimica Acta*. 51(5); 956-960 .
- Daini, O., Ogbolu, O., & Ogunledun, A. 2004. Quinolones Resistance and R-plasmids of some gram negative enteric Bacilli. *African Journal of clinical and experimental microbiology*. 6(1); 14-20 .
- Gross, W. 1994. Diseases due to *Escherichia coli* in poultry .
- Gupta, K., Hooton, T.M., & Stamm, W. E. 2001. Increasing antimicrobial resistance and the management of uncomplicated community-acquired urinary tract infections. *Annals of internal medicine*. 135(1); 41-50.
- Jafari, A., Ghane, M., & Arastoo, S. 2011. Synergistic antibacterial effects of nano zinc oxide combined with silver nanocrystales. *African Journal of Microbiology Research*. 5(30); 5465-5473 .
- Jafari, A., Ghane, M., Sarabi, M., & Siyavoshifar, F. 2011. Synthesis and antibacterial properties of zinc oxide combined with copper oxide nanocrystals. *Oriental Journal of Chemistry*. 27(3); 811-820.
- Kawashita, M., Tsuneyama, S., Miyaji, F., Kokubo, T., Kozuka, H., & Yamamoto, K. 2000. Antibacterial silver-containing silica glass prepared by sol-gel method. *Biomaterials*. 21(4); 393-398 .
- Kim, J.S., Kuk, E., Yu, K.N., Kim, J.H., Park, S. J., Lee, H.J., Hwang, C.Y. 2007. Antimicrobial effects of silver nanoparticles. *Nanomedicine: Nanotechnology, Biology and Medicine*. 3(1); 95-101 .
- Li, P., Li, J., Wu, C., Wu, Q., & Li, J. 2005. Synergistic antibacterial effects of  $\beta$ -lactam antibiotic combined with silver nanoparticles. *Nanotechnology*. 16(9); 1912 .
- Lin, Y.S. E., Vidic, R.D., Stout, J.E., & Victor, L. Y. 1996. Individual and combined effects of copper and silver ions on inactivation of *Legionella pneumophila*. *Water Research*. 30(8); 1905-1913 .
- Lok, C.N., Ho, C.M., Chen, R., He, Q.Y., Yu, W.Y., Sun, H., Che, C.M. 2006. Proteomic analysis of the mode of antibacterial action of silver nanoparticles. *Journal of Proteome research*. 5(4); 916-924 .
- Marshall, B., Petrowski, D., & Levy, S. B. 1990. Inter-and intraspecies spread of *Escherichia coli* in a farm environment in the absence of antibiotic usage. *Proceedings of the National Academy of Sciences*. 87(17); 6609-6613.
- Morones, J.R., Elechiguerra, J.L., Camacho, A., Holt, K., Kouri, J.B., Ramírez, J.T., & Yacaman, M. J. 2005. The bactericidal effect of silver nanoparticles. *Nanotechnology*. 16(10); 2346 .
- Österblad, M., Hakanen, A., Manninen, R., Leistevuo, T., Peltonen, R., Meurman, O., Kotilainen, P. 2000. A between-species comparison of antimicrobial resistance in enterobacteria in fecal flora. *Antimicrobial agents and chemotherapy*. 44(6); 1479-1484 .
- Park, S.J., & Jang, Y.S. 2003. Preparation and characterization of activated carbon fibers supported with silver metal for antibacterial behavior. *Journal of colloid and interface science*. 261(2); 238-432 .

- Reddy, K.M., Feris, K., Bell, J., Wingett, D.G., Hanley, C., & Punnoose, A. 2007. Selective toxicity of zinc oxide nanoparticles to prokaryotic and eukaryotic systems. *Applied physics letters*. 90(21); 213902 .
- Ruparelia, J.P., Chatterjee, A.K., Duttagupta, S. P., & Mukherji, S. 2008. Strain specificity in antimicrobial activity of silver and copper nanoparticles. *Acta Biomaterialia*. 4(3); 707-716.
- Sondi, I., & Salopek-Sondi, B. 2004. Silver nanoparticles as antimicrobial agent: a case study on E. coli as a model for Gram-negative bacteria. *Journal of colloid and interface science*. 275(1); 177-182 .
- Thirumurugan, G., Shaheedha, S., & Dhanaraju, M. 2009. In vitro evaluation of antibacterial activity of silver nanoparticles synthesised by using *Phytophthora infestans*. *Int J Chem Tech Res*. 1; 714-716 .
- Williams, D. N., Ehrman, S. H., & Holoman, T. R. P. 2006. Evaluation of the microbial growth response to inorganic nanoparticles. *Journal of nanobiotechnology*. 4(1); 3-9.
- Yang, L., Mao, J., Zhang, X., Xue, T., Hou, T., Wang, L., & Tu, M. 2006. Preparation and characteristics of Ag/nano-ZnO composite antimicrobial agent. *Nanoscience*. 11(1); 44-48.
- Yu-sen, E.L., Vidic, R.D., Stout, J.E., McCartney, C.A., & Victor, L.Y. 1998. Inactivation of *Mycobacterium avium* by copper and silver ions. *Water Research*. 32(7); 1997-2000.