RESEARCH ARTICLE / ARAŞTIRMA

DOI: 10.4274/mjima.galenos.2021.2021.50
Mediterr J Infect Microb Antimicrob 2021;10:50
Erişim: http://dx.doi.org/10.4274/mjima.galenos.2021.2021.50

Silver Nanoparticles: Green Synthesis Using Brain Heart Infusion Culture Medium and Evaluation of Antimicrobial and Antibiofilm Activity Against *Acinetobacter baumannii*

Gümüş Nanopartiküller: Beyin Kalp İnfüzyon Kültürü Besiyeri Kullanılarak Yeşil Sentezi ve *Acinetobacter baumannii*'ye Karşı Antimikrobiyal ve Antibiyofilm Aktivitelerinin Değerlendirilmesi

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Abstract

Introduction: In recent years, the use of nanoparticles (NPs) to eliminate pathogenic bacteria is increasing. The development of biological and nontoxic methods for synthesizing NPs is an important topic in nanotechnology to allow the use of these NPs for health and medical purposes. This study aimed to synthesize silver NPs (AgNPs) using bacterial culture medium and then evaluated the antibacterial activity of these NPs against *Acinetobacter baumannii*.

Materials and Methods: The synthesis of biocompatible AgNPs was performed by brain heart infusion culture medium. Synthesized NPs were observed using ultraviolet-visible spectroscopy and were characterized by Fourier transform infrared spectroscopy (FTIR). Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) were conducted to examine the morphology of AgNPs. The minimum inhibitory concentration (MIC) was determined to measure the antibacterial effect of AgNPs. Finally, antibiofilm activities of the synthesized AgNPs were assessed.

Results: Ultraviolet-visible spectroscopy showed that the highest absorption of the synthesized NPs was at 420 nm. Results of SEM and TEM revealed that the morphology of particles was spherical and irregular, and their mean diameter was 26 nm. In addition, FTIR results confirmed that the reducing, capping, and stabilizing agents of NPs were proteins. Minimum inhibitory concentration of AgNPs ranged from 1.56 to 12.5 µg/ml, and at 2 MIC concentration, the biofilm formation inhibitory activity of NPS increased.

Conclusion: Brain heart infusion medium was successfully applied for synthesizing stable AgNPs, and these NPs showed good antibacterial and antibiofilm activity against *A. baumannii*.

Keywords: Silver nanoparticles, synthesis, antibiofilm, antibacterial, *Acinetobacter baumannii*

Öz

Giriş: Son yıllarda patojenik bakterileri yok etmek için nanopartiküllerin (NP) kullanımı artmaktadır. Nanopartikülleri sentezlemek için biyolojik ve toksik olmayan yöntemlerin geliştirilmesi, nanoteknolojide bu NP'lerin sağlık ve tıbbi amaçlar için kullanımına izin vermek için önemli bir konudur. Bu çalışma, bakteri kültür ortamı kullanılarak gümüş NP'leri (AgNP) sentezlemeyi ve ardından bu NP'lerin *Acinetobacter baumannii*'ye karşı antibakteriyel aktivitesini değerlendirmeyi amaçladı.

Cite this article as: Solouki M, Moniri R, Fathizadeh H, Kashi FJ, Moosavi GA, Nazari-Alam A. Silver Nanoparticles: Green Synthesis Using Brain Heart Infusion Culture Medium and Evaluation of Antimicrobial and Antibiofilm Activity Against *Acinetobacter baumannii*. Mediterr J Infect Microb Antimicrob. 2021;10:50.

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Received/Geliş Tarihi: 21.04.2021 **Accepted/Kabul Tarihi:** 18.08.2021

Published: 19 August 2021

Gereç ve Yöntem: Biyouyumlu AgNP'lerin sentezi beyin kalp infüzyonu (BHI) kültür ortamı ile gerçekleştirildi. Sentezlenen NP'ler, ultraviyole-görünür spektroskopisi kullanılarak gözlendi ve Fourier transform kızılötesi spektroskopisi (FTIR) ile karakterize edildi. Gümüş NP'lerin morfolojisini incelemek için taramalı elektron mikroskobu ve transmisyon elektron mikroskobu (TEM) kullanıldı. Gümüş NP'lerin antibakteriyel etkisini ölçmek için minimum inhibitör konsantrasyon (MIC) belirlendi. Son olarak sentezlenen AgNP'lerin antibiyofilm aktiviteleri değerlendirildi.
Sonuçlar: Ultraviyole-görünür spektroskopisi, sentezlenen NP'lerin en yüksek absorpsiyonunun 420 nm'de olduğunu gösterdi. Taramalı elektron mikroskobu ve TEM sonuçları, parçacıkların morfolojisinin küresel ve düzensiz olduğunu ve ortalama çaplarının 26 nm olduğunu ortaya çıkardı. Ek olarak, FTIR sonuçları, NP'lerin indirgeyici, örtücü ve stabilize edici ajanlarının proteinler olduğunu doğruladı. Gümüş NP'lerin MIC değeri 1,56 ila 12,5 µg/ml aralığındaydı ve 2 MIC'de, NPS'nin biyofilm oluşumunu inhibe edici aktivitesi arttı.
Sonuç: Stabil AgNP'lerin sentezlenmesi için BHI ortamı başarıyla uygulandı ve bu NP'ler *A. baumannii*'ye karşı iyi antibakteriyel ve antibiyofilm aktivitesi gösterdi.
Anahtar Kelimeler: Gümüş nanoparçacıklar, sentez, antibiyofilm, antibakteriyel, *Acinetobacter baumannii*

Introduction

In recent years, the incidence of antibiotic resistance in various bacteria has increased. Annually, approximately 700,000 people globally die from diseases related to antimicrobial resistant pathogens[1]. *Acinetobacter baumannii* is one of the most important pathogens. It is a Gram-negative, strictly aerobic, nonmotile coccobacillus, and an opportunistic nosocomial pathogen. This bacterium is associated with serious diseases such as urinary tract infections, pneumonia, wound infections, septicemia, and severe cases of necrotizing fasciitis[2]. *Acinetobacter baumannii* can form biofilms after adhering to abiotic and biotic surfaces. As one of the most important virulence factors of *A. baumannii*, the ability to form biofilms plays an important role in the stability on different surfaces as well as in the resistance to various antibiotics. Therefore, it is difficult to control and eliminate infections caused by this bacterium[3].

Silver (Ag) is a natural antimicrobial agent, and the use of nanotechnology and nanoparticles (NPs) has increased its efficiency. Thus, AgNPs have more antibacterial properties than Ag metal[4-9]. Nanotechnology has a remarkable probable to control biofilm-associated infections produced by different pathogens[10]. Ag products are used as antimicrobial agents in many medical conditions, including traumatic wounds, burns, and diabetic ulcers as well as for coating catheters, wound dressing, and other devices implanted on or within the body[11]. Ag compounds of different concentrations and structures are used in clinical practice, e.g., Ag sulfadiazine is the gold standard for the treatment of topical burns[12,13]. Acute and chronic burns and wounds are treated using dressings containing Ag[11]. In recent years, researchers have paid special attention to AgNPs and their antimicrobial activity owing to special physical and chemical properties, biocompatibility, aqueous solubility, and biodegradable nature of these NPs[14]. To date, AgNPs have been used to treat injury, wounds, and burns as well as to treat cancer[11,15-17]. One study demonstrated the significant antibacterial effects of biosynthesized AgNPs against *Staphylococcus epidermidis* strains isolated from patients (pus, blood samples, and catheter tips) as well as against other pathogenic strains *(Vibrio cholerae, S. aureus, Salmonella typhi*, and *S. paratyphi)*; in addition, the study proved that this antibacterial property against *S. typhi* increased when AgNPs were combined with chloramphenicol[18]. Moreover, in several studies, the antibacterial effects of AgNPs against different strains of *Acinetobacter* have been indicated[19-22]. Silver NPs can be synthesized using different methods, such as physical, chemical, and biobase methods[23-27]. The advantage of biological methods over other methods is their eco-friendliness, cost-effectiveness, easy production ability. Biological methods are performed using plants, fungi, or bacteria[28,29]. In these methods, the stability and characteristics of NPs are dependent on the species of microorganisms used, their genetic properties, and the growth conditions of cells[30]. Therefore, extensive research is required to overcome these challenges that various methods of green synthesis NPs. Thus, the purpose of the present study was to synthesize AgNPs by a green method. In addition, the study assessed the antibacterial and antibiofilm effects of the synthesized AgNPs against *A. baumannii*.

Materials and Methods

Chemicals and Reagents

Chemicals required for experiments, including brain heart infusion (BHI); blood agar; MacConkey's agar; Triple Sugar Iron (TSI); Sulfide, Indole, Motility; were obtained from Hi-media (India), and Mueller-Hinton broth (MHB); Luria-Bertani (LB) liquid medium; and Ag nitrate, were purchased from Merck (Germany).

Study Design and Bacterial Isolates

In this cross-sectional study, a total of 70 clinical isolates of *A. baumannii* were collected from patients hospitalized at the teaching Shahid Beheshti hospital in Kashan during April to September 2017. Ethics committee approval (IR.KAUMS.MEDNT.REC.1396.071) was obtained before initiating the study. Isolates were recognized according to colony morphologies, Gram staining, growth on MacConkey's agar, TSI test, motility test, oxidase, and catalase reaction. For molecular confirmation, this method was used based on the presence of the blaOXA-51-

like carbapenemase gene. Clinical isolates were maintained in trypticase soy broth with glycerol (30%) at -70 °C[31].

Biological Synthesis of AgNPs and Ultraviolet–visible Spectra Analysis

Brain heart infusion medium was used for the reduction and synthesis of AgNPs. In the first stage, 37 g of BHI was dissolved in 1 L distilled water, and the solution was sterilized in the autoclave. Then, 10 ml of BHI medium was added to 10 ml of 0.5 mM Ag nitrate solution. The solution was then subjected to mechanical shaking at 22 °C for at least 24 h. Subsequently, the solution was kept of head-on small desk lamp at all time. A change in the color of the aqueous solution from light yellow to yellowish-brown indicated the formation of AgNPs. In the next step, reduction of Ag+ ions in the final yellowish-brown Ag nitrate solution was monitored by recording the ultraviolet-visible (UV-vis) spectrum between 300 and 800 nm using a spectrophotometer (Jenway, England).

Silver Nanoparticles Purification

The final solution was transferred to 2-ml sterile microtubes for centrifugation at 12,000 rpm for 15 min. After removal of the supernatant, pellets were collected. Then, the precipitate was carefully rinsed thrice with sterile distilled water. Subsequently, the pellets were rinsed twice with 96% ethanol. Purified pellets were dried to obtain a powder. The powder was then collected for final confirmatory tests to produce NPs.

Final Characterization of Synthesized AgNPs

Silver NPs were confirmed using several methods. Initially, Fourier transform infrared spectroscopy (FTIR) was conducted to assess the functional groups in synthesized AgNPs. Using an FTIR spectrophotometer (Nicolet, USA), a spectrum was obtained in the range of 4000-400 cm^{-1}. To evaluate the crystalline structure of AgNPs, X-ray diffraction (XRD) scanning was conducted in the 20°-60° range. Then, to study the shape and size of the synthesized AgNPs, images of NPs were obtained using a transmission electron microscope (TEM) (Leo, Germany) at accelerating voltages in the excess of 300 kV. In scanning electron microscopy (SEM), TESCAN BRNO-Mira3 LMU (Czech Republic) was used to confirm of the surface morphology of NPs. Images of AgNPs were obtained at an accelerating voltage of 10 kV. Energy-dispersive X-ray analysis (EDX) was used to identify the elemental composition of NPs. For this analysis, EDX Tescan device (Czech Republic) was used.

Biofilm Formation Assay

Microtiter plate examination for the quantitative detection of biofilm was conducted using a microtiter plate method, as explained previously[32]. In brief, *A. baumannii* isolates were cultured in LB medium and incubated at 37 °C for 24 h. The cultures were diluted (1:100) in fresh LB. Then, 200 ml of the diluted microbial solution was poured to the wells of a 96-well polystyrene microtiter plate. The plate was then incubated at 37 °C overnight. In the next stage, wells were carefully washed three times with 200 µl of phosphate-buffered saline (pH: 7.4). Then, biofilm structures were fixed by methanol for 20 min and then dried at ambient air and room temperature. Wells were stained with 200 µl of 2% crystal violet for 5 min. Subsequently, all wells were washed with distilled water and dried at room temperature. Afterward, 200 ml of acetic acid containing crystal violet was added to the wells. Lastly, the optical density (OD) of each well was calculated at 570 nm using an enzyme-linked immunosorbent assay reader (Awareness Technology, USA). The OD cut-off was determined as the mean OD of negative control + 3 × standard deviation of the negative control. The formation of biofilms by isolates was analyzed and arranged based on the absorbance of crystal violet-stained linked cells (Table 1). *Staphylococcus epidermidis* ATCC 35984 was used as a as reference strain of high-slime biofilm producer.

Table 1. Categorization to produce biofilms in *A. baumannii*

Biofilm formation abilities	Mean of OD values results	Cut-off value calculation
None	OD ≤ 0.059	OD ≤ 0.059
Weak	0.059 < OD ≤ 0.118	ODc < OD ≤ 2 × ODc
Moderate	0.118 < OD ≤ 0.236	2 × ODc < OD ≤ 4 × ODc
Strong	OD > 0.236	OD > 4 × ODc

OD: Optical density

Evaluation of Antimicrobial Susceptibility of AgNPs in A. baumannii

The antimicrobial susceptibility of AgNPs against 46 of a total of 70 *A. baumannii* isolates was determined by the broth microdilution method according to guidelines of the Clinical and Laboratory Standards Institute (CLSI)[33]. The following concentrations of AgNPs were used: 200, 100, 50, 25, 12.5, 6.25, 3.12, 1.56, and 0.78 µg/ml. Microplates were incubated at 37 °C in ambient air for 18-20 h. Briefly, bacterial inoculums in MHB medium were adjusted to 0.5 McFarland standard (1.5×10^8 CFU/ml). Then, this suspension was diluted (1:20) to yield a concentration of 10^6 CFU/ml. Ten microliter of each inoculum was added to wells containing 100 µl of MHB and AgNPs. Final test concentration of the microbes was 5×10^5 CFU/mL. Microplate containing suspension and NPs were incubated at 37 °C for 18-24 h. *Acinetobacter baumannii* ATCC 19606 strain was used as a quality control strain for susceptibility testing.

Evaluation of Minimum Inhibitory Concentration of AgNPs on Planktonic Form of *A. baumannii*

The minimum inhibitory concentration (MIC) of AgNPs against 46 planktonic forms of *A. baumanni*i was determined using the microdilution assay. Serial dilutions of AgNPs were prepared

based on the CLSI guideline. The wells containing AgNPs in MHB medium and strains at 10^5 CFU/ml were incubated at 37 °C. The MIC was determined after incubation for 24 h.

Evaluation of Biofilm Inhibition Activity of AgNPs

The microtiter assay was used to determine the biofilm inhibition activity, as previously described[21].

Statistical Analysis

All tests were replicated thrice. Results were analyzed using the Statistical Package for the Social Sciences 16 software and Kolmogorov-Smirnov test, Kruskal-Wallis test, and Mann-Whitney test. Results with $p \leq 0.001$ were regarded as statistically significant.

Results

Characterization of AgNPs Synthesized in Brain Heart Infusion Medium

Ultraviolet-visible Spectroscopy and XRD Pattern

Visual observation of the BHI medium incubated with $AgNO_3$ showed a color change from light yellow or from colorless to yellowish-brown, clearly indicating the formation of AgNPs (Figure 1A). The formation and stabilization of AgNPs in aqueous solutions were studied using UV-vis spectroscopy. Figure 1B shows a sharp peak at 420 nm in UV-vis spectrum that is attributed to the formation of AgNPs and the surface resonance. The exact nature of AgNPs can be deduced from the XRD spectrum of the sample, which is presented in Figure 2. The phase variety, grain size, and crystalline nature of NPs were determined by XRD studies. The diffraction peaks at 2θ values of 27.28°, 32.23°, 46.24°, 54.82°, 57.49°, and 67.46° were assigned to the (111), (200), (220), (311), (222), and (400) planes, respectively, of AgNPs. The peak at 38.07° was attributed to the diffraction of the (111) plane of metallic Ag.

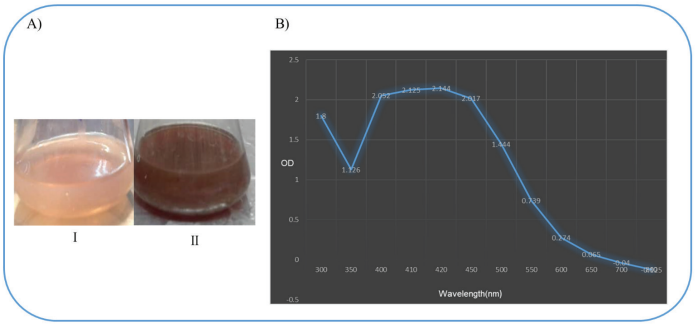

Figure 1. A) Aqueous solutions of (I) silver nitrate without addition of brain heart infusion (BHI) medium, (II) silver nanoparticles synthesized with BHI medium. B) Ultraviolet-vis spectrophotometric analysis of silver nanoparticles

Fourier Transform Infrared Spectroscopy

The FTIR spectrum identified the biomolecules responsible for capping and stabilization of AgNPs. Fourier transform infrared spectroscopy spectrum in the range of 400 to 4000 cm^{-1} showed absorption peaks at 3428.75, 1639.43, and 1384.36 cm^{-1}. The peaks at 3400-3500 cm^{-1} and 1560-1640 cm^{-1} indicated a stretch for N-H bond, and the peak at 1350-1520 cm^{-1} indicated a stretch for N-O bond (Figure 3). Thus, the synthesized AgNPs were capped by proteins and a nitro compound.

Scanning Electron Microscopy and EDX Analyses

The SEM images showed that the AgNPs had an irregular shape, with a mean particle size of 26 nm (Figure 4A). In addition, EDX analysis measured the elemental structure of the synthesized powder. As shown in Figure 4B, the EDX spectrum had the highest peak at around 3 keV, followed by that at approximately 2.6 KeV, which indicated that Ag and chlorine atoms, respectively, were the main elements. Furthermore, the EDX spectrum revealed that the weakest peaks corresponded to carbon and nitrogen. As shown in Figure 4C, TEM image of the AgNPs indicates spherical morphology with a size of 26 nm. The particle distribution diagram is shown in Figure 4D.

Antibacterial Activity of AgNPs

Among all *A. baumannii* isolates that were characterized by the phenotypic method and were confirmed by polymerase chain reaction of the blaOXA-51-like gene, a single band of the right size was obtained as shown in Figure 5. Of 70 clinical isolates of *A. baumannii*, 46 could form biofilms, of which 98% formed strong biofilms and 2% formed moderate biofilms. The antibacterial effect of AgNPs against 46 isolates of *A. baumannii* was determined, and the MICs was in the range of 1.56-12.5 µg/ml.

Antibiofilm Activity of AgNPs

Silver NPs have also been assayed for antibiofilm activity against biofilm-forming *A. baumannii*. The assay revealed that the biosynthesized AgNPs inhibited biofilm formation by *A. baumannii*, relative to the negative control used in the experiment. Figure 6 shows that 2 MIC, MIC, and 1/2 MIC concentrations of AgNPs inhibited biofilm formation of 46 isolates by 74.53%, 64.98%, and 58.27%, respectively.

Discussion

Biofilm formation is one of the important mechanisms that bacteria use to avoid accumulation of antimicrobial compounds and protect against them[34]. As a hospital opportunistic pathogen, *A. baumannii* has the ability to form biofilms on living and nonliving surfaces such as internal catheters[35]. Nanotechnology has been recently used to remove resistant bacteria and biofilm

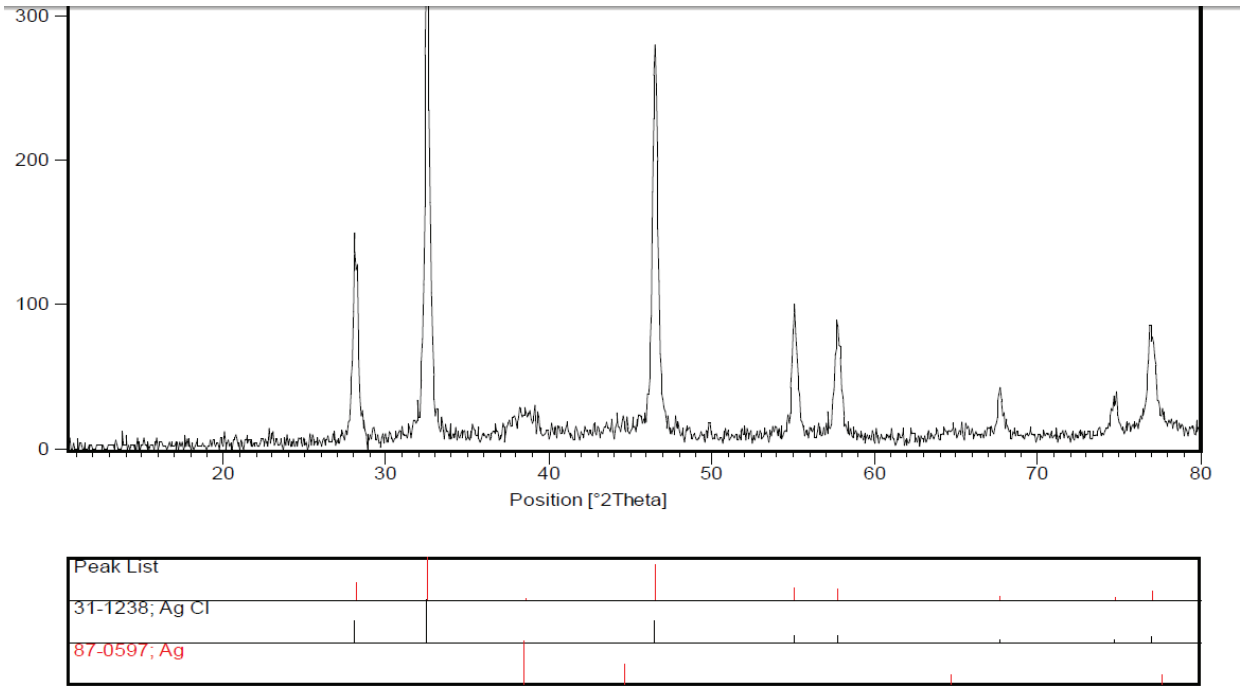

Figure 2. X-ray diffraction of silver nanoparticles

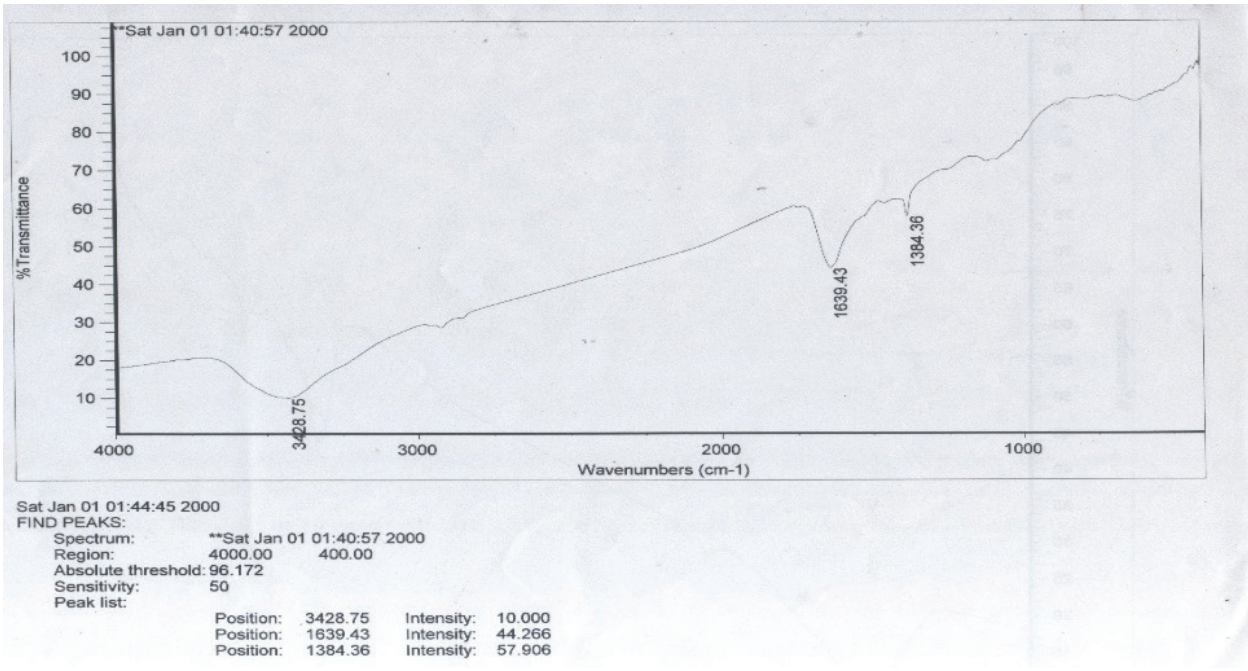

Figure 3. Fourier transform infrared spectroscopy analysis of silver nanoparticles

produced by them. Nanoparticles of metals are being proposed as new antimicrobial agents. Today, numerous NPs and nanocarriers are available as antimicrobial compounds[22,36,37]. The present study is the first to succeed in synthesizing AgNPs using a bacterial culture medium, and the method of synthesis was patented. The synthesized NPs were assessed using various methods, including UV-vis spectrophotometry, SEM, EDX, TEM, XRD, and FTIR spectroscopy. Then, antibacterial and antibiofilm activities of the synthesized NPs were assayed. Studies have reported on the role of Ag ions as antimicrobial agents in clinical cases[38,39]; however, the clinical use of these ions is limited owing to high toxicity, low stability, and the formation of complexes with salts and precipitation[40]. Thus, the clinical use of Ag compounds is subject to cytotoxicity studies in human cell lines as well as laboratory

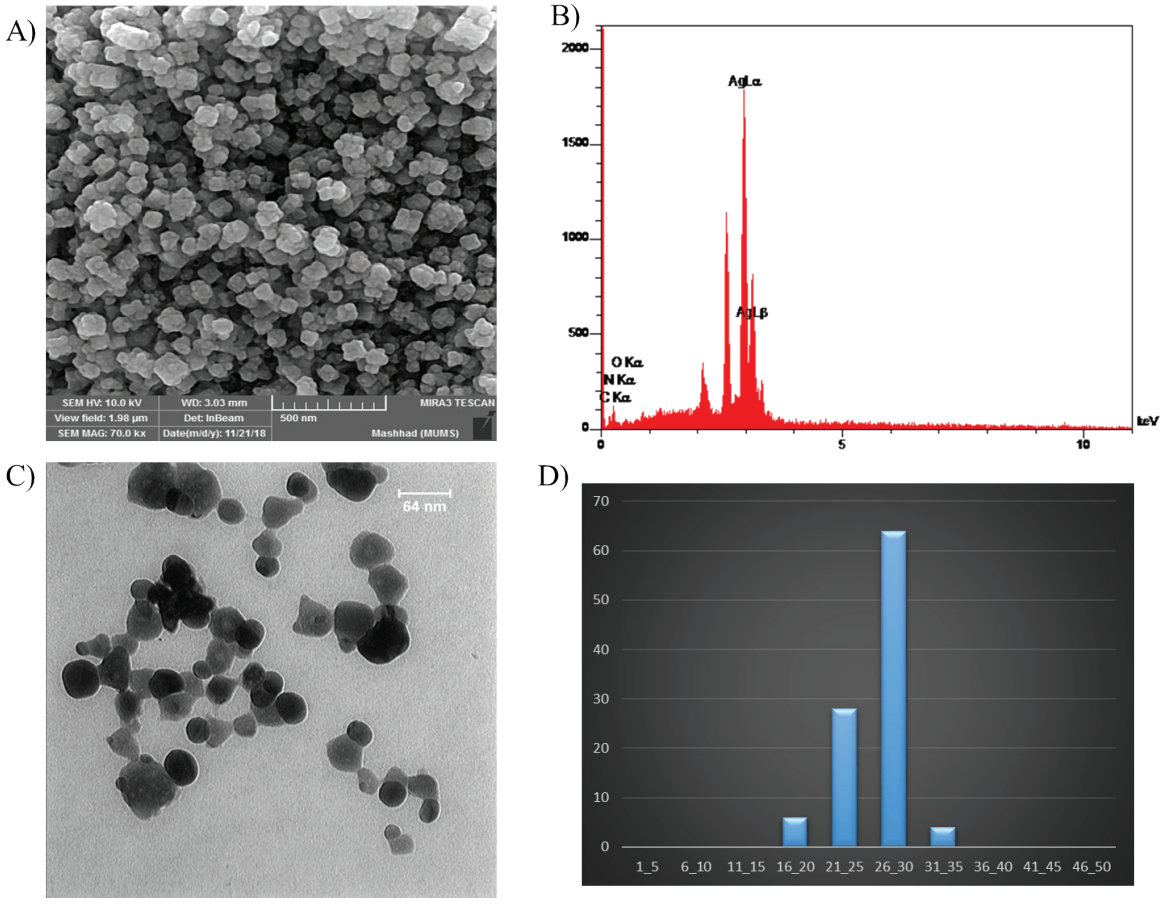

Figure 4. A) Scanning electron microscopy analysis of synthesized silver nanoparticles (AgNPs). B) Energy-dispersive spectroscopy spectrum of AgNPs. C) Transmission electron microscopy analysis of synthesized AgNPs. D) Size distribution of silver nanoparticles

animal models[11,41,42]. To overcome these limitations, AgNPs were awarded Ag ions. Silver NPs have been used in clinical cases owing to properties such as wide contact surface, surface plasmon resonance (SPR), and different physical, chemical, and mechanical properties[43]. There are several methods for synthesizing AgNPs and their derivatives, including chemical, physical, and biogenic methods. Biogenic methods are preferred to chemical and physical methods as they are economically viable, consume less energy, biocompatible, and environmentally friendly[43]. These include the use of plants, bacteria, and fungi in the synthesis of AgNPs. These methods are also associated with limitations such as the requirement of specific conditions for the production, collection, and storage of NPs[44]. In our study, AgNPs were synthesized using the BHI broth culture medium (green method), which is simple and inexpensive compared to other methods. The use of this method also led to the synthesis of good quality NPs in a short time. Notably, AgNPs were synthesized by the addition of 10 ml of BHI medium to 10 ml $AgNO_3$ solution. Color change of the solution to yellowish-brown indicates a special reaction between the compounds in the BHI medium with Ag ions, indicating the reducing power of BHI compounds and the formation of AgNPs.

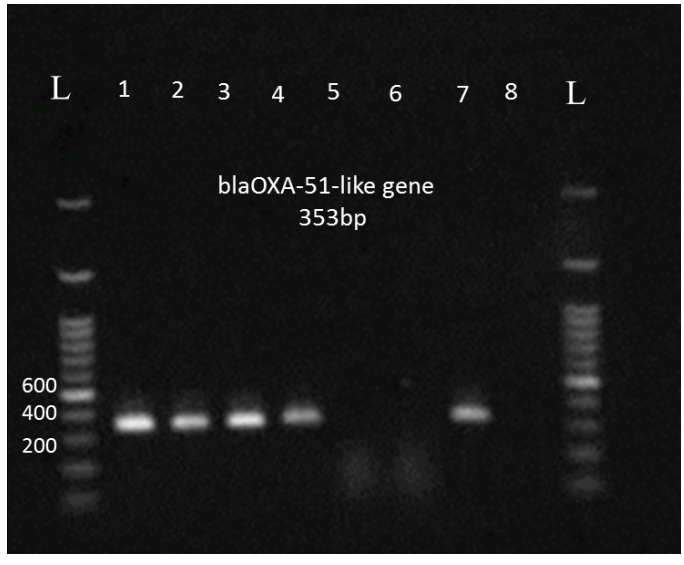

Figure 5. Polymerase chain reaction amplification of the blaOXA-51-like gene

We used UV-vis spectrophotometry to continuously monitor the synthesis of AgNPs. Ultraviolet-visible spectroscopy is an established method for the study of metal NPs. In our study, the

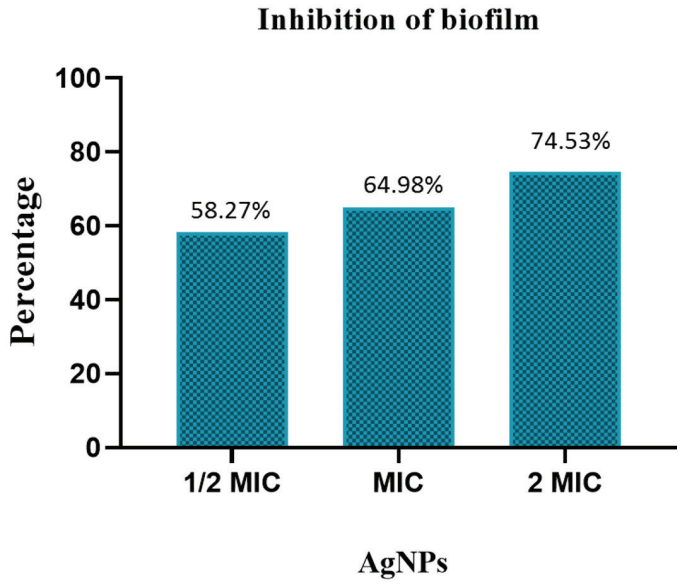

Figure 6. Antibiofilm activity of silver nanoparticles against *A. baumannii*

MIC: Minimum inhibitory concentration, AgNPs: Silver nanoparticles

highest absorption was noticed at 420 nm, which is the peak range characteristic for AgNPs. This characteristic and specific peak is attributed to the SPR of the particles, a characteristic that has been extensively established and recognized for various metal NPs in the 2-100 nm size range[45]. The absorption peak of AgNPs was clearly detected because of the combined vibration of AgNP electrons in resonance with the specific light wavelength. Notably, Mohanta et al.[46] synthesized AgNPs using medicinal plant extracts. Observation of the color showed a change to dark brown owing to the reducing power of plant extracts. Ultraviolet-visible spectrophotometry also showed a broad peak within the 420-430 nm range, indicating the formation of AgNPs. Similar results to our study have been reported in several studies on the synthesis of AgNPs[47-49]. The XRD pattern showed four peaks at 2θ values of 32°, 44°, and 52°. The XRD analysis indicated that the size of NP crystals was 28 nm. SEM and TEM measurements revealed that NPs were spherical, with sizes in the range of 16-35 nm. More than 60% of the particles were approximately 26 nm in size. This difference in particle size may lead to the formation of NPs at different time intervals. In this study, we used AgNPs we used AgNPs with extremely small size so more efficient other than similar study. Although irregular shapes were also observed in the spherical NPs, most of them had a spherical structure. Owing to the increased contact surface, smaller NPs are more effective than larger NPs. Nanoparticles function via binding to the cytoplasmic membrane of bacteria, impairing membrane permeability, and disrupting other cellular activities[5]. In a study by Shaker and Shaaban[50], AgNPs of 37-168 nm were synthesized by microbial methods. They stated that synthesized AgNPs are capable of inhibiting and eradicating biofilms made by multidrug-resistant strains isolated from individuals with uropathogenic infections. In our study, antibacterial analysis on planktonic and biofilm forms of 46 isolates of *A. baumannii* were conducted using synthesized AgNPs. The results indicated that AgNPs had an inhibitory effect on the planktonic and biofilm forms of *A. baumannii*. The range of MIC based on CLSI was 1.56-12.5 µg/ml, and at a 2MIC concentration, AgNPs had greater inhibitory activity against biofilm formation of 46 isolates. Similar to our study, a previous study analyzed AgNPs synthesized by green method and demonstrated the maximum optical absorption of NPs by UV–vis spectrophotometry at 454 nm. In addition, electron microscopy analysis revealed that the shape of NPs was oval and the size was 10-20 nm. Minimum inhibitory concentration range of this nanocomposite was 40-60 µg/ml for *Listeria monocytogenes*, *S. aureus*, *S. saprophyticus*, *Escherichia coli*, and *Pseudomonas potida*[51]. In 2017, de Jesús Ruíz-Baltazar et al.[52] synthesized AgNPs using *Melissa officinalis* leaf extract. The spherical shape of AgNPs was determined by TEM analysis, and the average size of the synthesized NPs was reported as approximately 12 nm. The antibacterial effect of these NPs on *E. coli* and *S. aureus* was investigated, and the diameter of the growth inhibition zone was 6-11.5 mm. In another study by Chauhan et al.[53], the antibacterial effect of Ag/AgCl NPs (size, 68 nm) produced by *Streptomyces* spp. JAR1 was measured by the disk diffusion method against *E. coli*. The diameter of the bacterial growth inhibition zone was approximately 12 mm. Biosynthetic and nontoxic synthesis, maximum light absorption at 420 nm, and the stability of produced NPs are some of the similarities between this study and our work. Overall, it can be concluded that the synthesis of AgNPs using the BHI broth culture medium, which was performed for the first time, can be a useful and effective method for the production of AgNPs. The advantage of this method over chemical and physical methods is low toxicity and time and cost savings. In addition, AgNPs that are synthesized in laboratory, chemically and physically tend to be unstable and accumulate; thus, they require a stabilizer. However, in our method, synthesis and stability are both achieved in one step by the culture medium. In addition, this culture medium, owing to its specific protein composition, can form a coating for AgNPs, which imparts stability to the NPs. Another advantage of this method over other methods, such as bacterial and fungal plant methods, is that it is simpler and takes less time to synthesize NPs. In addition, this method I not association with limitations such as collecting, maintaining, and creating optimal conditions. One of the limitations of this study was that as AgNPs could not be appropriately synthesized by existing methods, we invented a new method for the extraction of NPs via trial and error.

Conclusion

Brain heart infusion medium was successfully applied for the synthesis of stable AgNPs, and these NPs showed good antibacterial and antibiofilm activities against *A. baumannii*.

Overall, results of the antibacterial and antibiofilm tests suggest that AgNPs may serve as effective antimicrobial and antibiofilm agents against infections caused by *A. baumannii.*

Acknowledgments

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Ethics

Ethics Committee Approval: Ethics committee approval (IR.KAUMS.MEDNT.REC.1396.071) was obtained before initiating the study.

Informed Consent: As there were no human studies involved, there is no need for informed consent.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: A.N.A., Design: R.M., A.N.A., Data Collection or Processing: M.S., H.F., F.J.K., Analysis or Interpretation: R.M., Literature Search: G.A.M., Writing: M.S., H.F., G.A.M., A.N.A.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: This research was supported by the Kashan University of Medical Sciences (Grant no. 97038). The funding agency had no role in the design of the study and collection, analysis, and interpretation of data, and in writing the manuscript.

References

1. Mhondoro M, Ndlovu N, Bangure D, Juru T, Gombe NT, Shambira G, Nsubuga P, Tshimanga M. Trends in antimicrobial resistance of bacterial pathogens in Harare, Zimbabwe, 2012-2017: a secondary dataset analysis. BMC Infect Dis. 2019;19:746.
2. World Health Organization (WHO). Publishes List of Bacteria for Which New Antibiotics Are Urgently Needed, World Health Organization, 2017. Last accessed date: 15.09.2021. Available from: https://www.who.int/news/item/27-02-2017-who-publishes-list-of-bacteria-for-which-new-antibiotics-are-urgently-needed
3. Ramirez MS, Penwell WF, Traglia GM, Zimbler DL, Gaddy JA, Nikolaidis N, Arivett BA, Adams MD, Bonomo RA, Actis LA, Tolmasky ME. Identification of Potential Virulence Factors in the Model Strain *Acinetobacter baumannii* A118. Front Microbiol. 2019;10:1599.
4. El-Naggar MY, Gohar YM, Sorour MA, Waheeb MG. Hydrogel Dressing with a Nano-Formula against Methicillin-Resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa* Diabetic Foot Bacteria. J Microbiol Biotechnol. 2016;26:408-20.
5. Gurunathan S, Han JW, Kwon DN, Kim JH. Enhanced antibacterial and anti-biofilm activities of silver nanoparticles against Gram-negative and Gram-positive bacteria. Nanoscale Res Lett. 2014;9:373.
6. Wright JB, Lam K, Hansen D, Burrell RE. Efficacy of topical silver against fungal burn wound pathogens. Am J Infect Control. 1999;27:344-50.
7. Li WR, Xie XB, Shi QS, Duan SS, Ouyang YS, Chen YB. Antibacterial effect of silver nanoparticles on *Staphylococcus aureus*. Biometals. 2011;24:135-41.
8. Rai MK, Deshmukh SD, Ingle AP, Gade AK. Silver nanoparticles: the powerful nanoweapon against multidrug-resistant bacteria. J Appl Microbiol. 2012;112:841-52.
9. Shahverdi AR, Fakhimi A, Shahverdi HR, Minaian S. Synthesis and effect of silver nanoparticles on the antibacterial activity of different antibiotics against *Staphylococcus aureus* and *Escherichia coli*. Nanomedicine. 2007;3:168-71.
10. Pinto RM, Lopes-de-Campos D, Martins MCL, Van Dijck P, Nunes C, Reis S. Impact of nanosystems in *Staphylococcus aureus* biofilms treatment. FEMS Microbiol Rev. 2019;43:622-41.
11. Konop M, Damps T, Misicka A, Rudnicka L. Certain aspects of silver and silver nanoparticles in wound care: a minireview. Journal of Nanomaterials. 2016;2016.
12. Fox CL Jr, Modak SM. Mechanism of silver sulfadiazine action on burn wound infections. Antimicrob Agents Chemother. 1974;5:582-8.
13. Opasanon S, Muangman P, Namviriyachote N. Clinical effectiveness of alginate silver dressing in outpatient management of partial-thickness burns. Int Wound J. 2010;7:467-71.
14. Paladini F, Pollini M. Antimicrobial Silver Nanoparticles for Wound Healing Application: Progress and Future Trends. Materials (Basel). 2019;12:2540.
15. Hendi A. Silver nanoparticles mediate differential responses in some of liver and kidney functions during skin wound healing. Journal of King Saud University-Science. 2011;23:47-52.
16. Buttacavoli M, Albanese NN, Di Cara G, Alduina R, Faleri C, Gallo M, Pizzolanti G, Gallo G, Feo S, Baldi F, Cancemi P. Anticancer activity of biogenerated silver nanoparticles: an integrated proteomic investigation. Oncotarget. 2017;9:9685-705.
17. Prasher P, Sharma M, Mudila H, Gupta G, Sharma AK, Kumar D, Bakshi HA, Negi P, Kapoor DN, Chellappan DK. Emerging trends in clinical implications of bio-conjugated silver nanoparticles in drug delivery. Colloid and Interface Science Communications. 2020;35:100244.
18. Thomas R, Nair AP, Kr S, Mathew J, Ek R. Antibacterial activity and synergistic effect of biosynthesized AgNPs with antibiotics against multidrug-resistant biofilm-forming coagulase-negative staphylococci isolated from clinical samples. Appl Biochem Biotechnol. 2014;173:449-60.
19. Singh R, Wagh P, Wadhwani S, Gaidhani S, Kumbhar A, Bellare J, Chopade BA. Synthesis, optimization, and characterization of silver nanoparticles from *Acinetobacter calcoaceticus* and their enhanced antibacterial activity when combined with antibiotics. Int J Nanomedicine. 2013;8:4277-90.
20. Wan G, Ruan L, Yin Y, Yang T, Ge M, Cheng X. Effects of silver nanoparticles in combination with antibiotics on the resistant bacteria *Acinetobacter baumannii*. Int J Nanomedicine. 2016;11:3789-800.
21. Gaidhani S, Singh R, Singh D, Patel U, Shevade K, Yeshvekar R, Chopade BA. Biofilm disruption activity of silver nanoparticles synthesized by *Acinetobacter calcoaceticus* PUCM 1005. Materials Letters. 2013;108:324-7.
22. Łysakowska ME, Ciebiada-Adamiec A, Klimek L, Sienkiewicz M. The activity of silver nanoparticles (Axonnite) on clinical and environmental strains of *Acinetobacter* spp. Burns. 2015;41:364-71.
23. Ahmad S, Munir S, Zeb N, Ullah A, Khan B, Ali J, Bilal M, Omer M, Alamzeb M, Salman SM, Ali S. Green nanotechnology: a review on green synthesis of silver nanoparticles - an ecofriendly approach. Int J Nanomedicine. 2019;14:5087-107.
24. Lee PC, Meisel D. Adsorption and surface-enhanced Raman of dyes on silver and gold sols. J Phys Chem. 1982;86:3391-5.
25. Tanoue Y, Sugawa K, Yamamuro T, Akiyama T. Densely arranged two-dimensional silver nanoparticle assemblies with optical uniformity over vast areas as excellent surface-enhanced Raman scattering substrates. Phys Chem Chem Phys. 2013;15:15802-5.
26. Le A-T, Tam PD, Huy P, Huy TQ, Van Hieu N, Kudrinskiy A, Krutyakov YA. Synthesis of oleic acid-stabilized silver nanoparticles and analysis of their antibacterial activity. Materials Science and Engineering: C. 2010;30:910-6.

27. Suber L, Sondi I, Matijević E, Goia DV. Preparation and the mechanisms of formation of silver particles of different morphologies in homogeneous solutions. J Colloid Interface Sci. 2005;288:489-95.

28. He Y, Du Z, Lv H, Jia Q, Tang Z, Zheng X, Zhang K, Zhao F. Green synthesis of silver nanoparticles by Chrysanthemum morifolium Ramat. extract and their application in clinical ultrasound gel. Int J Nanomedicine. 2013;8:1809-15.

29. Ghosh S, Patil S, Ahire M, Kitture R, Gurav DD, Jabgunde AM, Kale S, Pardesi K, Shinde V, Bellare J, Dhavale DD, Chopade BA. Gnidia glauca flower extract mediated synthesis of gold nanoparticles and evaluation of its chemocatalytic potential. J Nanobiotechnology. 2012;10:17.

30. Iravani S, Korbekandi H, Mirmohammadi SV, Zolfaghari B. Synthesis of silver nanoparticles: chemical, physical and biological methods. Res Pharm Sci. 2014;9:385-406.

31. Alam AN, Sarvari J, Motamedifar M, Khoshkharam H, Yousefi M, Moniri R, Bazargani A. The occurrence of blaTEM, blaSHV and blaOXA genotypes in Extended-Spectrum β-Lactamase (ESBL)-producing *Pseudomonas aeruginosa* strains in Southwest of Iran. Gene Reports. 2018;13:19-23.

32. Fallah F, Yousefi M, Pourmand MR, Hashemi A, Nazari Alam A, Afshar D. Phenotypic and genotypic study of biofilm formation in Enterococci isolated from urinary tract infections. Microb Pathog. 2017;108:85-90.

33. Weinstein M PJ, Burnham C, Campeau SH, Conville P, Doern CH, Eliopoulos G, Galas M, Humphries R. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically, 11th Edition. CLSI. 2018;7:1-180.

34. Donlan RM, Costerton JW. Biofilms: survival mechanisms of clinically relevant microorganisms. Clin Microbiol Rev. 2002;15:167-93.

35. Peleg AY, Seifert H, Paterson DL. *Acinetobacter baumannii*: emergence of a successful pathogen. Clin Microbiol Rev. 2008;21:538-82.

36. Azad A, Rostamifar S, Modaresi F, Bazrafkan A, Rezaie Z. Assessment of the Antibacterial Effects of Bismuth Nanoparticles against *Enterococcus faecalis*. Biomed Res Int. 2020;2020:5465439.

37. Bindhu MR, Umadevi M. Antibacterial activities of green synthesized gold nanoparticles. Materials Letters. 2014;120:122-5.

38. Velázquez-Velázquez JL, Santos-Flores A, Araujo-Meléndez J, Sánchez-Sánchez R, Velasquillo C, González C, Martínez-Castañon G, Martinez-Gutierrez F. Anti-biofilm and cytotoxicity activity of impregnated dressings with silver nanoparticles. Mater Sci Eng C Mater Biol Appl. 2015;49:604-11.

39. Siegel HJ, Herrera DF, Gay J. Silver negative pressure dressing with vacuum-assisted closure of massive pelvic and extremity wounds. Clin Orthop Relat Res. 2014;472:830-5.

40. Mohanty S, Mishra S, Jena P, Jacob B, Sarkar B, Sonawane A. An investigation on the antibacterial, cytotoxic, and antibiofilm efficacy of starch-stabilized silver nanoparticles. Nanomedicine. 2012;8:916-24.

41. Pérez-Díaz M, Alvarado-Gomez E, Magaña-Aquino M, Sánchez-Sánchez R, Velasquillo C, Gonzalez C, Ganem-Rondero A, Martínez-Castañon G, Zavala-Alonso N, Martinez-Gutierrez F. Anti-biofilm activity of chitosan gels formulated with silver nanoparticles and their cytotoxic effect on human fibroblasts. Mater Sci Eng C Mater Biol Appl. 2016;60:317-23.

42. Burd A, Kwok CH, Hung SC, Chan HS, Gu H, Lam WK, Huang L. A comparative study of the cytotoxicity of silver-based dressings in monolayer cell, tissue explant, and animal models. Wound Repair Regen. 2007;15:94-104.

43. Whitesides GM. Nanoscience, nanotechnology, and chemistry. Small. 2005;1:172-9.

44. Ingale AG, Chaudhari A. Biogenic synthesis of nanoparticles and potential applications: an eco-friendly approach. J Nanomed Nanotechol. 2013;4:1-7.

45. Nayak D, Pradhan S, Ashe S, Rauta PR, Nayak B. Biologically synthesised silver nanoparticles from three diverse family of plant extracts and their anticancer activity against epidermoid A431 carcinoma. J Colloid Interface Sci. 2015;457:329-38.

46. Mohanta YK, Biswas K, Jena SK, Hashem A, Abd Allah EF, Mohanta TK. Anti-biofilm and Antibacterial Activities of Silver Nanoparticles Synthesized by the Reducing Activity of Phytoconstituents Present in the Indian Medicinal Plants. Front Microbiol. 2020;11:1143.

47. Krishnaraj C, Jagan EG, Rajasekar S, Selvakumar P, Kalaichelvan PT, Mohan N. Synthesis of silver nanoparticles using Acalypha indica leaf extracts and its antibacterial activity against water borne pathogens. Colloids Surf B Biointerfaces. 2010;76:50-6.

48. Iravani S, Zolfaghari B. Green synthesis of silver nanoparticles using Pinus eldarica bark extract. Biomed Res Int. 2013;2013:639725.

49. Gopinath V, MubarakAli D, Priyadarshini S, Priyadharsshini NM, Thajuddin N, Velusamy P. Biosynthesis of silver nanoparticles from Tribulus terrestris and its antimicrobial activity: a novel biological approach. Colloids Surf B Biointerfaces. 2012;96:69-74.

50. Shaker MA, Shaaban MI. Synthesis of silver nanoparticles with antimicrobial and anti-adherence activities against multidrug-resistant isolates from *Acinetobacter baumannii*. J Taibah Univ Med Sci. 2017;12:291-7.

51. Patil MP, Seo YB, Kim GD. Morphological changes of bacterial cells upon exposure of silver-silver chloride nanoparticles synthesized using Agrimonia pilosa. Microb Pathog. 2018;116:84-90.

52. de Jesús Ruíz-Baltazar Á, Reyes-López SY, Larrañaga D, Estévez M, Pérez R. Green synthesis of silver nanoparticles using a Melissa officinalis leaf extract with antibacterial properties. Results in physics. 2017;7:2639-43.

53. Chauhan R, Kumar A, Abraham J. A Biological Approach to the Synthesis of Silver Nanoparticles with Streptomyces sp JAR1 and its Antimicrobial Activity. Sci Pharm. 2013;81:607-21.