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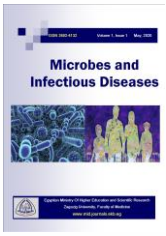

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Original article

Acinetobacter baumannii extracted silver nanoparticles: A study on the characterization and antibacterial activity

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ABSTRACT

Background: Antibiotic resistance is a worldwide problem, associated with increased mortality. Biosynthesized silver nanoparticles are promising solution. **Aim:** To improve the anti-microbial resistance using *Acinetobacter baumannii (A. baumannii)* biosynthesized silver nitrate. **Methods:** Optimization of biosynthesis condition and characterization by Ultraviolet-Visible spectroscopy (U-V-Vis) at a wavelength from 300 to 400 nm, ±1.67 nm. The *Acinetobacter baumannii* biosynthesized silver nitrate was examined using transmission electron microscope (TEM). Fourier transformed infrared spectroscopy was done to study the capping material, X-ray spectroscopy confirm silver nanoparticles synthesis with particle size match TEM measuring and testing its antibacterial effect was done. **Results:** The obtained U.V Spectroscopy and TEM characters of silver nitrate biosynthesized *A.baumannii*. *Acinetobacter baumannii* biosynthesized silver nitrate was obtained indicated. Minimum inhibitory concentration (MIC) was from 1-3 mg/ml against extreme drug resistance bacteria. the size of silver nanoparticles was found to be between 18 nm and 50 nm. The AgNPs were found to be stable for over 6 months at 37°C. The MIC for AgNPs formed from *A. baumannii* CFF was detected as 2.85 mg/ml, MBC (minimal bactericidal concentration) was 3 mg/ml for *K. pneumonia*, MIC was 1 mg/ml, MBC was 0.5 mg/ml for *P. aeruginosa* and MIC was 1.5 mg/ml and MBC was 1 mg/ml for *A. baumannii* isolates. Lethal concentration (LC) 50% was 60.584 ug/ml) assessed by Sulforhodamine-B assay on hepatocellular carcinoma cell line. **Conclusion:** *Acinetobacter baumannii* extracted silver nitrate has A promising antibacterial action that may help in solving the multidrug resistance bacteria.

Introduction

Highly resistant bacterial infections have dictated the development of alternate eco-protected antibacterial agents. Construction of metallic nanoparticles using biogenic substances is a promising potent anticancer and antibacterial agent [1]. Synthesis of nanoparticles using natural products like plants or microbes is an environmentally friendly technique [2]. Biosynthesis of silver nanoparticles (AgNPs) from bacteria is a known method as bacterial supernatant has high amount of reducing enzymes and secondary metabolites [3].

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Acinetobacter baumannii (*A. baumannii)* is a non-motile. Gram-negative bacillus, aerobic, pleomorphic, and known as highly resistant opportunistic pathogen which is highly dangerous for immunocompromised patients [4]. A study on antioxidant and prooxidant properties showed that *A. baumannii* produces a metal ion metabolite which perform an antioxidant function [4]. Silver nanoparticles was demonstrated as an effective mean against pathogenic organisms due to their cell membrane penetrative activity, cell wall attachment characteristics and their toxic effects to bacteria proved by their antibacterial action [5]. Other mechanism of AgNPs action as DNA and enzymes damage and destruction of the cell membrane via free radicals' formation were mentioned [2]. The cytotoxicity of AgNPs was observed via cell viability, oxidative stress, damage of mitochondria and production of reactive oxygen species (ROS) [6]. The transmission electron microscopic (TEM) analysis showed its intracellular action on mitochondria and DNA [7]. In this study, we aimed at improving the anti-microbial resistance using *A. baumannii* biosynthesized silver nitrate. giving the hope for decreasing bacterial resistance in the future.

Materials and Methods

Silver nanoparticles biosynthesis was done in the following steps (Figure 1):

A) Bacterial strain

The bacterial strains (*Klebsiella pneumonia, Pseudomonas aeruginosa, Acinetobacter baumannii)* were isolated from intensive care unit patients of The National Liver Institute Hospital, Menoufia university. Bacterial isolation and identification were done according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines. All isolates were cultured onto blood agar, Muller Hinton agar, and MacConkey agar (18 to 24 h at 35°C) to confirm the purity and viability. Microorganisms were tested with VITEK 2 system version 08.01 (BioMe rieux, France) cards (ID & AST) as shown in **table (1)** according to the manufacturer's guidance. The Bacterial isolates were suspended in 0.9% sodium chloride solution and adjusted to 0.50 McFarland standard by Densicheck system ***(Biom rieux, France)*** [8]. The bacterial strains were stored in nutrient broth agar with 20% glycerol at – 20 ^{O}c [9].

B) Preparation of bacterial supernatant

Fresh growth of bacterial isolates *A.baumannii* was inoculated in nutrient broth (NB) tubes. 16 rRNA was extracted using primers (Fw-TTTAAGCGAGGAGGAGG and Rv-ATTCTACCATCCTCTCCC) [10,11], PCR extraction and purification kits (Thermoscientific gene JET genomic kit #k0721 and #k702, Thermofisher, USA) by adding 20 µl of proteinase reagent to 200µl of bacterial suspension then Adding 200 µl Buffer AL to the sample, mixing by pulse-vortexing for ≥15 seconds, Incubating at 56°C ± 3°C for 10 minutes ± 1 minute. Centrifugation of the 1.5 ml microcentrifuge tube to remove drops from the inside of the lid. We Added 200 µl of ethanol (96–100%) to the sample and mixed again by pulse-vortexing for ≥15 seconds followed by centrifugation in the 1.5 ml microcentrifuge. We applied 750 µl Buffer AW1 to the QIAamp Mini column and adding 750 µl Buffer AW2 without wetting the rim of the QIAamp Mini column followed by adding 200 µl elution Buffer (AE) equilibrated to room temperature. Incubate at room temperature for 1 minute, and then centrifuge at approximately 6000 x *g* for ≥1 minute and stored for further processing then visualized using gel electrophoresis **(Figure 2)** adjusted with one control (without inoculation). Cell free filtrate (CFF) was obtained from the supernatant.

C) Extracellular synthesis of AgNPs

Silver nitrate 1mM (from **sigma Aldrich** company) was added and observed after 48 incubations for colour changes. It was lifted to dry and AgNPs was obtained as precipitate which used for description and antimicrobial studies [11] **(Figures 3, 4).**

D) Optimization of silver nanoparticles synthesis

Microbial synthesis of AgNPs in the supernatants (CFF) have been investigated under different conditions to detect the most suitable condition for Bio reduction of silver ions. Changing volume of supernatant was done by adding 10, 25, 50 % of total mixture volume, different concentrations of silver nitrate (1, 2 and 4 mM $AgNO_3$) were used, at different pH range (4, 7, 8) by NaOH (0.2 M) and HCl (0.1 M). Incubation temperature were (28° C - 37^{O} C and 50^{O} C) for 48 h incubation with pH 7.0 of final mixture [12].

E) Description of silver nitrate

The volume of supernatant is parameter that affect property of NPs. It has effects on reaction time and color of reaction mixture. changing bacterial supernatant volume by adding 10, 25, 50 % of total volume as 1:9 ,2.5:7.5 and 5:5 ratio respectively. **(Figure 5)** shows the visual color of the final

mixture at different volumes of supernatant which show that reaction mixture contains 25% of supernatant had darkest color intensity then 50 % of supernatant volume then 10%

The absorbance beaks of *A. baumannii* at supernatant volume 10%, 25% and 50 % are 0.629 nm, 1.67 nm, and 0.955 nm, UV - visible absorption intensity was noted higher using 25% supernatant volume than 50 % with absorbance beak 1.67 nm and 0.955 nm respectively as in **(Figure 6)** and **(Figure 7)**

F) Ultra-Violet– visible spectrophotometry analysis

The reaction mixture aliquots (2 ml) were monitored intervals, and reduction of the Ag^+ ions were measured and recorded on Ultraviolet-Visible spectroscopy (T80+UV/VIS Spectrometer, England) (200 - 800 nm) at Genetic Engineering and Biotechnology Research Institute (GEBRI), Egypt

G) Transmission electron microscopy (TEM)

TEM analysis (JEOL JEM-2100) of silver nitrate characters and compositions was done at Faculty of Science Menoufia university Egypt. Sterilized dispersed AgNPs solution was added onto carbon coated TEM grids.

H) X-ray diffraction (XRD)

X -ray diffractometer (D8 Advance, Bruker, Germany), was used. The samples were prepared in powdered form [13].

I)Fourier transform -infrared (FT-IR) spectroscopy

The spectrum of AgNPs stabilization and capping was done by Perkin Elmer Fourier transform infrared spectrometer in the range of 400–4000 cm [14] with are solution of 4 cm [1] after freeze drying the samples.

J) Antimicrobial activity of AgNPs

Antimicrobial activity of AgNPs was done by well diffusion assay method [15]. All the pathogenic microbes *Klebsiella pneumonia, Pseudomonas aeruginosa* and *A. baumannii* were grown over night in Luria broth (LB),100 µ L culture of each strain was spread on a nutrient agar plate. Each well (8mm) containing 100 µ L of the AgNPs (0.5 mg/ml dissolved in water). AgNPs formed with silver nitrate concentration 1mM and PH 7 at well A, 2 mM, and PH 7 at well B, 4Mm and PH 7 at well C, 1 Mm and PH 8 at well D, well E for silver nitrate solution as control. The plates were incubated at 37 °C for 24 h.

The action of nanoparticles against bacteria was compared to antibiotics alone and in combination with formed nanoparticles by disc diffusion method, paper discs [16] (6mm) containing 50 µ L of the Ag NPs (0.5 mg/ml in water) and other antibiotic disc such as ciprofloxacin, cefotaxime and cefazolin was tested alone and in combination with AgNPs then the plates were incubated at 37 °C for 24 h, zones of inhibition were measured.

K) Minimum inhibitory concentration

The lowest Ag-NPs concentration which stop bacterial growth defined as the minimum inhibitory concentration (MIC) [17]. The concentration inhibits growth of bacteria to 99.9% known as Minimum bactericidal concentration (MBC)[17]. Each tube was inoculated with one of the organisms (*Acinetobacter baumannii, P. Aeruginosa and K. Pneumoniae*).

Cytotoxicity assay

Sulforhodamine B dye (SRB assay). Was used to detect viability by using 96-well plates containing 10µL Hep G2 hepatocellular carcinoma cell line cell suspension (5x10^3cells) and incubated for 24h. 100 µL media containing AgNPs was used to treat cells at various concentrations ranging from (10 ug/ml, 100 ug/ml). 10 % trichloroacetic acid (TCA) (50 ml) was used to fix cells after 72 h and incubated for 1 h at 4° C. The cells were incubated 10 min in dark place at room temperature after treatment with 70 µL SRB solution (0.4 % w/v) and washing 5 times with distilled water. the absorbance was measured at 540 nm using a BMGLABTECH®- FLUO star Omega microplate reader (Rotenberg, Germany) [18].

Statistical analysis

The mean and standard deviation was calculated using IBM SPSS statistics version 21. A significant p-value < 0.05. Minimum inhibition concentration and inhibition zone values were calculated.

Results

Bacterial strain

Phenotypic identification was done by Vitek Ⅲ automated system and result in **table (1)**.

Silver nanoparticles formulation

The biosynthesis of biosynthesized silver nitrate was determined by a color change of the reaction substrates in **(Figure 3)**, since the colour transition from the yellow color of bacterial supernatant to brown indicates the biotransformation of Ag^+ ion to

Ag^0. There is no change of colour for the control tube. That is confirmed by UV - visible absorbance **(Figure 4)** illustrated the formation of AgNPs surface plasmon peak from 300 to 400 nm, 1.67 nm for *A. baumannii* at adding 1 mM of silver nitrate to 1:4 mixture of supernatant.

Optimization of silver nanoparticles synthesis

The condition of synthesis has great effect on final synthesized nanoparticles. Here we will see the effect of supernatant volume added to total mixture, silver nitrate concentration, final mixture PH and incubation temperature, **Figure 5** shows the color of final mixture after incubation with different condition silver nitrate concentration is changed in three different concentration 1mM, 2mM and 4mM. there are no significant visual changes in colour of final prepared mixture as shown in Fig. 4. but UV–visible absorbance intensity is 1.67 nm ,0,8 nm and 0.75 nm for *A. baumannii*. for the three concentrations respectively. The maximum absorbance is for 1Mm silver nitrate concentration as in **figure (7).**

The optimization of condition by changing final mixture PH to PH 4, PH 7 and PH 8 of final has direct effect on biosynthesis of AgNPs. by visual appearance there is great difference in final mixture colour after incubation as in **figure (5)**. And by measuring the UV- absorbance after incubation for 72 hours was 0.15 nm, 1.67 nm, 0.83 nm respectively. **Figure 8** indicates that neutral PH is the most suitable PH for extracellular biosynthesis of nanoparticles by bacterial filtrate. the highest intensity was seen at wavelength 400-450 nm is for PH 7 and PH 8 is lower intensity at PH 4 which is not suitable for biosynthesis of nanoparticles no visual color change was detected as and lowest UV-absorbance beak.

Characterization of silver nanoparticles

Transmission electron microscopy (TEM)

Characterization of the nanoparticles state was monitored by using TEM analysis (JEOL JEM-2100) at Faculty of Science, **Menoufia University, Egypt**.

X-ray diffraction (XRD)

X-ray diffraction study on *A. baumannii* AgNPs formed diffractions patterns at 2 θ values 32.125◦, 37.976◦, 43.911◦, 54.378◦ and 64.359◦, indicated (111), (200), (210), (321), (310) respectively. The calculated particle size was obtained by Debye–Scherrer 's formula are 6 nm and 14.8 nm, the size detected by XRD was like the size of AgNPs measured by TEM analysis **(Figure 9)**

Fourier Transform -Infrared (FT-IR) Spectroscopy: results were illustrated in **figure (10)**

Antimicrobial activity of Ag NPs.

Well diffusion assay

Antibacterial action was evaluated, and the result illustrated in **table (2)**. The inhibition zone is 15, 16, 17 and 12 mm against *A. baumannii* for, the inhibition zone is 16.17, 17, 9 for well A, B, C, D respectively against *K. Pneumonia* and 18, 18, 16, 9 mm for well A, B, C and D against *P. aeruginosa* respectively. The result approves the antibacterial action of biosynthesized AgNPs formed from *A. baumannii* and there is no added benefit on increasing silver nitrate concentration information of nanoparticles as no great difference between well A, B, C and changing PH from PH 7 decreasing antibacterial activity as shown by decreasing inhibition zone in well D, while there is no antibacterial action for 1Mm silver nitrate solution alone **(Figure 11)**.

Combination of antibiotics and AgNPs show inhibition zone diameter for combination disc more than antibiotic and nanoparticles alone as antibiotic show no antibacterial activity against extreme drug resistance bacteria in the results is shown in **table (3)** and **(Figure 12)**.

The minimum inhibitory concentration (MIC) and minimum bacterial concentration (MBC)

The MIC for AgNPs formed from *A. baumannii* CFF was detected as 2.85 mg/ml, MBC was 3 mg/ml for *k. pneumonia*, *MIC* was 1 mg/ml, MBC was 0.5 mg/ml for *p. aeruginosa* and MIC was 1.5 mg/ml and MBC was 1 mg/ml for *A. baumannii* isolates **(Table 4)**.

Cytotoxicity assay of silver nanoparticles in vitro

Cancer treatment by nanomedicine is promising, hepatocellular carcinoma (HCC) Incidence is increasing around all the world. This study investigates the cytotoxic effect of AgNPs synthetized from *A. baumannii* CFF in HepG2 cells Many studies reported the inhibitory effects of AgNPs on cells. Cytotoxic effect at concentration 10 ug/ml for *A. baumannii* CFS AgNPs is 99.4408, concentration 100 ug/ml has higher cytotoxic effect for with cell viability 17.470 by calculation LC 50 % will be 60.584 ug/ml **(Table 5)**.

Table 1. Vitek 2 identification result of *Acinetobacter baumannii.*

2	**APPA**	**-**	**3**	**ADO**	**-**	**4**	**PYRA**	**-**	**5**	**LARL**	**-**	**7**	**DCEL**	**+**	**9**	**BGAL**	**-**
10	H2S	**-**	**11**	BNAG	**-**	**12**	AGLTp	**+**	**13**	dGLU	**+**	**14**	GGT	**-**	**15**	OFF	**-**
17	BGLU	**-**	**18**	dMAL	**-**	**19**	dMAN	**-**	**20**	dMNE	**+**	**21**	BXYL	**-**	**22**	BAlap	**-**
23	ProA	**+**	**26**	LIP	**-**	**27**	PLE	**-**	**29**	TyrA	**+**	**31**	URE	**-**	**32**	dSOR	**-**
33	SAC	**-**	**34**	dTAG	**-**	**35**	dTRE	**-**	**36**	CIT	**+**	**37**	MNT	**-**	**39**	5KG	**-**
40	lLATk	**+**	**41**	AGLU	**-**	**42**	SUCT	**+**	**43**	NAGA	**-**	**44**	AGAL	**-**	**45**	PHOS	**-**
46	GlyA	**-**	**47**	ODC	**-**	**48**	LDC	**-**	**53**	lHISa	**+**	**56**	CMT	**+**	**57**	BGUR	**+**
58	O129 R	**+**	**59**	GGAA	**-**	**61**	lMLTa	**+**	**62**	ELLM	**-**	**64**	lLATa	**+**			

Table 2. Well diffusion assay, inhibition zone of *Acinetobacter baumannii* biosynthesized silver nanoparticles different formed mixtures measured by mm.

Inhibition Zone mm *Acinetobacter baumannii* AgNPs concentration							
		Silver nanoparticles	**1Mm A**	**2mM B**	**4 mM C**	**PH 8 D**	**$AgNO_3$ E**
Bacterial Strain	***Acinetobacter baumannii***	Mean	17	16	15	12	8
		SD	0.193	0.47	0.683	0.15	0.01
	Klebsiella Pneumonia	Mean	**16**	**17**	**17**	**9**	**8**
		SD	0.204	0.76	0.17	0.5	0.01
	Pseudomonas aeruginosa	Mean	**18**	**18**	**16**	**9**	**8**
		SD	0.1	0.87	0.23	0.16	0.01

Table 3. Inhibition zone of combination of nanoparticles and antibiotics ± SD.

Inhibition Zone of Combination of Nanoparticles and Antibiotics by mm								
Bacterial strains	**CIP**	**CIP + AgNPs**	**CTX**	**CTX + AgNPs**	**ERY**	**ERY + AgNPs**	**CZ**	**CZ + AgNPs**
Acinetobacter baumannii	**6**	**11 ± 0.5**	**6**	**10 ± 0.4**	**6**	**11 ± 0.9**	**6**	**11± 1**
Klebsiella	**6**	**13 ± 0.1**	**6**	**12 ± 0.5**	**6**	**11 ± 0.5**	**6**	**10 ± 0.6**
Pseudomonas	**6**	**12 ± 0.4**	**6**	**13 ± 1**	**6**	**12 ± 0.6**	**6**	**11 ± 0.5**

CIP Ciprofloxacin, CTX Cefotaxime, CZ Cefazolin

Table 4. MIC and MBC *Acinetobacter baumannii* AgNPs concentration by mg/ml on *Klebsiella pneumonia*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii*

Bacterial strains	***Klebsiella pneumonia***		***Pseudomonas aeruginosa***		***Acinetobacter baumannii***	
	MIC	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
***Acinetobacter baumannii* AgNPs concentration mg /ml**	2.85	3	0.5	1	1	1.5

Table 5. Cell viability percent of hepatocellular carcinoma cell line treated with nanoparticles.

Nanoparticles		**Cell Viability %**
Concentration	10 ug/ml	99.4408
	SD	0.42745
	100 ug/ml	17.4702
	SD	0.6879
LC 50%	60.584	50

Figure 1. Diagram of biosynthesis method and characterization of silver nanoparticles.

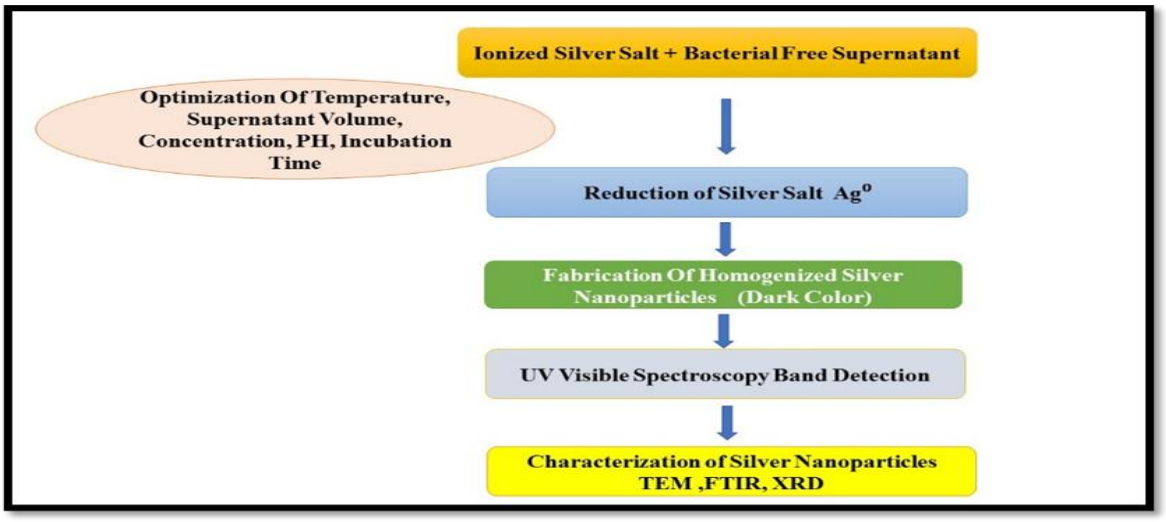
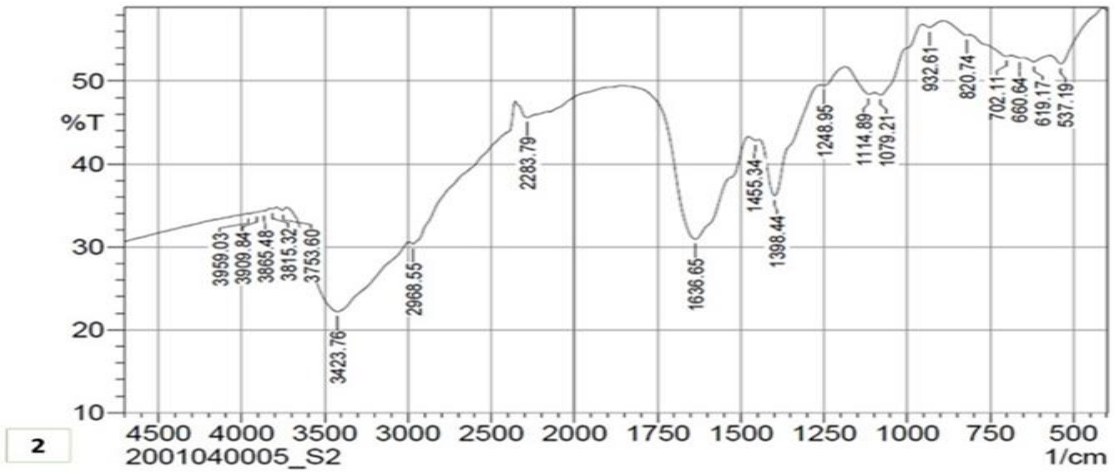


Figure 2. Shows gel electrophoresis of *Acinetobacter baumannii* 16S rRNA. Lane 1 shows positive control and tested Acineto at Lane 2 at 600 bp.

Figure 3. The bacterial supernatant with 25% of supernatant and 1mM of silver nitrate in scroller A, The bacterial supernatant with 50% of supernatant and 1mM of silver nitrate in scroller B, The bacterial supernatant with 50% of supernatant and 2 mM of silver nitrate in scroller C before and after incubation for 48 h.

Figure 4. XRD of silver nanoparticles formed by *Acinetobacter baumannii* supernatant.



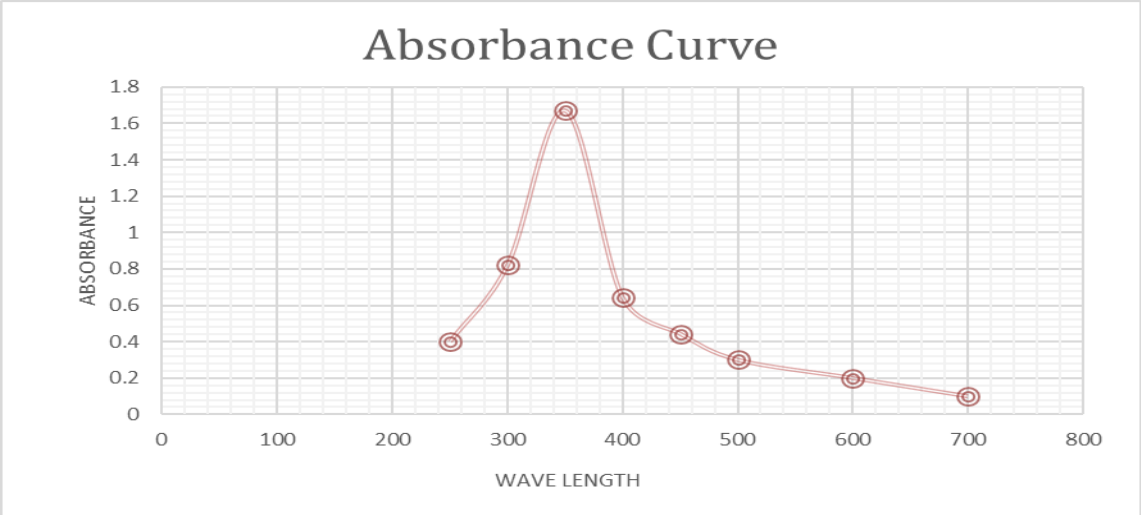


Figure 5. Optimization of nano particles synthesis was done by changing the volume of supernatant, silver nitrate concentration and changing final mixture PH.

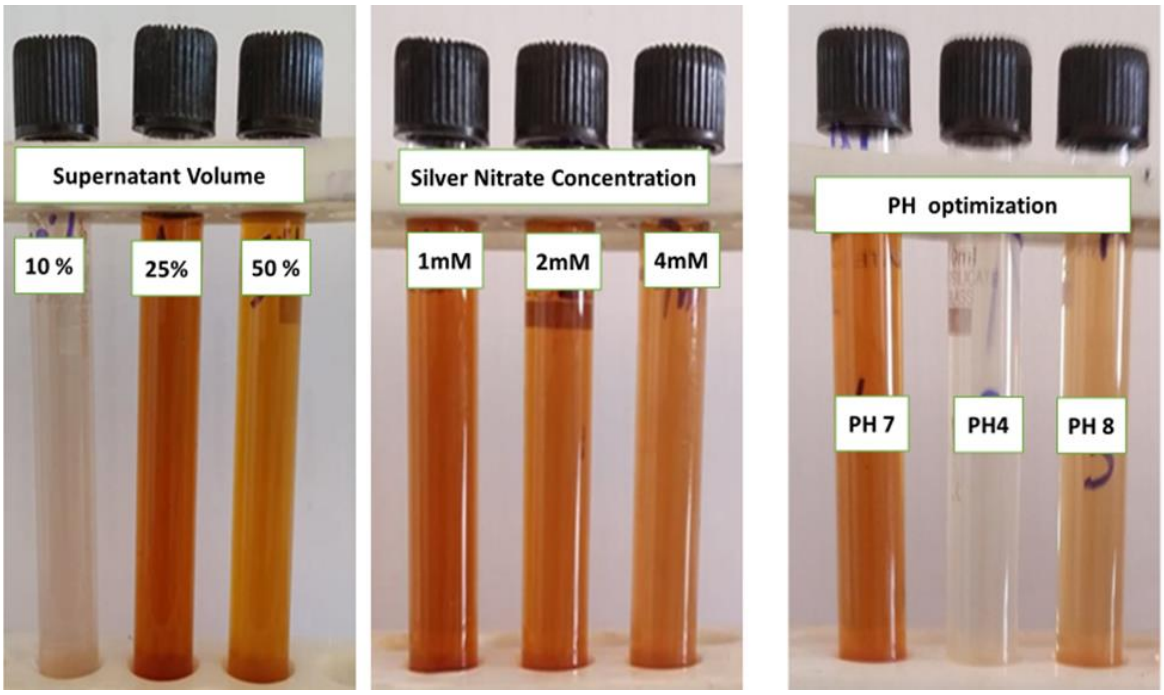


Figure 6. UV absorbance curve of final mixture after incubation with different supernatant volume 10, 25, 50 % of total mixture.

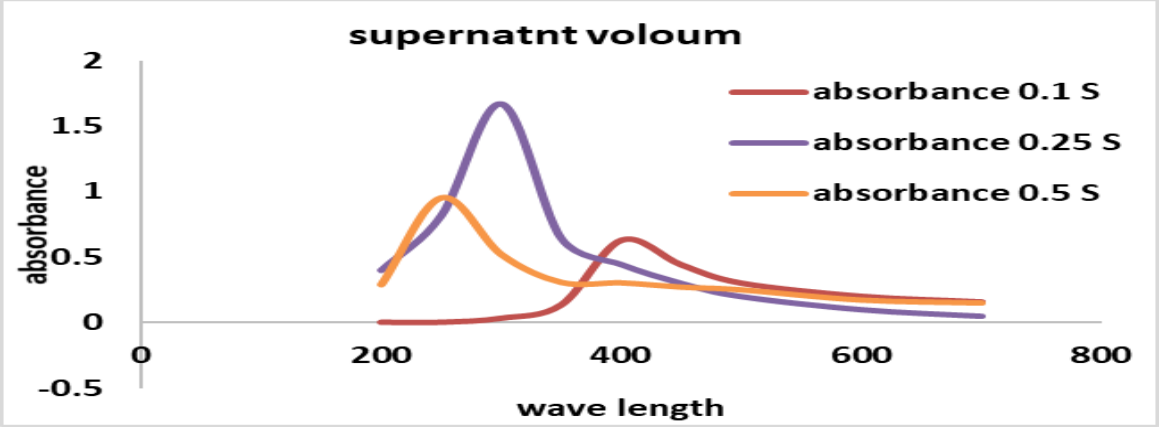


Figure 7. UV absorbance chart of final mixture after incubation with different supernatant volume 10, 25, 50 % of total mixture.

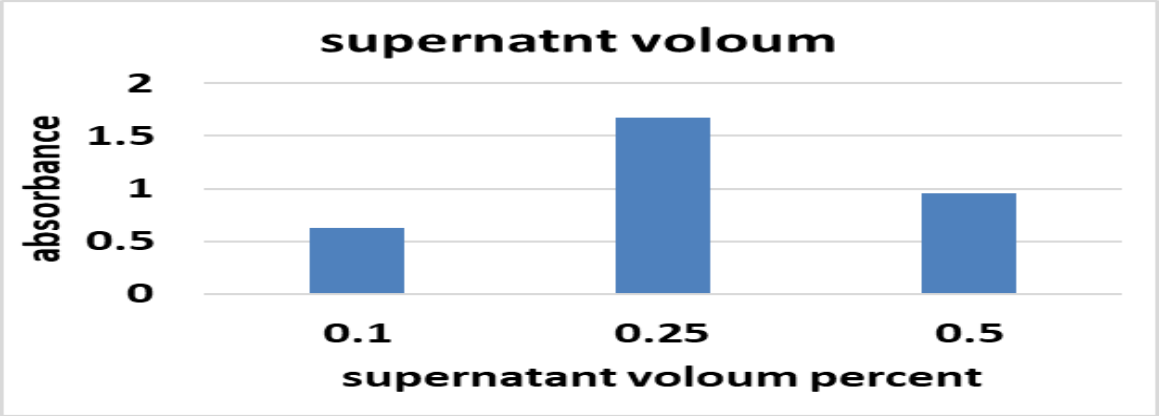


Figure 8. The optimization of nano particles synthesis by changing the silver nitrate concentration, by changing the final mixture PH.

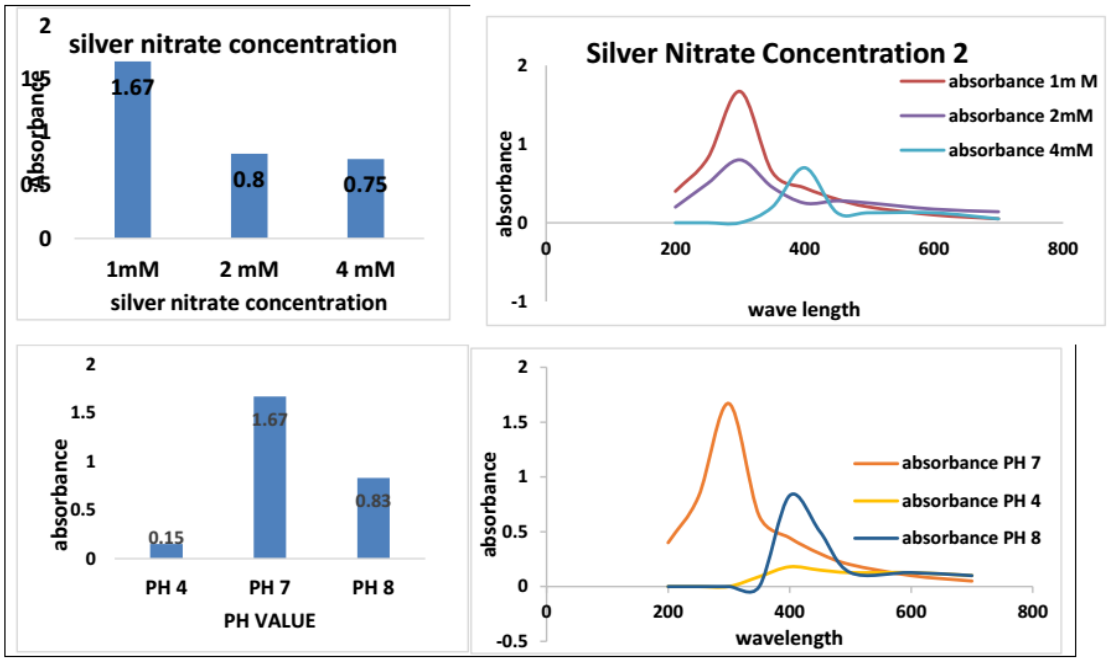


Figure 9. TEM pictures of silver nanoparticles synthesized by *Acinetobacter baumannii* supernatant.

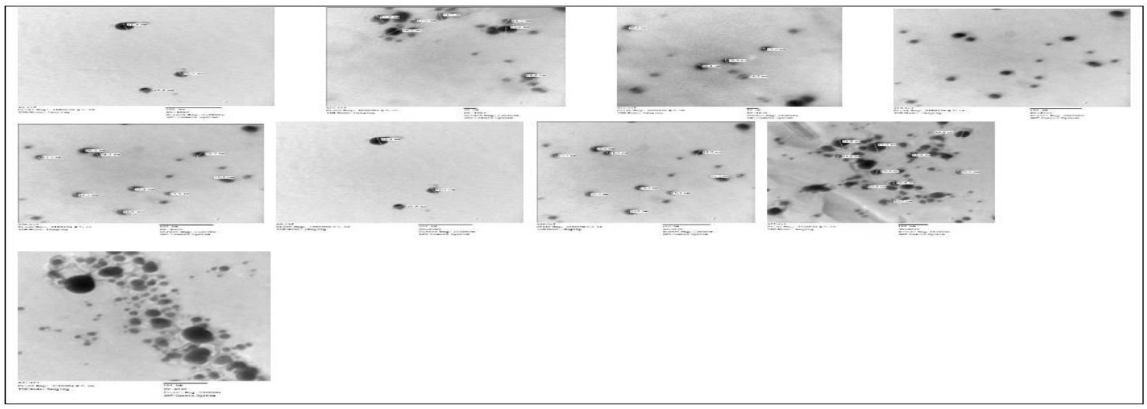


Figure 10. FTIR of silver nanoparticles formed by *Acinetobacter baumannii* supernatant.

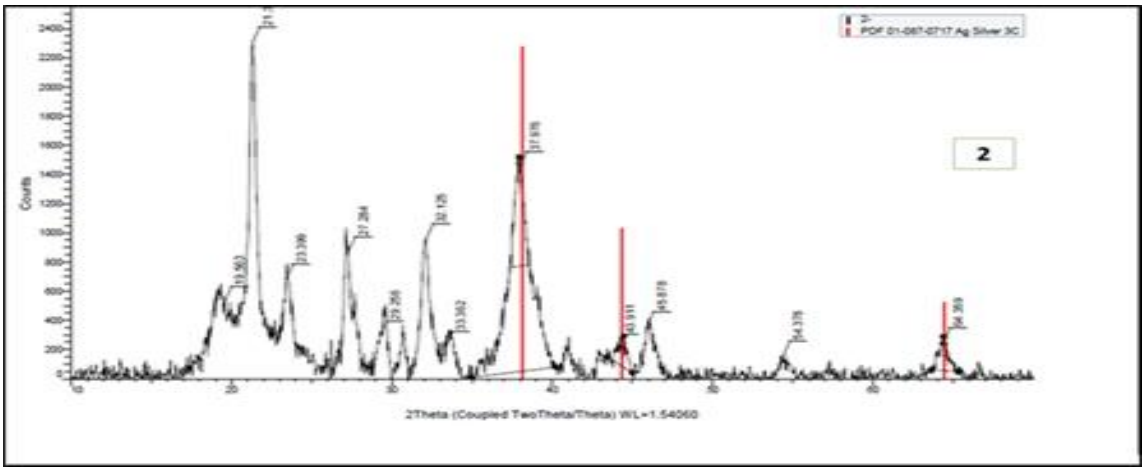


Figure 11. Effect of AgNPs on pathogenic bacteria *Klebsiella pneumonia, Pseudomonas aeruginosa*.

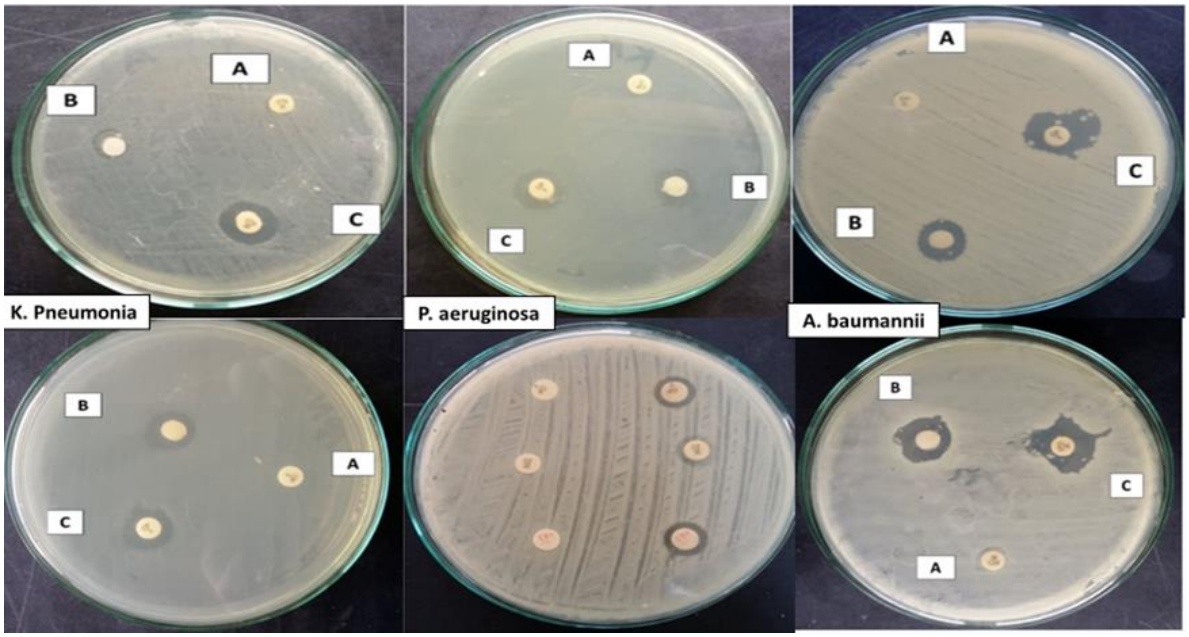
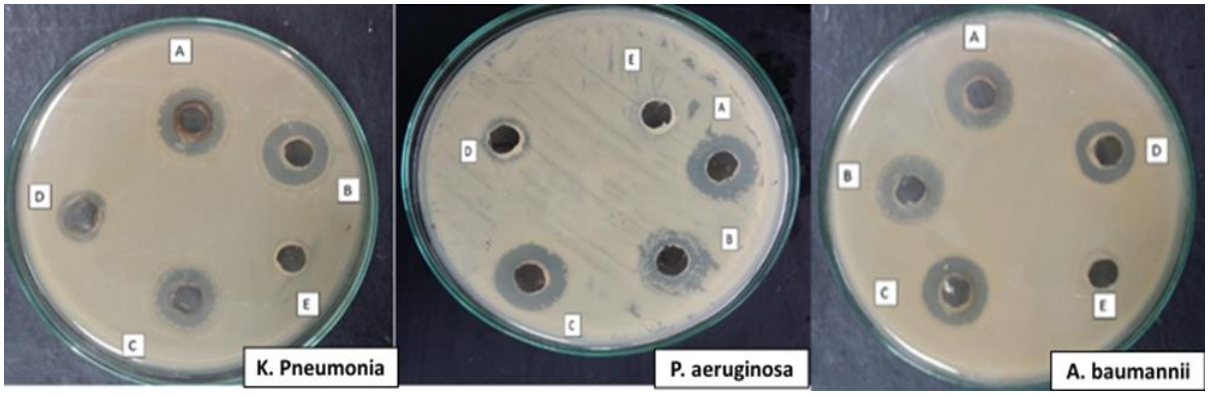


Figure 12. Effect of *Acinetobacter baumannii* nanoparticles on pathogenic bacteria *Klebsiella pneumonia, Pseudomonas aeruginosa, Acinetobacter baumannii*, A for AgNPs alone, B for antibiotic alone, C for antibiotic combination with AgNPs.



Discussion

Nanomaterials provides a solution for many causes of increasing mortality and morbidity as cancer and antibiotic resistant bacteria. The detection of AgNPs is confirmed in the current study by measuring UV - visible absorption intensity as in **figure (3)**. That is matched with the (UV–vis) spectra reported in a study [18]. As well as absorption peak at 390 nm from *Acinetobacter baumannii* [19], and *Acinetobacter calcoaceticus*, the UV-Vis spectrum, was detected at 440 nm [20] as well as the silver nanoparticle characteristic absorption peak using *Klebsiella pneumonia* [21].

In our study, the broad absorption band detected at about 420 nm is indicating the biosynthesized Ag-NPs presence by using the *P. aeruginosa* culture supernatant. The synthesis of silver nanoparticles was observed in a similar study [22]. This also confirmed by **Monowar et al.** study [4], that explained the reduction ability of A baumannii strain in formation of nano silver particles from silver nitrate solution.

In this study, optimization of nanoparticles synthesis was done by changing the volume of supernatant as it has effects on reaction time and color of the reaction mixture. As the concentration of culture supernatant increased the synthesis was also observed to be higher, which could be related to the enzyme and secondary metabolites concentration but in cell free supernatant of *A. baumannii.*

Our study entails optimization of Nano particles synthesis by changing silver nitrate concentration the absorption intensity was observed

higher for the AgNPs which agreed with studies [20].

Current study shows that the optimization of condition by changing PH 4, PH 7 and PH 8 of final mixture has direct effect on biosynthesis of AgNPs as each enzyme has a specific pH range. changing the pH will affect enzyme activity. **Alqadi et al.** conclude that raising the final pH of the solution leads to the formation of smaller size nanoparticles and vice versa [23].

In the present study, TEM analysis of the nanoparticles showed that nanoparticles were homogeneous with spherical morphology, the size of AgNPs was between 12 nm and 50 nm. This result agreed with **Wadhuani et al.** [20]. On the other hand, **Shaker & Shaaban** mentioned that the AgNPs were dispersed in the supernatant, spherical shape and the particle size was 37-168 nm [19], Also, **Wan et al**. mention that AgNPs were 5–12 nm in diameter, with a size average 8.4 nm. The nanoparticles were found to be stable for over 6 months, even at 37°C [18]. Our study revealed that, X-ray diffraction on *A.baumannii* AgNPs formed at 2 θ values 32.125◦, 37.976◦, 43.911◦, 54.378◦ and 64.359◦, indicated (111), (200), (210), (321), (310) respectively. Our result is agreed with **Wadhwani et al.** [20].

In this study, FT-IR spectrum analysis of the compound matched with a study revealed that proteins were responsible for stabilizing the AgNPs synthesized by using the culture filtrate of *A. baumannii* [20].

In the present study, we found that antimicrobial activity of biosynthesized AgNPs against pathogenic bacteria *A. baumannii, K. pneumonia and P. aeruginosa* by well diffusion assay show god antibacterial efficacy and show higher antibiotic efficacy when combined with antibiotic disc due to its destruction of cell wall and facilitation of antibiotics entrance inside bacterial cell. AgNPs may penetrate different bacterial cell parts such as peptidoglycan, DNA and protein stop cell replication which cause cell damage [24]. Other studies reported that their relation to the generation of oxidative stress (OR) in bacteria, **Quinteros et al.** report that AgNPs generated oxidative stress in *S. aureus, E. coli,* and *P. aeruginosa* mediated by the increase of reactive oxygen species and that was correlated with better antimicrobial activity [25]. Extracellular biosynthesis of AgNPs using a *P. aeruginosa 25* and *E. coli* [26]. Also, an evaluation of the antimicrobial activity of the biosynthesized AgNPs against opportunistic microorganisms and, human pathogens such as *Staphylococcus epidermidis, S. aureus, Acinetobacter baumannii, Enterococcus faecalis, Proteus mirabilis, P. aeruginosa, E. coli, S. typhi, Klebsiella pneumoniae, Bacillus subtilis, Vibrio* [27]. Our result agreed with the MICs of AgNPs were determined to be 3.125 mg/ml against *E. coli*, 1.56 mg/ml against *P. aeruginosa* and 3.125 mg/ml against *K. pneumoniae* [19,20] which mention that the MIC. For AgNPs, was in the range of 150–600 mg/L against Gram-negative bacteria.

In our study, the anticancer and cytotoxicity efficacy of AgNPs is evaluated by (SRB assay) on Hep G2 Hepatocellular carcinoma cell line and LC 50% is 60.584 µg/ml, our result is agreed with *Vijayakumar et al* and in *wan et al*. results approved that the cytotoxicity effect of AgNO3 is higher than AgNPs [28]. that is approved in our study as concentration 10 µg/mL show very small cytotoxic effect about 0.6 % In other study done by **Raj** [29] investigates the cytotoxic potential of β-sitosterol-assisted silver nanoparticles (BSS-SNPs) in HepG2 cells, the silver nanoparticles have very high cytotoxic activity as The IC50 of BSS-SNPs in HepG2 cells was found at 7 ng/mL [27, 30].

We conclude that, *A. baumannii* cell free filtrate contain reducing agent can reduce silver nitrate to AgNPs with significant antibacterial activity alone and synergistic action with other antibiotics and has moderate cytotoxic activity which considered as promising therapeutic option as anticancer in this study examined with hepatocellular carcinoma cell line and moderate number of pathogenic bacteria. in further studies we will need to examine its activity on another cell lines and bacteria.

Conflict of interest

All authors declare no has no conflict of interest.

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Author contribution

MA, MK, and **RA** conceived and designed research. **MA, FK,** and **AB** conducted experiments. **MA and FK** describe results and wrote the manuscript. All authors approved the manuscript.

Ethical statement

Informed consent was obtained from all individual participants included in the study before obtaining their culture sample. All procedures

performed in studies involving human participants were in accordance with the ethical standards of the National Liver Institute**,** Menoufia University and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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