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Biological and Chemical Synthesis of Silver Nanoparticles: Characterization, MIC and Antibacterial Activity against Pathogenic Bacteria

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ABSTRACT

This study was practical for evaluating the difference in characterization and effects on pathogenic bacteria between AgNPs synthesis by biological method by using marine green alga Ulva fasciata and chemical method by sodium borohydride (NaBH4). Also examines the synergistic or antagonisms that applied by loading AgNPs synthesis by biological and chemical methods on antibiotics. The results point to that there is difference in characterization of AgNPs synthesis by various methods that determined by UV-vis absorbance, Transmission electron microscope (TEM), Zeta potential and X-ray Diffraction techniques (XRD). Antibacterial action of AgNPs experienced against eclectic pathogenic bacteria such as Gram negative bacteria (E. coli O157 (KY797670), Aeromonas hydrophila and Salmonella enteric subsp. salamae (Em.1-EGY015) and Gram positive bacteria (Bacillus cereus and Staphylococcus aureus); meanwhile, Biological synthesis AgNPs had more effective against pathogenic bacteria than chemical synthesis AgNPs. The minimum inhibitory concentration (MIC) of AgNPs creation by biological method is less than that created by chemicals methods. The synergetic or antagonism of AgNPs loading in antibiotics (Norfloxacin, Cefepime, Levofloxacin, Amoxicillin/Clavulanic Acid, Ampicillin/Sulbactam, Cephalexin, Ofloxacin, Neomycin, Cefoperazone and Amikacin) had different effects according to antibiotics, pathogenic bacteria and methods of synthesis AgNPs.

Keywords: Silver nanoparticles; Chemical synthesis; Biological synthesis; Bacteria; Antibiotics

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INTRODUCTION

Biosynthesis of nanoparticles that are produced by extracts that are manufactured from plant sources are stimulating area in nanotechnology with cost-effective plus environmental [1]. Silver nanoparticles have accepted as

antimicrobial means and have an actual as antimicrobial agent [2]. Silver nanoparticles creation by green are less expensive cost and less dangerous than chemicals [3]. Antibacterial commotion of silver nanoparticles in contrast to pathogenic bacteria such as *Bacillus subtilis, Enterococcus faecalis, Escherichia coli, Salmonella typhimurium* and *Candida albicans* remained described [4] Additionally, *Streptococcus pyogenes,* methicillin which resist to *Staphylococcus epidermidis* and methicillin that attack to *Staphylococcus aureus* were sensitive toward silver nanoparticles with size ranges from 160-180 nm [5]. Chemical creation of nanoparticles has several defects in consuming lethal solvents and manufacture risk by-products, but the biomolecules complicated in the biological creation of nanoparticles remain less lethal also playing roll as functional ligands, so biological creation of nanoparticles remains extra appropriate than chemical creation [6]. Some biological systems such as microorganisms, plant, marine organisms and micro-fluids are acting like reducing means for the biological creation of silver nanoparticles [7]. Also biological creation of silver nanoparticles via algae indicated further beneficial above other bioprocesses by fungi and bacteria, for it is extra proper for huge scale manufacture of silver nanoparticle through different shapes also size and it removes the cell culture preserving method [8]. Even though chemical creation necessitates short time for production of big amount of nanoparticles, this manner needs capping agents for size maintenance of the nanoparticles. Chemicals are used for creation of nanoparticles and maintenance is dangerous and lead to unsafe byproducts. The requirement for safe synthetic protocols for production of nanoparticles leads to the increase in natural methods which are avoiding the consumption of dangerous chemicals and this leading to increase request for green nanotechnology [9]. According to the silver nanoparticles and microbes get connected in the direction of the cell barrier, so cause distressing the cellular respiration and penetrability of cell barrier. The nanoparticles enter inside the cell barrier, consequently, producing cellular destruction by connecting using sulfur in addition to phosphorus having compounds like DNA in addition to protein which are considered existing inside the cell. The bactericidal action of silver nanoparticles is due to the discharge of silver ions origination the particles, which give the antimicrobial act [10]. Owing to the increase of bacterial resistance to common antibiotics, the studies of the antibacterial activities of silver nanoparticles are increased [11]. Several studies prove the idea that silver classes release Ag^+ ions and they are connected with the thiol groups in proteins of bacteria leading to disturbance in the duplication of DNA [12]. The goal of this consideration was tocompare between characterization besides antibacterial activity of silver nanoparticles (AgNPs) synthesis by biological method by marine alga *Ulva fasciata* and chemical method by sodium borohydride (NaBH4) and estimate antibacterial activity of AgNPs in contradiction of Gram negative bacteria (*Escherichia coli O157(KY797670*), *Aeromonas hydrophila* and *Salmonella enterica subsp. salamae (Em.1-EGY015)*) and Gram positive bacteria (*Staphylococcus aureus* also *Bacillus cereus*) and tested the synergistic effects of AgNPs loading on various antibiotics against pathogenic bacteria.

EXPERIMENTAL SECTION

This inquiry was carried out in microbial biotechnology department, Genetic Engineering and Biotechnology Research Institute, university of Sadat city, Sadat city, Egypt at 2018.

Chemicals

Silver nitrate, sodium borohydride, sodium hydroxide, sodium dodecyl sulfate, Norfloxacin (NOR), Cefepime (FEP), Levofloxacin (LEV), Amoxicillin/Clavulanic Acid (AMC), Ampicillin/Sulbactam (SAM), Cephalexin (CL), Ofloxacin (OFX), Neomycin (N), Cefoperazone(CEP), Amikacin; AK from the Sigma pharmaceutical industries in Egypt and Sterile distilled water was used throughout the experiments

Pathogenic Bacteria

Staphylococcusaureus, *Salmonellaenterica subsp. salamae (Em.*1EGY015), *Aeromonas hydropila*, *Escherichia coliO157(KY797670)* and *Bacillus Cereus* were isolated in Bacteriology, Mycology and Immunology Department faculty of veterinary medicine, University of Sadat City.

Microbial Media Used

Nutrient agar was used where simple technique used and was still usually used in the bacteriological investigation of selection of materials and is also recommended by standard methods. Nutrient Broth has the method initially planned for procedure in the Standard Technique and non-selective use in predictable cultivation of microorganisms [13].

Biological Synthesis of (AgNPs)

***Ulva fasciata*:** Alga was obtained from shallow water near the beach of Abu-qir coast Alexandria Egypt and was recognized [14].

Elaborations of *Ulva fasciata* aqueous quotations: One gram of *Ulva fasciata* that prepared in dry powder form was additional to 100 ml DD water boiled for one hour then filtrated to obtain algal aqueous extract.

Biosynthesis of silver nanoparticles (AgNPs): According methods of Hamouda et al. [8] ten ml of prior ready extract was additional gently to 90 ml of recently prepared 0.1 mM of $AgNO_3$ with stirring and warming at 40^oC for 30 minutes until the color change to brown.

Chemical Synthesis of AgNPs

Add 30 mL of 0.002M sodium borohydride ($NaBH_4$) to an Erlenmeyer flask and place a snow bath on a stirring plate. Snow bath is used to go slow the reaction also give good controller to last particle size/shape. Mixing and cooling the liquid for approximately 20 minutes. Drop 2 mL of 0.001M silver nitrate ($AgNO_3$) into the mixing $NaBH_4$ resolution at around 1 droplet per second. Stop mixing as soon as all the $AgNO_3$ is additional [15].

Characterization of Silver Nanoparticles in Two Methods

The creation of AgNPs was examined by Ultraviolet–Visible absorbance. Absorbance for produced AgNPs is in the range of 201 to 801 on Ultraviolet-Visible spectroscopy (T80+UV/VIS Spectrometer) at Genetic Engineering and Biotechnology Research Institute (GEBRI), Egypt. The external morphology besides particle size of the sample were described via Transmission Electron Microscope (JEOL JEM-2100). To detect the efficient groups of created silver nanoparticles FTIR (Perkin Elmer) spectroscopy was achieved. Zeta potential value and Size distribution of the nanoparticles remained examined by a zeta potential analyzer (Malven Zeta size Nano-Zs90). XRD analysis to study the physico-chemical character of silver nanoparticles, the mineralogists and hard state chemists practice chiefly the Powder X-ray Diffraction performances which are the most significant characterization devices used in hard state chemistry besides material science. The shape, size, lattice parameter purpose and period fraction analysis

of the unit cell designed for any compound can be evaluated basically by XRD. The data of translational replicated size and shape of the unit cell are attained from peak positions of Diffraction design.

Antimicrobial Assay

In this study there are five bacteria were experienced two of them are Gram positive (*Bacillus cereus* and *Staphylococcus aureus*) and three are Gram negative bacteria (*Aeromonas hydrophila*, *Escherichia coliO157(KY797670***)** and *Salmonella enteric subsp. salamae* (*Em.1-EGY015*) and, the antibacterial activity of nanoparticles were studied according the methods [16].

Serial Dilution Assay

Minimal inhibition concentration (MIC) is the smallest concentration that obstructs the visible growing of bacteria [17]. Disk diffusion method was used for assessment of MIC where values of AgNPs were determined in the MIC based on making serial dilution of AgNPs [18]. The initial concentration of AgNPs were 5mg/ml and serial dilution 14 times occurred and each concentration tested by disk diffusion test.

Disk Diffusion Test

Active cultures were prepared by transmitting a loop-full of culture from each pathogenic strain to five ml of nutrient broth then incubated (at 37°C) for24hrs then the suspension having10^6 CFU ml^{-1} of the check microbes existed swabbed equally on nutrient agar. The discs with six mm diameter were each disk saturated by different concentration of AgNPs solution which prepared previously and located on the agar plate. The inoculated dishes were incubated at 37 °C for 24 hrs. and inhibition zones were measuring. Each assay in the experiment was done in a triplicate [19]. Also by the same technique applied with antibiotic discs only and discs of antibiotic loaded with biological and chemical solutions of AgNPs [20]**.**

RESULT AND DISCUSSION

The appearance of the biosynthesis silver nanoparticles was became yellowish brown, while the color of silver nanoparticles which synthesized by chemical method was turned from bright to bale yellow.

Characterization of Silver Nanoparticles has Biosynthesized

Ultraviolet-visible spectroscopy of AgNPs by two methods: UV-Vis spectroscopy of the silver nanoparticles stayed described by one of the most commonly applied procedure [21]. A single peak was observed at 400 nm in biological synthesis and 420 nm in chemical synthesis, which corresponded to plasmon excitation of silver nanoparticles as shown in Figure 1. Several investigators require identified absorption of a broad peak of colloidal silver in resolution between 400 and 450 nm, which is due to surface plasmon stimulation of the metal nanoparticles [22]. The peaks at 400 nm and 420 nm are approval with the theoretical excitation of SPR using Mie's theory [23].

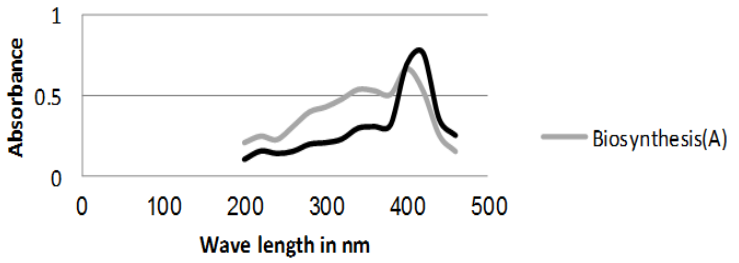

Figure 1. UV-Vis absorption spectra of silver nanoparticles synthesized by: A) Biosynthesis B) Chemical synthesis

Transmission Electron Microscope

The consequences achieved from TEM micrograph noted from the silver nanoparticles precipitated on carbon layered copper TEM net was presented in Figure 2. Silver nanoparticles micrograph seen spherical shaped in chemical and biological synthesis, well distributed in solution and in a range of 8-17 nm in chemical Ag-NPs, while 9-21 nm in size in biological Ag-NPs. Characterization of silver nanoparticles by TEM has been described by Sondi and salopek-Sondi [24].

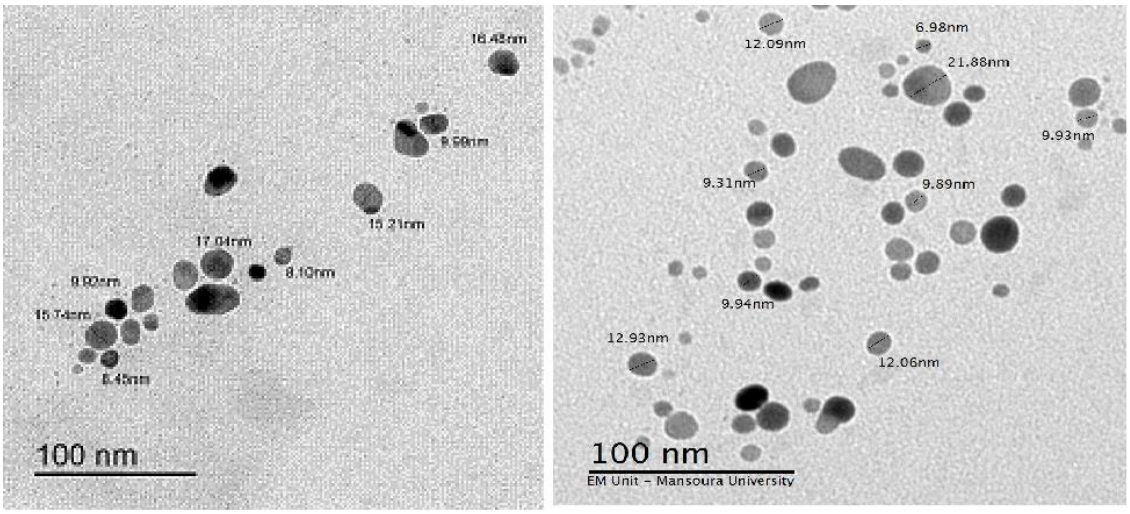

Figure 2. Transmission Electron Microscopic image of silver nanoparticles A) chemical and B) biological synthesis

Fourier-Transform Infrared (FTIR)

Fourier transform infrared measurements were carried out to identify the active groups in molecules in charge of the stabilization in addition coating of the newly synthesized silver nanoparticles which synthesized via biological besides chemical methods. The FTIR spectrum of silver nanoparticles is presented in Figure 3 which recorded from the powdered sample. In biological synthesis, the absorption peaks at 1635 and 1380 cm-1 showed the presence of NO_2which may be from $AgNO_3$. C-H stretch weak peaks represents at 2962, 2921and 2854 cm^{-1}. The peak at 3445 cm^{-1} is located assigned to OH stretching. These results may be due to phenols compound [25], the absorption peak at 1635 and 1380 cm^{-1}which showed that the existence of amide bond in proteins. These crests points to reducing and stabilizer $AgNO_3$ by alga extract to (Ag^0) [26]. The primary amines of proteins in plant extracts have a chief role in the reduction of $AgNO_3$ to AgNPs [27], absorption strong peak at 1532 cm^{-1} indicates C=O amide, broad peak at 1446 cm^{-1} represented CH_2 bend and two strong peaks at 1245 $cm^{-1}$and 1105 cm^{-1}represented C-O-C stretch and C-OH respectively. While in chemical synthesis there are three peaks only where the strong peak at 3433 cm^{-1} was given to OH stretching, the absorption peaks at 1638 cm^{-1} showed the presence of NO_2which may be from $AgNO_3$ and the band is moved to lower frequency at 671cm^{-1} which represent C-H bending vibration where C-H bending shaking of 671 $cm^{-1}$the spectrum recorded for AgNPs. An FTIR band of silver nanoparticles which was synthesized by biological method approved the existence of protein in the silver nanoparticles, which more check that biological synthesis of AgNPs coated with biotic molecules. This study additional verifies that capping protein stabilizes AgNPs and avoids accumulation of AgNPs [28-30].

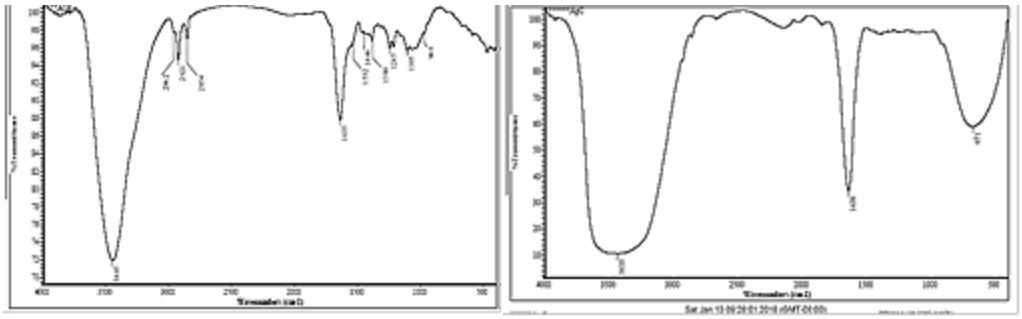

Figure 3. Fourier transform infrared spectroscopic data of AgNPs synthesized by A) Biology B) Chemical

Zeta Potential

The zeta potential is point to surface charge of silver nanoparticles (AgNPs) also it is beneficial considerations to investigate the stability of nanoparticles [29]. Zeta potential is a vital consideration to guess the stability of Ag NPs. Zeta potential less than -20 mV also more than +20 mV expects physical stability. The zeta potential assessment of bio created silver nanoparticles was -25 mV and by chemical synthesis was -23 mV. The results in Figure 4 show that AgNPs synthesis by biological method is a good stability than synthesis by chemical method [31] because the repulsive forces avoid aggregation with aging.

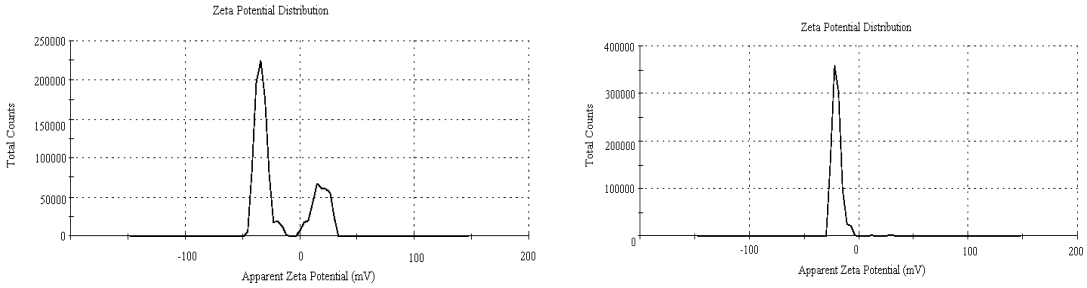

Figure 4. Zeta-potential analyses of silver nanoparticles A) biology and B) chemical

X-ray Diffraction Analysis

Peak indexing: On or after the peak placing the unit cell dimensions are confirmed this practice is named indexing which is the principal step in diffraction pattern investigation. Miller guides (hkl) are essential to be apportioned for each peak to index. It is not mere simple reverse of scheming peak positions from the unit cell dimensions and wavelength [32]. XRD investigation of the ready sample of silver nanoparticles was completed by a Bruker D8 progressive X-ray diffractometer by means of CuKα radiation (λ=1.5418 Å), under 40 kV/30Ma-X-ray, 2Θ/Θ Scanning manner, Stable Monochromator. Records were taken for the 2Θ range of 25 to 80 degrees in biological synthesis of Ag-NPs while from 20 to 50 degrees in chemical synthesis of Ag-NPs with a stage of 0.02 degree. Data used for some 2Θ range consumes each peak was given in a chief step. Diffractogram of the whole data is in Figure 5. Indexing has been done in two different methods and data are in Table 1 for biological Ag-NPs and Figure 2A and 2B for chemical Ag-NPs. Tables 1 and 2 one essential to find a dividing constant also values in the 3rd column suits integers (approximately). Here, the constant is 20(77.9-58.2=20) in Ag-NPs biological synthesis while is 10(49.90-30.90=19) in Ag-NPs chemical synthesis. Additionally, the great intense peak for cubic materials is common (111) reflection, which is detected in the sample. Five peaks at 2Θ values of 27.925, 32.409, 46.333, 57.582and 76.618 degree corresponding to (111), (200), (220), (222) and (331) in Ag-NPs biological synthesis while

in Ag-NPs were synthesized values of 2Θ 20.238, 25.802, 30.52, 39.789 and 45.336 degree corresponding to (110),(111),(200),(211) and (220). The XRD study approves that the consequential particles are (FCC) silver nanoparticles **[**33].

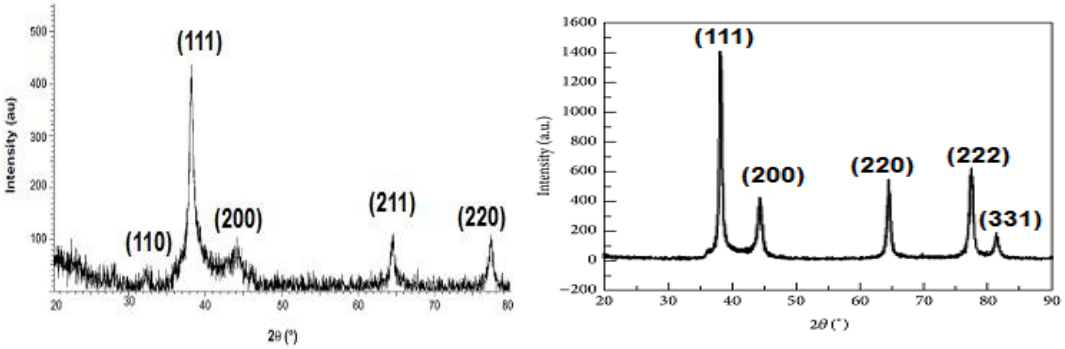

Figure 5. X-ray diffraction analysis of silver nanoparticles synthesized by (A) Biological and B) chemical

Table 1. Ag-NPs biosynthesis characterise X-ray diffraction analysis, simple peak index and Peak indexing from d-spacing

Peak position 2Θ	1000*sin^{2}Θ	1000*sin^{2}Θ/20	Reflection	Remarks	d	1000/d^2	1000*d^2/33	hkl
27.925	58.2	2.91	111	12+12+12=3	3.19241	98.126	2.97	111
32.409	77.9	3.89	200	22+02+02=4	2.76025	131.251	3.98	200
46.333	154.8	7.74	220	22+22+02=8	1.95805	260.213	7.89	220
57.582	231.9	11.59	222	22+22+22=12	1.59942	390.93	11.85	222
76.618	384.3	19.22	331	32+32+12=19	1.24261	647.668	19.26	331

Table 2. Ag-NPs Chemical synthesis characterise X-ray diffraction analysis, simple peak index and Peak indexing from d-spacing

Peak position 2Θ	1000*sin^{2}Θ	1000*sin^{2}Θ/19	Reflection	Remarks	d	1000/d^2	1000*d^2/32	hkl
20.238	30.9	1.63	110	12+12+02=2	4.3844	52.03	1.63	110
25.802	49.9	2.63	111	12+12+12=3	3.45018	84.03	2.63	111
30.52	69.3	3.65	200	22+02+02=4	2.9267	116.69	3.65	200
39.789	115.8	6.09	211	22+12+12=6	2.26364	195.31	6.1	211
45.336	148.5	7.82	220	22+22+02=8	1.98491	253.81	7.93	220

Particle size calculation: From this study, seeing the peak at degree, average particle size has been valued via Debye-Scherrer formula [34]:

$D=0.9\lambda/\beta \cos\theta$ (1)

Where"β" is FWHM (full width at half maximum), "λ" is wave length of X-ray (0.1541 nm), "θ" is the diffraction angle and "D" is particle diameter for AgNPs were synthesized by biology.

According to this calculation of particle size where found particle size of AgNPs synthesized with biological from 9 nm to 35 nm while by chemical method from 9 nm to 35 nm where AgNPs synthesis by biological method are less in size than AgNPs synthesis by chemical method.

Antimicrobial Assay of Biological and Chemical Synthesis of Silver Nanoparticle

In this research, silver nanoparticles were synthesized by biological and chemical methods were experienced for antibacterial action as recommended [35] against Gram positive bacteria (*Bacillus cereus* and *Staphylococcus aureus*) and Gram negative bacteria (*Aeromonas hydrophila*, *Escherichia coliO157 (KY797670*), and *Salmonella enteric subsp. salamae (Em.1-EGY015*)) by disk diffusion method where the inhibition zones around each well with AgNPs synthesized by biological and chemical method are noticed in Figure 6. The maximum antimicrobial action of nanoparticles was detected with *Staphylococcus aureus* and *E. coli* then after that *Aeromonas hyrophila*, *Bacillus cereus* and *Salmonella enteric subsp. salamae* in biological synthesis. While in chemical synthesis, the maximum antimicrobial action was detected with *Aeromonas hydrophila* and *E. coli* after that *Staphylococcus aureus*, *Bacillus cereus* and lowest zone in *Salmonella enterica subsp. salamae*. Where AgNPs synthesized by biological method achieved the highest antimicrobial activity than AgNPs synthesized by chemical method. Several concepts prove actuality on the act of silver nanoparticles on microorganisms to reason bacteriological action. Silver nanoparticles have ability quick to penetrate bacterial cell barrier, and in the long term, pass in it; by this way, it reasons physical changes in the cell surface [36].

MIC of the Silver Nanoparticles

The evaluation of minimum inhibitory concentrations (MIC) of AgNPs which synthesis by biological and chemical against *S. aureus, Salmonella enterica subsp. salamae, Aeromonas hydrophila, E. coli O157 and Bacillus cereus* occurred by disk diffusion method as shown in a Table 3. The MIC for biological AgNPs were 0.5, 1, 0.5, 2 and 1mg while in chemical AgNPswere 4,4,2,3 and 4 mg for *S. aureus, Salmonella enterica subsp. salamae, Aeromonas hydrophila, E. coli O157 and Bacillus cereus* respectively. The result of MIC was stated that the biological synthesis of AgNPs give more inhibition zones with pathogenic bacteria was tested in this study in low concentration than AgNPs synthesis by chemical.

Table 3. MIC of bacterial pathogens treated with biological and chemicals synthesis of silver nanoparticles

	S. aureus	***Salmonella***	***Aeromonas***	***E. coli***	***B. cereus***
AgNPs bio(mg/ml)	0.5	1	0.5	2	1
AgNPs chemical(mg/ml)	4	4	2	3	4

Estimation of Synergistic Antimicrobial Activity

The synergism and the antagonism effect of the AgNPs synthesis by biology and chemical were estimated as shown by the decrease or increase in diameter of inhibition zone (cm) around the different antibiotic disk (NOR, FEP, LEV, AMC, SAM, CL, OFX, N, CEP and AK) conjugated with AgNPs synthesis by biology and chemical. The presence zone of inhibition that was indicated the antimicrobial effect of AgNPs conjugated with antibiotic disk (Table 3). The size of inhibition zone was diverse due to the type bacteria by disk diffusion assay [37]. Inhibition zone results were obtained for ten types of antibiotics with synthesized AgNPs biological and chemical synthesis against five

species (*Staphylococcus aureus, Salmonella enterica subsp. salamae, Aeromonas hydrophila, E. coli O157 and Bacillus cereus*) as shown in Figure 6.

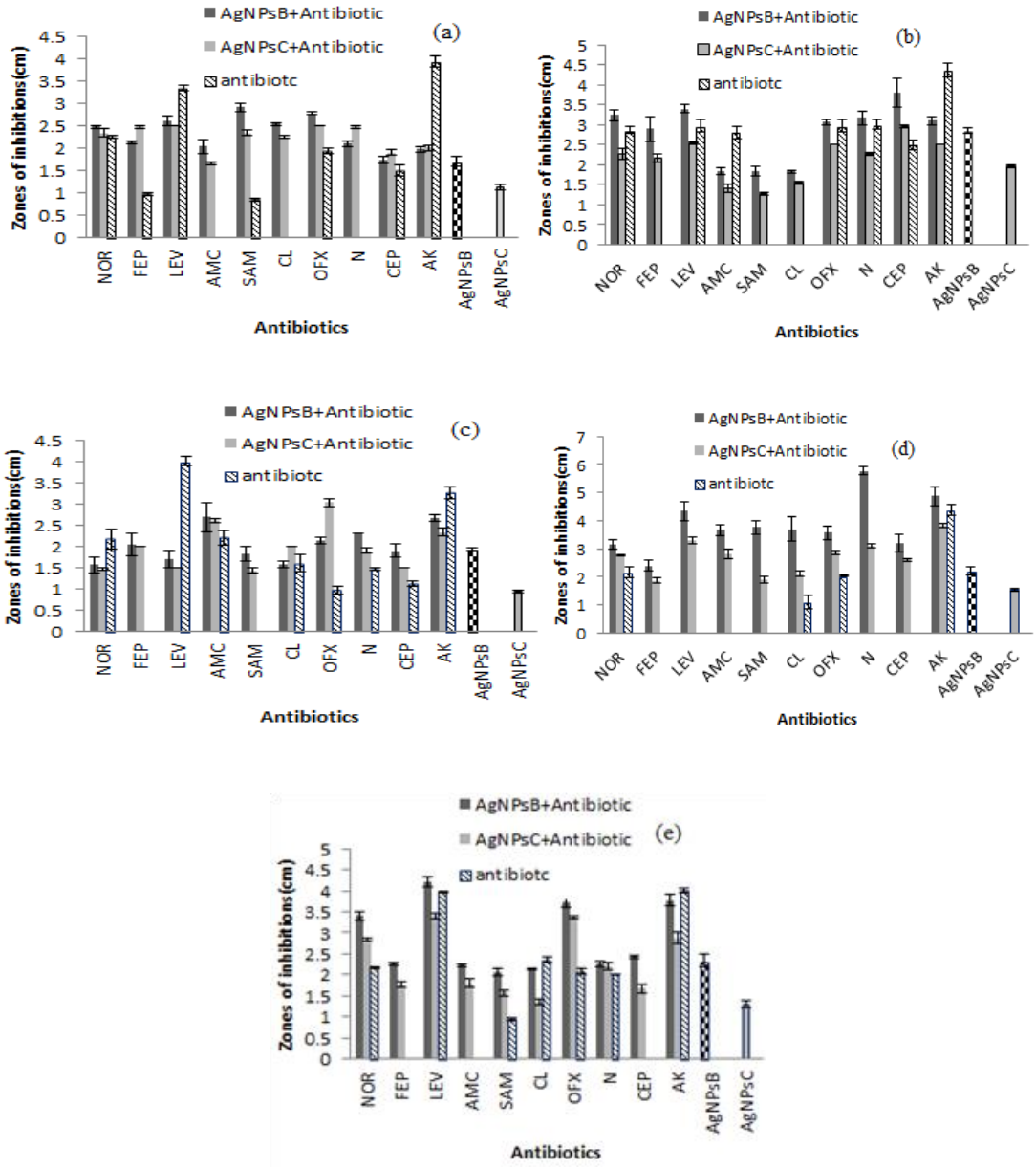

Figure 6. Effect of different antibiotics, and antibiotics conjugated with AgNPs synthesis by biological and chemicals methods against pathogenic bacteria (a) *Staphylococcus aureus,* (b*) Salmonella enterica* (c) *Aeromonas hydrophila,* (d) *E. colli* O157 and (e) *Bacillus cereus*

The antibiotics were Norfloxacin; NOR, Cefepime; FEP, Levofloxacin; LEV, Amoxicillin/Clavulanic Acid; AMC, Ampicillin/Sulbactam; SAM, Cephalexin; CL, Ofloxacin; OFX,Neomycin; N, Cefoperazone; CEP and Amikacin; AK

The synergism and the antagonism effect of the Silver nanoparticles synthesis by two methods were observed by the increase or decrease in length of inhibition region (cm) [38] round the different antibiotic disks (NOR, FEP, LEV, AMC, SAM, CL, OFX, N, CEP and AK) loaded with AgNPs synthesis by biological and chemical methods.

Table 4. Effect of different antibiotics, and antibiotics conjugated with AgNPs synthesis by biological and chemical methods against Pathogenic bacteria

	Staphylococcus aureus			***Salmonella enterica subsp. salamae***			***Aeromonas hydrophila***			***E. colli O157***			***Bacillus cereus***		
Treat.	a	a+b	a+c	a	a+b	a+c	a	a+b	a+c	a	a+b	a+c	a	a+b	a+c
NOR	S*	S+	S	S*	S+	S	S*	A	A	S*	S+	S	S*	S+	S
FEP	S*	S	S+	R	S+	S	R	S+	S	R	S+	S	R	S+	S
LEV	S*	A	A	S*	S	A	S*	A	A	R	S+	S	S*	S	A
AMC	R	S+	S	S*	A	A	S*	S+	S	R	S+	S	R	S+	S
SAM	S*	S+	S	R	S+	S	R	S+	S	R	S+	S	S*	S+	S
CL	R	S+	S	R	S+	S	S*	A	S	S*	S+	S	S*	A	A
OFX	S*	S+	S	S*	S	A	S*	S	S+	S*	S+	S	S*	S+	S
N	R	S	S+	S*	S	A	S*	S+	S	R	S+	S	S*	S+	S
CEP	S*	S	S+	S*	S+	S	S*	S+	S	R	S+	S	R	S+	S
AK	S*	A	A	S*	A	A	S*	A	A	S*	S	A	S*	A	A

S*, sensitive; R, resistance; S, synergism; A, antagonism; S^+, synergism more than S; a, Antibiotics; a+bAntibiotic+ Bio AgNPS; a+c Antibiotic+ chemical AgNPs.

All tested antibiotics were shown synergetic effect with silver nanoparticles synthesis by biological and chemical methods except Levofloxacin and Amikac in with AgNPs biological and chemical synthesis are antagonism with *Staphylococcus aureus* are antagonism as shown in Table 4. The antagonism were present when loaded Levofloxacin, Amoxicillin/Clavulanic Acid, Ofloxacin and Amikacin with chemical of AgNPs with *Salmonella enteric*, and also Amoxicillin/Clavulanic Acid and Amikacin when loading with biological synthesis AgNPs were caused antagonisms with *Salmonella entericas subsp. salamae* shown in Table 4. When, loading two types of AgNPs on Norfloxacin, Levofloxacin and Amikacin and loading biological AgNPs only on Cephalexin were made antagonism with *Aeromonas hydrophila* are as shown in Table 4. With *E. coli O157* were noticed antagonism only when chemical AgNPS was loaded on Amikac in as shown in Table 4, while with *Bacillus cereus* noticed antagonism in two types of AgNPs were loaded on Cephalexin and Amikacin and when chemical AgNPs only loaded on Levofloxacin as shown in Table 4. The synergism, antagonism, resistance, and sensitive differ according to antibiotics, bacteria, and the methods of synthesis AgNPs.

CONCLUSION

This study compare between Silver nanoparticles synthesis by biological and chemical methods. The AgNPs synthesis by biological and chemical methods were spherical and size in a range of 8-17 nm in chemical while 9-21nm in size in biological according to TEM and characterized by FTIR, Zeta potential and XRD that proved that AgNPs had formed by both biological and chemical methods differ in characteristics and the formation of chemical silver nanoparticle was found more cost, also the use of different toxic chemicals for its creation makes the biotic

creation is extra preferable than chemical synthesis and the chemical method cause environmental pollution. Biotic creation techniques are conducted in ecological conditions also they are secure enough. The inhibition growth was higher in biological synthesis of AgNPs than chemical and also Biological AgNPs showing more synergetic effect than chemical AgNPs when loading on antibiotics, while loading AgNPs synthesis by chemical on antibiotics showing antagonistic effect than biological AgNPs against Gram negative bacteria (*Escherichia coli O157(KY797670), Aeromonas hydrophila* and *Salmonella enteric subsp. salamae* (*Em.1-EGY015*) and Gram positive bacteria (*Staphylococcus aureus and Bacillus cereus*). The differences in sensitivity of bacteria due to the resistant of bacteria to tested antibiotics, also the type of synthesis of AgNPs and the differences in cell physiology, cell wall structure and cell contributed to antimicrobial agent against pathogenic microorganisms. The current study would recommend using Silver nanoparticle synthesis by biological method where it more effective than Silver nanoparticle synthesis by chemical method, and the possible application of AgNPs combined with antibiotics to stop the fatal diseases produced by pathogenic bacteria.

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