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Silver and zinc oxide nanoparticles as potential weapons for enhancement of antibiotics activity against multi drug resistant microorganisms

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ABSTRACT

Some microorganisms became less sensitive to several ordinary used antibiotics, causing the outbreak of infectious diseases. Therefore, the present study is carried out to investigate the antibacterial properties of silver and zinc oxide nanoparticles. Their physicochemical properties as well as the combined effects of antibiotics previously impregnated with the metal and metal oxide nanoparticles. Silver nanoparticles (AgNPs) were obtained using the chemical reduction method, while zinc oxide nanoparticles (ZnONPs) were synthesized using the precipitation method followed by calcination process. The formed nanoparticles (Nps) were characterized using UV-Visible spectroscopy, Fourier Transforms Infrared Spectroscopy (FT-IR), X-ray diffraction analysis (XRD) and Transmission Electron Microscopy (TEM). The antibacterial effect of AgNPs and ZnONPs was tested against four different genera: *Staphylococcus aureus* (Gram positive), *Shigella boydii* (Gram negative), *Klebsiella pneumoniae* (Gram negative) and *Escherichia coli* (Gram negative) and showed strong effect against all. Also, the synthesized nanoparticles were evaluated for their role in increasing the antibacterial activity of ten tested antibiotics which cover most antibiotics groups according to their mechanism of action. Enhancement in the antibiotic activities was recognized via increasing inhibition zone diameters (mm).

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1. Introduction

Since the discovery, the use of antibiotics is most probable choice of physicians for treating most of the infectious diseases, as they were considered the wonder discoveries of the 20th century [1]. They have been the effective weapon for the last 60 years. Over time, about 70 % of the bacteria that cause infections in hospitals have become resistant to at least one of the antibiotic drugs most commonly used for their treatment [2,3]. The biggest problem is that some organisms are resistant to all approved antibiotics and can only be treated with experimental and potentially toxic drugs [2, 4]. The process of producing a new antibiotic, however, is long and expensive, requiring approximately ten years and $300 million to bring a new antibiotic to market which make the problem more complicated [5]. So scientists are now searching for effective and unconventional antimicrobial agents that can be used as enhancers for the present antibiotics for solving the problem [6]. New and promising enhancers that have a strong antimicrobial effect are nanoparticles [7-10]. Nanoparticles (NPs) are a form of nanomaterials that are very small particles with size ranging from 1nm to100 nm so having a larger surface area to volume ratio and unique chemical and physical properties [11,12]. Also, nanoparticles have a lower propensity to stimulate microbial resistance than many other antimicrobial agents [13,14]. The mechanisms through which antibiotics prevent microbial growth are quite different from that of nanoparticles [15-17]. Therefore, nanoparticles have the potential to serve as an enhancer or even as an alternative to antibiotics. Currently; the metallic nanoparticles are thoroughly being explored and extensively investigated as potential antimicrobials [18-20]. Due to these properties, silver and ZnO nanoparticles are being used widely in the antimicrobial field. Many investigations have been carried out for indicating their antimicrobial effect, but their combined effect with antibiotics has not been known yet.

The objective of this study was to prepare both silver and zinc oxide nanoparticles by simple and fast methods using the standard laboratory equipment without the requirement of highly expensive and specialized instruments as well as to investigate the antibacterial effect of these prepared NPs alone and in combination with some selected antibiotics.

2. Experimental

2.1. Materials

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All chemicals were of the analytical grade and used as received. Silver nitrate ($AgNO_3$), $Zn(CH_3COO)_2$,Starch and sodium hydroxide NaOH were obtained from Sigma(Sigma, St. Louis, MO, USA). Solutions were prepared in double-distilled deionized water just before use. All determinations were performed at room temperature and at pH = 7.0. The different antibiotics discs used during this investigation were procured from Oxoid Ltd, Basingstoke, Hampshire, England.

2.2. Preparation of silver nanoparticles (AgNPs)

The most common method used for the synthesis of silver nanoparticles is chemical reduction method [21-24]. In this study we obtained uniform silver nanoparticles by reduction of silver nitrate (5 mM) at 90 °C under atmospheric pressure with 1% starch solution which was used also as a stabilizer, the mixture was stirred at room temperature for 30 min. The transparent colorless solution was converted to pale yellow and then to reddish brown solution after continuous stirring at 90 °C for 60 minutes indicating the formation of silver nanoparticles [25]. The silver nanoparticles thus prepared in starch were stable for two months without any change in the surface plasmon resonance as indicated from the absorption spectra at room temperatures, showing that the starch was a good reducing and stabilizing agent for the silver nanoparticles.

2*.3. Preparation of ZnO nanoparticles (ZnONPs)*

Synthesis of ZnO nanopowder was achieved by the precipitation method followed by calcination process, that by adding slowly (200 mM) sodium hydroxide (NaOH) solution to (100 mM) zinc acetate $Zn(CH_3COO)_2$ solution while stirring. The resulting precipitate of $Zn(OH)_2$ was washed with deionized water several times, and then underwent calcination treatment to obtain ZnO nanopowder. The washing process was repeated several times until the pH of the solution was 7 that which was detected by using pH- meter. Finally the product was dried in the oven at 150 °C for 8 hours to allow complete dehydration. Zinc Oxide formed by the dehydration was suspended in distilled water to obtain suspension of Zinc oxide nanoparticles.

2.4. Instrumentation

The resulting nanoparticles were characterized by Ultraviolet-visible Spectroscopy (UV-Vis) which was performed at room temperature with samples in a quartz cuvette using a spectrophotometer (SHIMADZU UV-1650PC, Columbia, MD, USA). The studies of size, morphology and composition of the nanoparticles were performed by means of transmission electron microscopy (TEM; JEOL 1210, JEOL Ltd., Tokyo, Japan). Electron microscope was operated at an accelerating voltage of 90 KV. The samples were prepared by drop-coating the NPs solutions onto the carbon-coated copper grid and were loaded onto a specimen holder. X-ray diffraction was done using equipment Siemens D-5000 with CuKα radiation operating at 40 kV and 40 mA. Fourier transform Infrared (FT-IR) specter were recorded at room temperature on a Bruker Tensor 27 FT-IR spectrometer.

2.4.1. UV-Visible spectroscopy

The formation of silver and zinc oxide nanoparticles was preliminarily confirmed by visual observation of color change from pale yellow color to deep reddish brown in case of AgNPs and from clear colorless to turbid white in case of ZnONPs. The produced nanoparticles were subjected to characterization by UV-Vis. spectra at different time intervals. Sharp peak given by UV-Vis. spectra at the 420 and 370 nm for silver and zinc oxide nanoparticles respectively confirms the nanoparticles formation.

2.4.2. Transmission electron microscopy (TEM)

Characterization of the synthesized nanoparticles was carried out by TEM to determine the size and shape of nanoparticles. For the TEM measurements; a drop of a solution containing the particles was deposited on a copper grid covered with amorphous carbon. After allowing the film to stand for 2 minutes, the extra solution was removed by means of blotting paper and the grid was allowed to dry before the measurement. From the TEM micrographs sizes and shape of silver and zinc oxide NPs were detected. Histograms of size distribution were calculated from the TEM images by measuring the diameters of at least 50 particles.

2.4.3. X-ray diffraction analysis (XRD)

The synthesized ZnO nanoparticles were characterized by x-ray diffraction using equipment Siemens D-5000 with CuK_α radiation operating at 40 kV and 40 mA to determine formed phases, lattice parameters, and relative crystallinity. The observed diffraction peaks in all the recorded XRD patterns are in agreement with those of the JCPDS card 89-7102 for hexagonal ZnO with wurtzite structure. No peaks of any other phase were detected.

2.4.4. Fourier transforms infrared spectroscopy (FT-IR)

For FT-IR measurements of synthesized nanoparticles, an appropriate amount of the formed nanoparticles were mixed with KBr salt to obtain FT-IR spectrum. Silver and zinc oxide nanoparticles were subjected to FT-IR analysis in the range of 4000 to 400 cm^{-1}

2.5. Preparation of inocula

Isolates of *Staphylococcus aureus* (NCMB 6571), *Shigilla boydii* (ATCC9207), *Klebsiella pneumoniae* (clinical culture) and *Escherichia coli* were provided by bacteriology laboratory, faculty of science, Suez Canal University. Firstly, a single colony of the tested organisms were grown overnight in a nutrient broth (NB) medium at 30 °C. The inocula were prepared by diluting the overnight cultures with 0.9% NaCl and detected by UV spectrophotometer at 600 nm to obtain a 0.5 McFarland standard. A 200 µL of these bacterial suspensions was added to the sterile nutrient agar on plates and spread by a glass spreader.

2.5.1. Antibacterial activity of the synthesized AgNPs and ZnONPs

The antibacterial activity of synthesized silver and zinc oxide nanoparticles were evaluated by using the disc-diffusion method. Different volumes (10, 20 and 30 µL) of freshly prepared AgNPs and ZnONPs were injected in sterile filter paper discs using sterile micropipette and placed in the bacterial lawn. The combined effect of AgNPs and ZnO nanoparticles with antibiotics was tested along with 10 various antibiotics covering most of antibiotics groups according to mechanism of action. Each standard antibiotic disc was impregnated with three different volumes (10, 20 and 30 µL) of the two metallic nanoparticles and placed in the bacterial lawn. After incubation at 30 °C for 24 hours, the zone of inhibition was measured, which appear as a clear area around the discs. The assays were performed in triplicate.

3. Results and discussion

Silver nanoparticles were synthesized by chemical reduction method with starch at 90 °C as mentioned above.

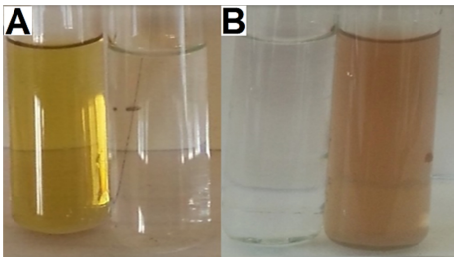

Figure 1. Silver nanoparticles obtained (A) after 30 min and (B) after 60 min.

$$2AgNO_3\ (aq) + (C6H_{10}O_5)n\ (aq) + H_2O\ (l) \rightarrow 2Ag\ (s) + 2HNO_3\ (aq) + C_6H_{12}O_7\ (aq) \quad (1)$$

The change of the colorless solution to yellow, yellowish-brown and finally to reddish-brown in the reaction vessels indicated the formation of AgNPs (Figure 1). The synthesis process can be summarized in the redox reaction (1).

In this reaction, starch $(C_6H_{10}O_5)n$ which is a polymer of glycose reduces the silver cations from the silver nitrate. As the silver metal forms, starch coats the outsides of the particles, preventing them from aggregating and forming larger particles. Synthesis of ZnO nanoparticles was achieved by the precipitation method as the reaction between sodium hydroxide and zinc acetate leads to the formation of zinc hydroxide $Zn(OH)_2$. Calcination process (150 °C) was followed to convert the formed $Zn(OH)_2$ to ZnO nanoparticles (Figure 2) according to the following reaction:

$$Zn(CH_3COO)_2 + 2NaOH \rightarrow Zn(OH)_2 + 2CH_3COONa \quad (2)$$

$$Zn(OH)_2 \rightarrow ZnO + H_2O \quad (3)$$

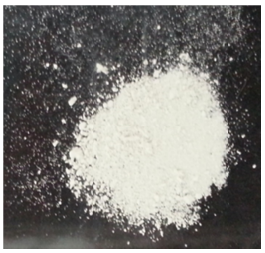

Figure 2. ZnO nanoparticles obtained after calcination.

3.1. Characterization studies of synthesized nanoparticles

3.1.1. UV-Visible spectroscopy

Synthesis of AgNPs exhibits strong absorption in the visible range due to the surface Plasmon resonance (SPR) [26]. UV-Vis spectra of the synthesized samples showed a maximum absorbance peaks at 420 nm for AgNPs (Figure 3A) and at 370 nm for ZnONPs (Figure 3B). Observation of this strong but broad surface Plasmon peak has been well documented for various AgNPs, with sizes ranging all the way from 2 to 100 nm [26-28]. On the other hand ZnONPs showed single narrow peak indicating the synthesis of spherical nanoparticles. There is a relationship between the UV-Vis absorbance spectrum, size and shape of nanoparticles [29]. With the increase in the particle size, the optical absorbance spectra of metal nanoparticles that are dominated by surface Plasmon resonance shift towards longer wavelengths (red shift) [29]. Small blue shift or red shift in the wavelength of the absorbance peak could be related to obtaining NPs in different shapes and sizes [29].

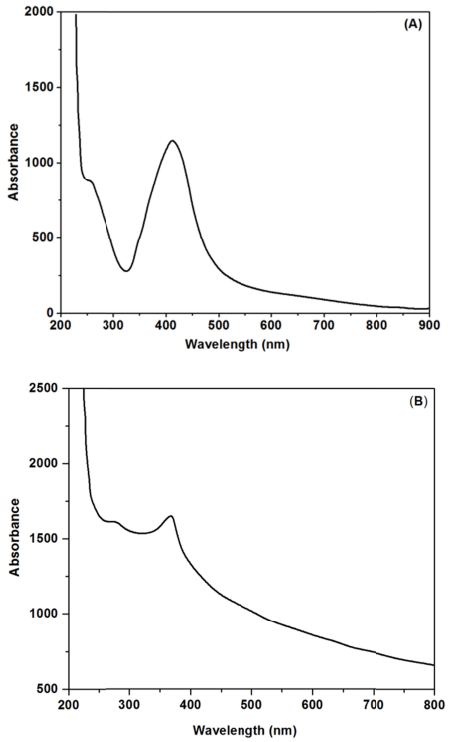

Figure 3. UV-Vis spectra of synthesized (A) AgNPs and (B) ZnONPs.

3.1.2. Transmission electron microscopy (TEM)

The TEM measurements were carried out to determine the morphology and shape of the formed nanoparticles. Transmission electron microscope micrograph of silver nanoparticles (Figure 4A) revealed that they were spherical and well dispersed without agglomeration, their polycrystalline nature was confirmed by the selective area electron dispersion (SAED) pattern (Figure 4B). Figure 4C shows representative nanoparticles size histograms of silver nanoparticles which contained 25 particle unit/mL in sizes ranging between 10 to 30 nm. Most of particles were between 15-20 nm in size, and possess an average size of 17 nm. Various reports have provided evidence of synthesis of AgNPs by TEM images that also revealed spherical and polydispersive AgNPs ranging from 10 to 40 nm [26].

Transmission electron microscope micrograph of synthesized zinc oxide nanoparticles denoted by (Figure 5A) revealed that they were spherical with little agglomeration. Most of particles were between 10-15 nm in size and possess an average size of 12 nm (Figure 5B).

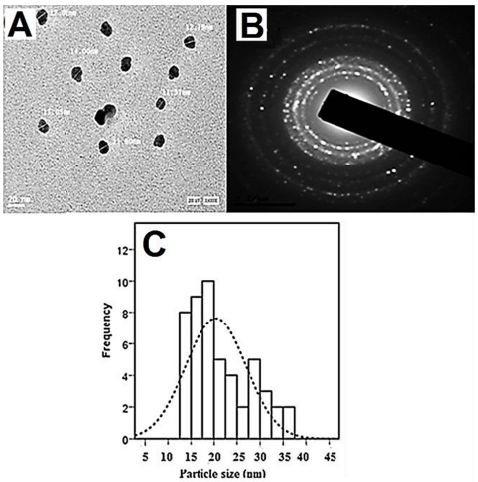

Figure 4. (A) Transmission electron microscope image shows spherical shape of AgNPs. (B) Selective area electron diffraction (SAED) confirms the polycrystalline nature of AgNPs (C) Histogram shows the sizes of the synthesized AgNPs.

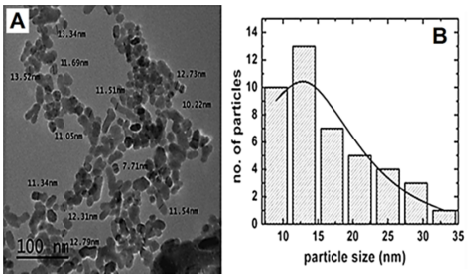

Figure 5. (A) Transmission electron microscope image shows shape of ZnONPs. (B) Histogram shows the sizes of ZnONPs.

3.1.3. X-Ray diffraction studies

XRD pattern of the prepared Zinc oxide nanoparticles showed strong and narrow diffraction peaks of ZnO at 2θ = 31.72, 34.38, 36.26, 47.54 and 56.58 ° are associated with (100), (002), (101), (102) and (110) (as shown in Figure 6). The strong and narrow diffraction peaks indicate high purity and good crystallinity of the grown nanostructures. All the reflections can be assigned to the standard powder pattern for the pure hexagonal phase of ZnO with lattice constants a = 3.2516 Å, c = 5.2000 Å. The hkl values are agreed well with the standard card of ZnO powder sample [30].

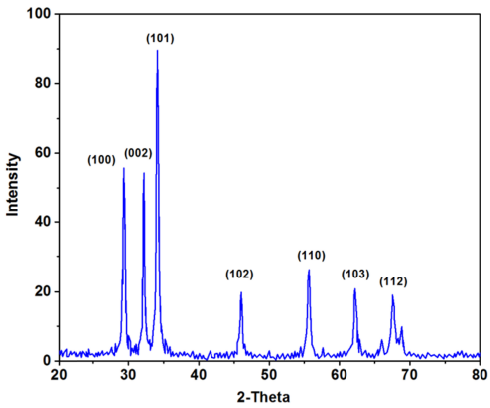

Figure 6. X-Ray diffraction patterns of synthesized ZnONps.

3.1.4. Fourier transforms infrared spectroscopy (FT-IR)

Various vibrational frequencies, in the range of 4000-400 cm^{-1} of AgNPs and ZnONPs were shown in (Figure 7A and B). FT-IR spectrum revealed the presence of bands in case of AgNPs at 3455, 2256, 1638 and 542 cm^{-1} (Figure 7A), while ZnONPs spectrum showed bands at 3420, 1632, 1380, 1116, 1035 and 650 cm^{-1} (Figure 7B).

The well-defined peak, in Figure 7A, observed at 3455 cm^{-1} could be due to (i) stretching vibration (symmetric and asymmetric) of aliphatic hydroxyl (OH) group and (ii) the stretching vibrational band of H_2O molecules. Band observed at 2256 cm^{-1} is assigned for aliphatic (C–H) stretching , while the peak observed at 1638 cm^{-1} could be assigned as a bending band of H_2O and a stretching vibrational band of carbonyl group (C=O) resulting from the residue of the starch molecule.

FT-IR spectrum of the synthesized ZnO nanoparticles showed in Figure 7B exhibited bands at 3420 cm^{-1} which corresponds to stretching vibration (symmetric and asymmetric) of aliphatic hydroxyl (OH) group as well as asymmetric and symmetric stretching H-O-H vibration while the bending H-O-H vibration band is observed at 1632 cm^{-1}. Peak observed at 1632 cm^{-1} may also be corresponds to stretching vibrational band of carbonyl group (C=O). Bands observed at 1035, 1116 and 1380 cm^{-1} are due to the C-O stretching vibration. The band at 650 indicates the stretching vibrations of ZnO nanoparticle.

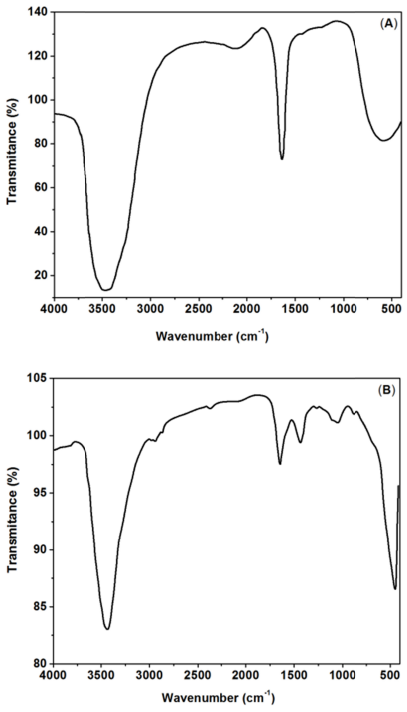

Figure 7. FT-IR spectra of (A) AgNPs and (B) ZnONPs.

3.2. Antibiotic susceptibility pattern

Both silver metal and zinc oxide nanoparticles are known to have antimicrobial role since many years. In this study the antibacterial effect of AgNPs was investigated against four different genera *Staphylococcus aureus* (NCMB 6571) (Gram positive), *Shigella boydii* (ATCC9207) (Gram negative), *Klebsiella pneumoniae* (clinical culture) (Gram negative) and *Escherichia coli* (Gram negative) while ZnONPs was tested only against *Staphylococcus aureus* (NCMB 6571) and *Klebsiella pneumonia* (clinical culture).

Table 1. The antibacterial effect of AgNPs against *S. aureus* and their combined affect with ten various antibiotics.

Antibiotic	Inhibition zone diameter (mm)			
	Different concentrations of AgNps			
	0 µL	+10 µL	+20 µL	+30 µL
No antibiotic	-	11	14	16
Norfloxacin 10	34	38	40	42
Ciprofloxacin 5	35	37	38	39
PenicillinG 10	27	31	34	37
Cefotoxime 30	30	32	34	35
Erythromycin 15	20	22	23	24
Tetracyclin 30	11	14	16	21
Gentamycin 10	20	23	25	25
Nalidixic acid 30	24	26	27	30
Ampicillin 10	20	22	23	25
Amoxycillin 10	9	10	12	14

Table 2. The antibacterial effect of AgNPs against *Shigella boydii* and their combined affect with ten various antibiotics.

Antibiotic	Inhibition zone diameter (mm)			
	Different concentrations of AgNps			
	0 µL	+10 µL	+20 µL	+30 µL
No antibiotic	-	14	16	17
Norfloxacin 10	23	25	27	31
Ciprofloxacin 5	24	26	28	30
PenicillinG 10	16	17	18	22
Cefotoxime 30	12	13	14	15
Erythromycin 15	15	17	19	20
Tetracyclin 30	26	30	33	36
Gentamycin 10	14	14	15	16
Nalidixic acid 30	23	25	26	27
Ampicillin 10	10	12	13	14
Amoxycillin 10	10	11	12	14

Table 3. The antibacterial effect of AgNPs against *K. pneumoniae* and their combined affect with ten various antibiotics.

Antibiotic	Inhibition zone diameter (mm)			
	Different concentrations of AgNps			
	0 µL	+10 µL	+20 µL	+30 µL
No antibiotic	-	9	11	12
Norfloxacin 10	00	12	14	15
Ciprofloxacin 5	14	16	18	20
PenicillinG 10	00	11	13	14
Cefotoxime 30	26	28	30	31
Erythromycin 15	00	13	14	16
Tetracyclin 30	15	18	20	22
Gentamycin 10	15	17	17	19
Nalidixic acid 30	21	23	25	26
Ampicillin 10	00	9	11	12
Amoxycillin 10	00	9	11	12

Table 4. The antibacterial effect of AgNPs against *E. coli* and their combined affect with ten various antibiotics.

Antibiotic	Inhibition zone diameter(mm)			
	Different concentrations of AgNps			
	0 µL	+10 µL	+20 µL	+30 µL
No antibiotic	-	9	11	14
Norfloxacin 10	21	24	26	29
Ciprofloxacin 5	29	30	31	32
PenicillinG 10	00	9	11	14
Cefotoxime 30	19	20	21	21
Erythromycin 15	00	11	14	16
Tetracyclin 30	11	12	12	13
Gentamycin 10	12	14	15	16
Nalidixic acid 30	15	19	21	23
Ampicillin 10	00	9	11	14
Amoxycillin 10	10	11	12	14

The antibacterial effect was observed by measuring the inhibition zone diameters around the discs impregnated with different volumes. The combined effect of AgNPs and ZnONPs with 10 various antibiotics using disc-diffusion method was also examined. The enhanced effect was also observed with the increase in the inhibition zone diameters around the antibiotics discs that previously impregnated with nanoparticles.

The antibiotic susceptibility test showed that the application of silver and ZnO nanoparticles increase the antibacterial effect of all antibiotics used against both Gram (+ve) and Gram (-ve) bacteria up to varying extents. This showed that the method is applicable for both kinds of bacteria. Their enhancement in the combined effect was preferably due to the difference in the mechanism of inhibition followed by nanoparticles and antibiotics.

3.2.1. Silver nanoparticles antibacterial and combined effect results

Tables 1-4 and Figures 8 and 9 represent the antibacterial effect of AgNPs which was observed by using disc-diffusion method and measuring the inhibition zone diameters around the discs impregnated with different volumes and also their combined affect with ten various antibiotics.

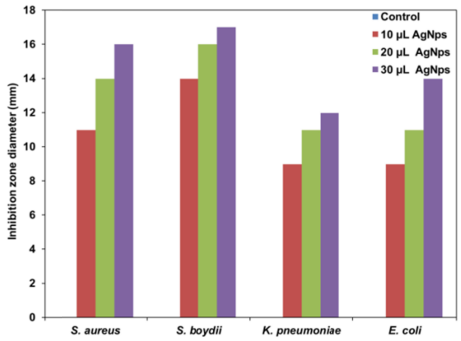

Figure 8. Effect of different AgNPs concentrations against the four isolates.

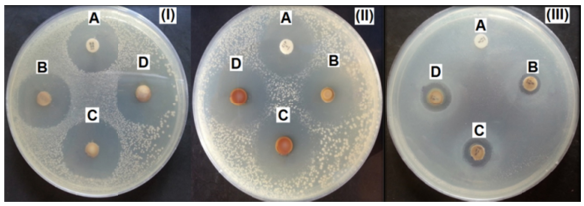

Figure 9. (I) Antimicrobial activity of AgNPs against *S. aureus* (A) Nalidixic acid, (B) Nalidixic acid +10 µL AgNPs, (C) Nalidixic acid + 20 µL AgNPs and (D) Nalidixic acid + 30 µL AgNPs. **(II)** Antimicrobial activity of AgNPs against *S. boydii* (A) Ciprofloxacin, (B) Ciprofloxacin + 10 µL AgNPs, (C) Ciprofloxacin + 20 µL AgNPs and (D) Ciprofloxacin + 30 µL AgNPs. **(III)** Antimicrobial activity of AgNPs against *E.coli* (A) Erythromycin; (B) Erythromycin + 10 µL AgNPs, (C) Erythromycin + 20 µL AgNPs and (D) Erythromycin + 30 µL AgNPs.

3.2.2. Zinc oxide nanoparticles antibacterial and combined effect results

Tables 5 and 6, and Figures 10 and 11 represent the antibacterial effect of ZnONPs which was observed by using disc-diffusion method and measuring the inhibition zone diameters around the disc impregnated with different volumes and also their combined affect with ten various antibiotics.

According to the results of this study antimicrobial activity of silver nanoparticles differs according to the volumes used and the tested isolates. Increasing the AgNPs volumes (10, 20 and 30 µL) increases the antimicrobial activity gradually. The highest antimicrobial activity of AgNPs was observed against *S. boydii* followed by *S. aureus* and its effect on *K. pneumoniae* and *E. coli* is nearly equal. In very small concentrations, silver is harmless to human cells but it is biocidal to microbial cells [31]. The exact mechanism which silver nanoparticles employ to cause antimicrobial effect is not clearly known and still under discussion, however there are many theories for their action against microbes [32,33]. Silver nanoparticles is known to act on the surface area of the microorganisms [19,34], where silver nanoparticles react with sulfur-containing proteins in cell membrane, as they are known to have high affinity to sulfur compounds, affecting cell membrane viability. According to electron spin resonance spectroscopy studies, silver nanoparticles release free radicals specially Ag^{+} ions [35,36]. These ions have appositive charge so interact with the negatively charged microbial cell membrane, making it porous and subsequently disrupt its main functions; permeability and respiration, then react with DNA moieties and thiol groups of many enzymes inactivating them, all leading to bacterial cell death [37]. Also this study shows that the activity of all antibiotics has increased in the presence of silver nanoparticles against the tested organisms except for Gentamycin which was not enhanced when combined with 10 µL AgNPs against *S. boydii* but the largest volumes did.

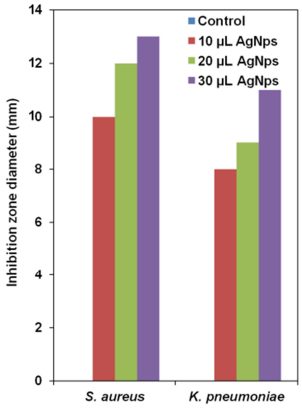

Figure 10. Effect of different ZnONPs concentrations against the two tested isolates.

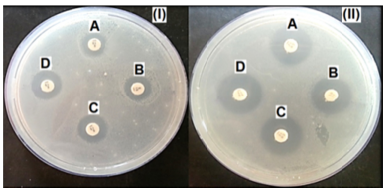

Figure 11. (I) Antimicrobial activity of ZnONPs against *S. aureus* (A) Erythromycin, (B) Erythromycin + 10 µL ZnONPs, (C) Erythromycin + 20 µL ZnONPs and (D) Erythromycin + 30 µL ZnONPs. **(II)** Antimicrobial activity of ZnONPs against *K. pneumonia* (A) Gentamycin (B) Gentamycin + 10 µL ZnONPs (C) Gentamycin + 20 µL ZnONPs and (D) Gentamycin + 30 µL ZnONPs.

Zinc oxide (ZnO) is listed as "generally recognized as safe" (GRAS) by the U.S. Food and Drug Administration (21CFR182. 8991) as many studies have shown that these nanoparticles have selective toxicity to bacteria but exhibit minimal effects or even no effects on human cells [38]. The antimicrobial activity of ZnO nanoparticles differs according to the volumes used and the tested isolates. Increasing the ZnONPs volumes (10, 20 and 30 µL) increases the antimicrobial activity gradually. The antimicrobial activity of ZnONPs against *S. aureus* was higher than its antimicrobial activity against *K. pneumoniae*. The mechanism of the antimicrobial activity of ZnO nanoparticles is not well understood but there are several mechanisms which have been proposed to explain their antimicrobial activity. Studies suggested that ZnONPs can affect bacterial cell in two levels, one of them is the direct contact of ZnONPs with the microbial cell wall. As ZnONPs adhere to the microbial cell wall so membrane morphology changes, resulting in increasing permeability and disruption in cell membrane transport system and finally leading to the cell death [39,40]. The other level is the oxidative stress by releasing of hydrogen peroxide (H_2O_2) from its surface, Zn^{2+} ions and release of superoxide ions (O_2^{-}) which can damage cell membrane and interact with intracellular contents such as phosphorus and sulfur containing compounds like DNA causing the microbial cell death as represented by the following equations:

$$ZnO + O_2 \rightarrow O_2^{-} \quad (4)$$

$$O_2^{-} + 2H^{+} \rightarrow H_2O_2 \quad (5)$$

The activity of all antibiotics has increased in the presence of ZnONPs against the two tested organisms except for Penicillin G and Ampicillin when combined with 10 µL ZnONPs against *S. aureus* and Ciprofloxacin was not enhanced at the same volume against *K. pneumoniae* but the largest volumes did.

Table 5. The antibacterial effect of ZnONPs against *S. aureus* and their combined affect with ten various antibiotics.

Antibiotic	Inhibition zone diameter (mm)			
	Different concentrations of ZnONps			
	0 µL	+10 µL	+20 µL	+30 µL
No antibiotic	-	10	12	13
Norfloxacin 10	34	36	38	40
Ciprofloxacin 5	35	36	37	38
PenicillinG 10	27	27	28	29
Cefotoxime 30	30	32	34	36
Erythromycin 15	20	23	25	27
Tetracyclin 30	11	14	15	17
Gentamycin 10	20	21	22	23
Nalidixic acid 30	24	25	26	27
Ampicillin 10	20	20	21	22
Amoxycillin 10	9	11	13	15

Table 6. The antibacterial effect of ZnONPs against *K. pneumoniae* and their combined affect with ten various antibiotics.

Antibiotic	Inhibition zone diameter (mm)			
	Different concentrations of ZnONps			
	0 µL	+10 µL	+20 µL	+30 µL
No antibiotics	-	8	9	11
Norfloxacin 10	0	12	13	14
Ciprofloxacin 5	14	14	15	16
PenicillinG 10	0	14	16	20
Cefotoxime 30	26	27	29	31
Erythromycin 15	0	10	11	12
Tetracyclin 30	15	16	17	21
Gentamycin 10	15	17	19	21
Nalidixic acid 30	21	24	25	27
Ampicillin 10	0	8	9	11
Amoxycillin 10	0	8	9	11

The results show that cell wall synthesis-inhibitors-antibiotics Penicillin G, Cefotoxime, Ampicillin and Amoycillin were all effective against *S. aureus* and *S. boydii*. They were enhanced by adding AgNPs and ZnONPs volumes. *Klebsiella pneumoniae* was sensitive only to Cefotoxime but not sensitive to the other three antibiotics. With penicillin G synergism occurred as the inhibition zone diameter was wider than NPs volumes only. On the other hand *E.coli* was not sensitive to Penicillin G and Ampicillin and the effect was only for AgNPs volumes, but was sensitive to Cefotoxime and Amoycillin and enhancement occurred. Also, *S. aureus*, *S. boydii* and *E. coli* were sensitive to all DNA synthesis-inhibitors-antibiotics represented by Norfloxacin, Ciprofloxacin and Nalidixic acid and enhancement occurred for all. *K. pneumoniae* was not sensitive to Norfloxacin and synergism occurred.

The three antibiotics that inhibit Protein synthesis Erythromycin, Tetracycline and Gentamycin were effective against *S. aureus*, *S. boydii* and enhancement occurs for all. Tetracycline and Gentamycin were effective against *K. pneumoniae* and *E.coli* but Erythromycin was not effective. Synergism occurred with Erythromycin for both the two organisms as shown in Figures 12-14.

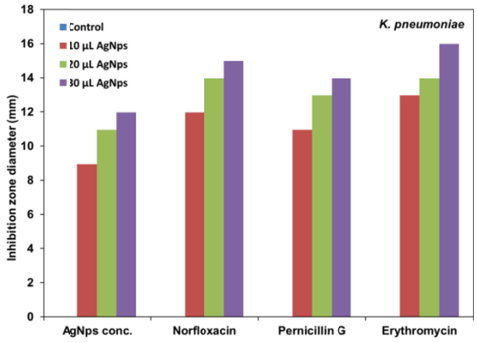

Figure 12. Synergism between antibiotics and AgNPs against *K. pneumonia.*

K. pneumoniae and *E. coli* are the two main bacteria that produce extended-spectrum beta-lactamase enzymes enabling them to be resistant to pencillins, cephalosporins and often to other types of antibiotics. Results showed that *K. pneumoniae* was resistant to pencillin G, Erythromycin and Norfloxacin and synergism occurred with these antibiotics when treated with different volumes of AgNPs and ZnONPs. While in case of *E.coli* synergism occurred with penicillin G, Erythromycin and Ampicillin when treated with different volumes of AgNPs.

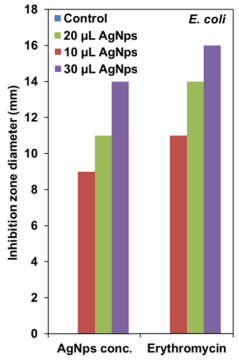

Figure 13. Synergism between antibiotics and AgNPs against *E. coli.*

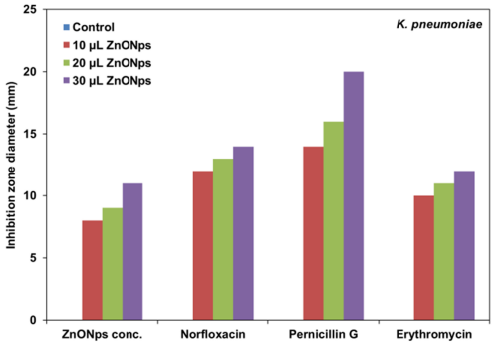

Figure 14. Synergism between antibiotics and ZnONPs against *K. pneumoniae.*

From results data AgNPs and ZnONPs may inhibit the beta-lactamase enzymes causing disruption of the resistance mechanism of *K. pneumoniae* and *E. coli* and enables antibiotics to be functional against this two types of bacteria.

4. Conclusion

In this study silver and zinc oxide nanoparticles were synthesized by chemical methods. The formation of these nanoparticles was confirmed by UV-vis spectroscopy, Transmission electron microscope (TEM), X-Ray diffraction analysis and Fourier Transform Infrared Spectroscopy (FT-IR). Also the antimicrobial activity of the synthesized nanoparticles against four different genera *Staphylococcus aureus* (NCMB 6571), *Shigella boydii* (ATCC9207), *Klebsiella pneumoniae* (clinical culture) and *Escherichia coli* alone and in conjugation with 10 various antibiotics representing most of antibiotics groups according to function were studied. The antibacterial activity of different volumes (10, 20 and 30 µL) of synthesized silver and zinc oxide nanoparticles were evaluated by using the disc-diffusion method. Also the combined effect of different volumes (10, 20 and 30 µL) of Ag and ZnO nanoparticles with antibiotics were tested along with ten various antibiotics covering most of antibiotics groups according to mechanism of action. Antimicrobial activity of AgNPs and ZnONPs differ according to the volumes used and the tested isolates. The highest antimicrobial activity of AgNPs was observed against *S. boydii* followed by *S. aureus* and its effect *on K. pneumoniae* and *E.coli* was nearly equal. In case of ZnONPs the antimicrobial activity against *S. aureus* was higher than that against *K. pneumonia.*

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