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Cytotoxic Effect of Biogenic Selenium Nanoparticles Using Bacteriocin of *Acinetobacter baumannii* Isolated from Burns and Wound Infections

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Abstract:

Recently, advancements in nanotechnology have opened up new avenues for enhancing healthcare outcomes by leveraging the unique properties of nanomaterial. Scientists have previously developed complexes of bacteriocins with nanomaterials or bacteriocin-nanoconjugates for diverse biomedical applications that utilize bacteriocins. The usage of bacteriocin–nanoconjugates has several benefits, including enhanced stability over extended use, defense against proteolytic enzyme degradation, and synergistic efficacy. The requirement to provide new antibiotic alternatives is becoming more pressing in this regard. Because of their special qualities, metal oxide nanomaterials are currently attracting a lot of attention as possible antimicrobial and anti-cancer agents. The bacteriocin-like inhibitory substance (BLIS) from *Acinetobacter bumannii* used to synthesiss selenium nanoparticles. Atomic Force Microscopy (AFM), Field Emission Scanning Electron Microscope (FESEM), X-Ray Diffraction (XDR) analysis and UV visible spectrometry were used to characterize the biosynthesized nanoparticles and ascertain the product's chemical and physical properties XRD verified the hexagonal structure, AFM analysis used to reveal the roughness and distribution of nanoparticles indicated the size range at 81,23 nm, and FESEM validated the surface morphology. The UV-visible spectroscopic analysis revealed that the synthesized nanoparticles exhibited their highest absorption peaks at a wavelength of 270 nm. Minimum inhibitory concentration (MIC) at 250 mg/ml Biogenic selenium nanoparticles demonstrated comparable in order to ascertain the optimal antimicrobial activity against pathogenic microorganisms through the use of a well diffusion method, pathogenic microorganisms were isolated clinically infections in Iraqi patients and identified using the VITEK-2 Compact. The cytotoxicity assays demonstrated that the treatment with biologically synthesized selenium nanoparticles did not markedly compromise the viability of human lymphocytes.

Keywords: Bacteriocin-like inhibitory substance, Human lymphocytes, Sodium selenite.

تأثير السمية الخلوية لجزيئات السيلينيوم النانوية الحيوية باستخدام البكتريوسين للبكتيريا الراكدة البومانية المعزولة من اصابات الحروق والجروح

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الخلاصة

في الآونة الأخيرة، فتحت التطورات في تكنولوجيا النانو آفاقًا جديدة لتعزيز نتائج الرعاية الصحية من خلال الاستفادة من الخصائص الفريدة للمواد النانوية. سبق للعلماء أن طوروا مجمعات من البكتريوسينات مع مواد نانوية أو تجمعات بكتريوسين نانوية لتطبيقات طبية حيوية متنوعة تستخدم البكتريوسينات. استخدام تجمعات البكتريوسن مع النانو له فوائد عديدة، بما في ذلك تعزيز الاستقرار على مدى الاستخدام الممتد، والدفاع ضد تدهور الإنزيم المحلل للبروتين، والفعالية التآزرية. أصبحت الحاجة إلى توفير بدائل جديدة للمضادات الحيوية أكثر إلحاحًا في هذا الصدد. نظرًا لصفاتها الخاصة، تجتذب المواد النانوية لأكسيد الفلز حاليًا الكثير من الاهتمام باعتبارها عوامل محتملة مضادة للميكروبات ومضادة للسرطان. المادة المثبطة الشبيهة بالبكتريوسين BLIS من بكتريا الراكدة البومانية استخدمت في تصنيع جسيمات السيلينيوم النانوية. تم استخدام مجهر القوة الذرية AFM والمجهر الإلكتروني لمسح الانبعاثات الميدانية FESEM، وتحليل حيود الأشعة السينية XDRوالقياس الطيفي المرئي للأشعة فوق البنفسجية لتوصيف الجسيمات النانوية المُصنّعة حيويًا والتأكد من الخواص الكيميائية والفيزيائية للمنتج، وقد تحقق XRD من البنية السداسية، . أشار AFM التحليل المستخدم للكشف عن خشونة وتوزيع الجسيمات النانوية إلى نطاق الحجم عند 81،23 نانومتر، وقام FESEM بالتحقق من صحة مورفولوجيا السطح. أظهر التحليل الطيفي للأشعة فوق البنفسجية المرئية أن الجسيمات النانوية المحضرة أظهرت أعلى قمم امتصاص عند طول موجي قدره 270 نانومتر. اما عن الحد الأدنى للتركيز المثبط MICعند 250 ملجم/مل أظهرت جسيمات السيلينيوم النانوية الحيوية قابلة للمقارنة من أجل التأكد من النشاط الأمثل المضاد للميكروبات ضد الكائنات الحية الدقيقة المسببة للأمراض من خلال استخدام طريقة الانتشار بالحفر، وتم عزل الكائنات الحية الدقيقة المسببة للأمراض سريريًا عند المرضى العراقيين وتم تشخيصها باستخدام جهاز VITEK −2 المدمج. أظهرت فحوصات السمية الخلوية أن العلاج باستخدام جسيمات السيلينيوم النانوية المُصنَّعة بيولوجيًا لم يؤثر بشكل ملحوظ على قدرة الخلايا الليمفاوية البشرية على البقاء.

Introduction

Acinetobacter baumannii has emerged as a highly formidable pathogen in contemporary healthcare due to its remarkable capacity to develop resistance to antibiotics. Numerous strains of A. baumannii exhibit exceptional resistance to the majority of antibiotics commonly used in clinical settings [1]. Bacteriocin is a short polypeptide or ribosomally synthesized protein that has antibacterial activity against bacteria that are closely related to the strain that produces it or against other bacteria [2]. A microbial community's cells may be susceptible to any bacteriocin, resistant to all bacteriocins, or bacteriocinogenic (generate bacteriocin. When three cell types coexist and compete for limited resources, only a fraction of bacteriocinogenic cells become activated to synthesize and secrete bacteriocins [3]. Metallic nanoparticles show great promise in applications such as biological diagnostic probes, components of optoelectronic and display devices, and catalysts [4].

SeNP biosynthesis typically involves the addition of microorganisms or plant extracts to a sodium selenite solution. The microorganisms produce organic materials on the surface of SeNPs, such as proteins, polysaccharides, and lipids, to stabilize the structure, while also using their own metabolism to convert the high-valent selenium to SeNP [5]. To get around some of these restrictions, bacteriocin-nanoparticales have shown to be a workable solution and the most potent antibacterial agent *in vitro* so the antibacterial activity of nanoparticles in combination with bacteriocins is a new strategy against pathogenic bacteria [6]. The primary objective of the present investigation is to synthesize SeNPs using bacteriocin derived from Acinetobacter baumannii, which was isolated from burn wounds. Subsequently, the study focuses on characterizing the biosynthesized SeNPs employing a range of analytical techniques. To use nanoparticles as an alternative to antibiotics, which are ineffective, and finally evaluated their cytotoxic activities on lymphocyte viability.

Materials and methods

A. baumannii isolation and identification

In this study, one hundred fifty specimens from *burns and wounds* infections were collected during September 2023 to December 2023 from patients in Baghdad medical city hospital in Baghdad, Iraq. The specimens were cultured on *Acinetobacter* agar base then incubated for 24-48 hrs at 37°C under aerobic conditions. Isolates were subsequently confirmed *A. baumannii* by using the Vitek 2 compact system (BioMe`rieux/France) [7].

Antibiotic sensitivity test

The antibiotic sensitivity test was done for all isolates with positive growth with *A. baumannii* only (44 isolates) by VITEK- 2 -the antibiotic Sensitivity Test Card (ASTC) AST-N222 [8].

Biosynthesis of bacteriocin-like inhibitory substance (BLIS)

The agar well diffusion assay (AWD) method was employed to screen all Acinetobacter baumannii isolates for their inhibitory activity against a panel of indicator strains, with the aim of identifying the isolate exhibiting the highest production of the bacteriocin-like inhibitory substance (BLIS) bumanniicin. The indicator strains included gram positive and negative bacteria [9].

Optimization of BLIS

Synthesis of BLIS is optimized numerous factors were examined to determine the ideal settings for high-level bumanniicin synthesis. Determination of optimum pH, determination of optimum incubation time and effect of nitrogen source & carbon source (sugars).

Partial purification of bumanniicin-like inhibitory substance

Partial purification of crud bacteriocin was prepared via precipitation with ammonium sulfate at different concentrations.

Estimation of protein concentration

Concentration was determined according to Bradford, (1976) [10].

Biosynthesis of Selenium Nanoparticles

For green synthesis of SeNPs, 2 grams of sodium selenite (Na2SeO3) was added to a volume of 10 mL of *A. baumannii* BLIS solution (baumanniicin) concentration 10.65 µg/ml the flasks were incubated at 30 °C to 35 °C for three days, and each color change was recorded. After incubation, the reaction mixture was centrifuged at 6000 revolutions per minute for 25 min at 4 ˚C in order to separate out the supernatant. The supernatant was then replaced with deionized water and the centrifugation process was repeated three more times under the same conditions to remove the remaining supernatant. The collection of nanoparticles in the form of pellets was transferred to a hot air oven set at 120 °C to evaporate all liquid contents. The dried powder was carefully collected and stored for further analysis [11].

Characterization of biosynthesized Selenium Nanoparticles

According to [12]. UV-VIS spectroscopy, Atomic force microscopy AFM, Energy-dispersive X-ray analysis EDX analysis Bruker/Germany, Field emission scanning electron microscope, Fourier transform infrared (FTIR) spectroscopy.

Antibacterial application of SeNPs by agar well diffusion method:

The antibacterial activity of SeNPs was evaluated using the AWD method against gram-positive and negative bacterial strains and determine the minimum inhibitory concentration

(MIC) of SeNPs on them, isolates were inoculated into nutrient broth and cultured at 37°C for 24 hours. Then Mueller Hinton agar was used for the agar diffusion assay. Sterilized cotton swabs were used, isolates suspensions (0.5 McFarland) onto Muller hinton agar plates. A sterilecork borer was used to create wells, which were then filled with 100 µl of SeNPs solution with 5 concentrations (1000, 500,250,125and 62.5) µg/ml were used. The plates were subsequently incubated for an additional 24 hours at 37 ° C, after which the zones of inhibition were determined the following day [13].

Isolation of human lymphocytes:

In assessing the cellular viability of different concentrations of SeNPs (1000, 500, 250, 125 µg/ml), the MTT assay, as mentioned in prior studies, was employed on normal lymphocytes. The percent viability of cells exposed to treatments was calculated using the following equation and the Concentration that inhibits 50% of cell growth was used as a parameter for cytotoxicity
Cell Viability (%) = (Mean OD of Treated Cells/Mean OD of Control Cells) × 100
The lymphocytes were obtained from the blood of a healthy, non-smoking donor who had no symptoms of an infectious disease at the time of blood collection. Then treated according to. The cytotoxic effect of SeNPs was performed by using MTT ready to usekit [14].

MTT Protocol:

1- In 96 flat well microtiter plates, cells ($1x104^{-1}x106$ cells/ml) were grown in a final volume of 200□l of complete culture medium (RPMI-1640) per well. Sterilized parafilm was placed over the microplate, and it was meticulously shaken. The plates were incubated for 24 hours at 37°C with 5% CO_2.
2- After incubation, the medium was removed and two-fold serial dilutions of SeNPs (1000, 500, 250, 125, 62.5 µg/ml) were added to the wells containing lymphocytes. For every concentration, triplicates were employed along with the controls, which were cells cultured in serum-free medium. For the chosen exposure duration of 24 hours, plates were incubated at 37°C with 5% CO2.
3- Ten microliters of the MTT solution were added to each well following exposure. The plates were further incubated for four hours at 37°C with 5% CO2. After taking care to remove the media, 100 µl of the solubilization solution was added to each well and left for five minutes.
4- Absorbance was determined using an ELISA reader at a wavelength of 575 nm. The optical density data statistically analyzed and the viability factor and IC50 (concentration of compound required to cause a 50% reduction in cell viability) were calculated for cell line.

Results and Discussion

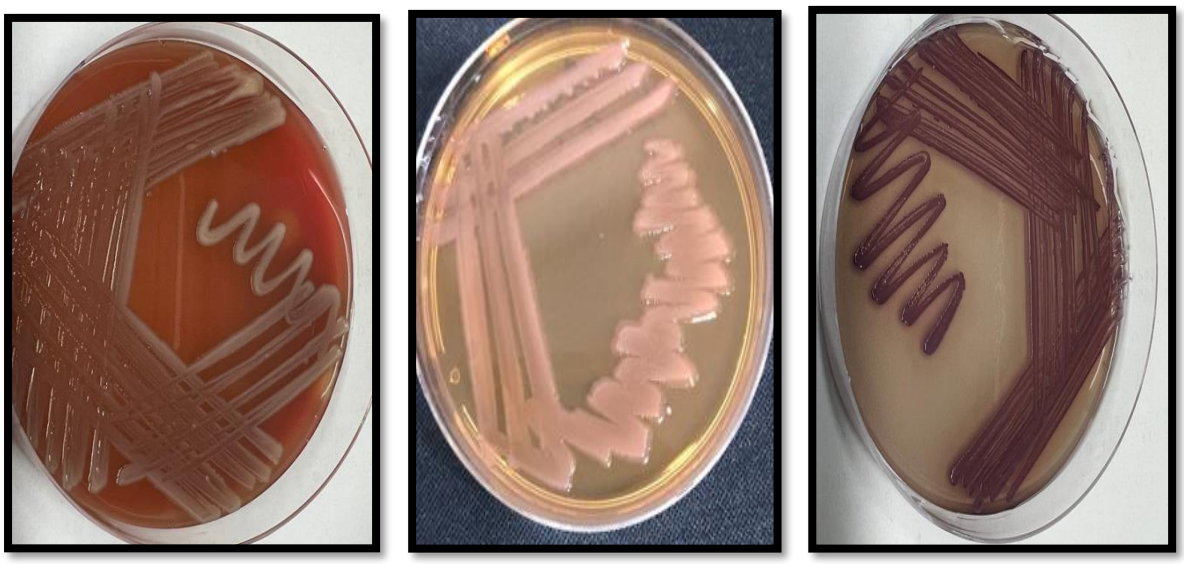
Isolation one hundred fifty Specimens from *burns and wounds* infections samples, were examined and only 44 (MF1-MF44) isolates were identified *A. baumannii* as shown in (Table 1).

Table 1: Prevalence of *Acinetobacter baumannii* isolates among clinical Samples

Source of Samples	Other gram- negative bacteria No. Isolates	No. of *Acinetobacter baumannii* (%)
Burns	66	**27 (61.36%)**
Wounds	40	**17 (38.63%)**
Total	106 (70.66%)	**44(29.33%)**

A. baumannii is non-fastidiousand grows well on ordinary culture media like Blood agar plates it was appearing as creamy or white and mucoid or smooth colonies, non-hemolytic after 18-24 hours of incubation at 37°C and non-lactose fermentive on MacConkey agar and colonies are appear as slight lavender color [15]. The Acinetobacter agar medium has the advantage of using pure chemical substances, such as sodium citrate and sodium deoxycholate, which inhibit

the growth of cocci and Gram-positive bacilli. The crystal violet, in the used concentration, increases the inhibitory action for the *Streptococcus* and *Staphylococcus* species, without changing the sensitiveness of the medium for the *Acinetobacter* genus bacteria [16].
After 24 hours of incubation at 30°C in air as illustrated in figure, *Acinetobacter* spp. typically produce circular, convex, smooth, opaque colonies with entire margins of 1 to 2 mm in diameter. Shown in Figure 1.



(a) (b) (c)

Figure 1: *A. baumannii* on (a) *Acinetobacter* agar base, (b) *MacConkey* agar) and (c) Blood agar at 37°C for 24 hrs

A. baumannii is a Gram-negative (G-ve), coccobacillus, non-motile, strictly aerobic and non-glucose fermenting. Acinetobacter baumannii exhibits positive results in catalase and citrate tests, while producing negative results in indole, oxidase, and urease tests. It is noteworthy that A. baumannii is the sole species within the genus that can thrive at a temperature of 44°C [17].

Antibiotic Sensitivity Test:

The current findings indicate that clinical isolates of *A. baumannii* exhibited high resistance to most of the tested antibiotics. Specifically showed complete resistance (100%) to the following antibiotics: Piperacillin, Ceftazidime, Cefepime, Cefotaxime, Ceftriaxone, Ampicillin–sulbactam, Piperacillin–tazobactam, Ticarcillin–clavulanic acid, and Gentamicin. *A.baumannii* has become one of the most successful pathogens in modern healthcare because of its surprising ability to gain antibiotic resistance. Several strains of *A. baumannii* are extremely resistant to most clinically available antibiotics [18]. To describe antimicrobial resistance of *Acinetobacter spp.* various nomenclatures have been used, such as Multidrug Resistance (MDR), Extended Drug Resistance (XDR), pan-drug resistance (PDR), and XDR *Acinetobacter spp*., which is also resistant to polymyxins and tigecycline. MDR Acinetobacter strains that are additionally resistant to carbapenems are referred to as XDR *Acinetobacter spp*. MDR Acinetobacter spp. can relate to resistance to at least three classes of antimicrobial medications, such as all cephalosporins and penicillins, aminoglycosides and fluoroquinolones [19].

Optimum conditions for bacteriocin production:

The results indicate that pH 4 the best value for production. Lower inhibition zone 15 mm at pH 8 [20]. As shown in Figure 2.

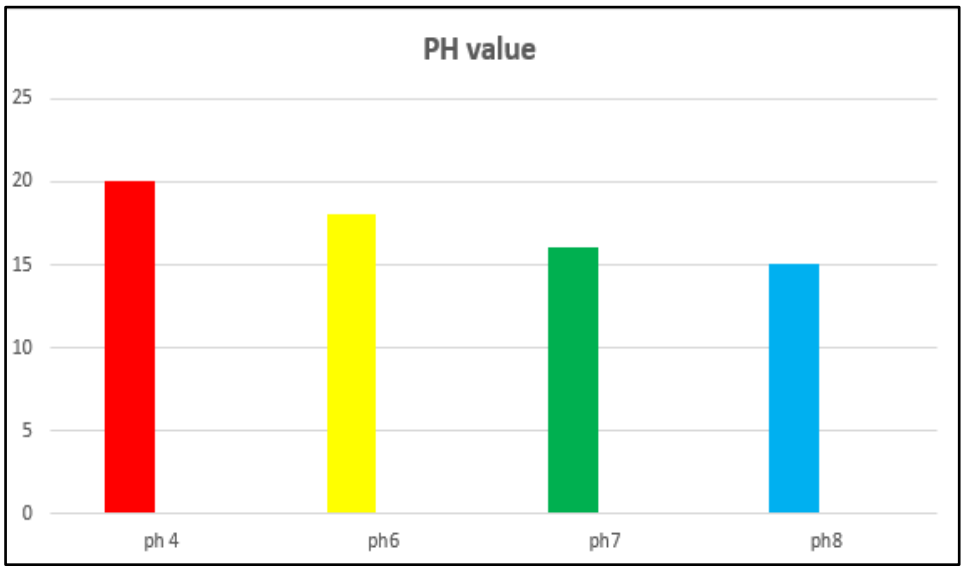


Figure 2: Relationship between diameter of the inhibition zone in mm with different pH values.

At various incubation times it was noted that the highest production occurred after 48 hours of incubation, resulted in the largest inhibition zone, measuring 24 mm [21]. As shown in Figure 3.

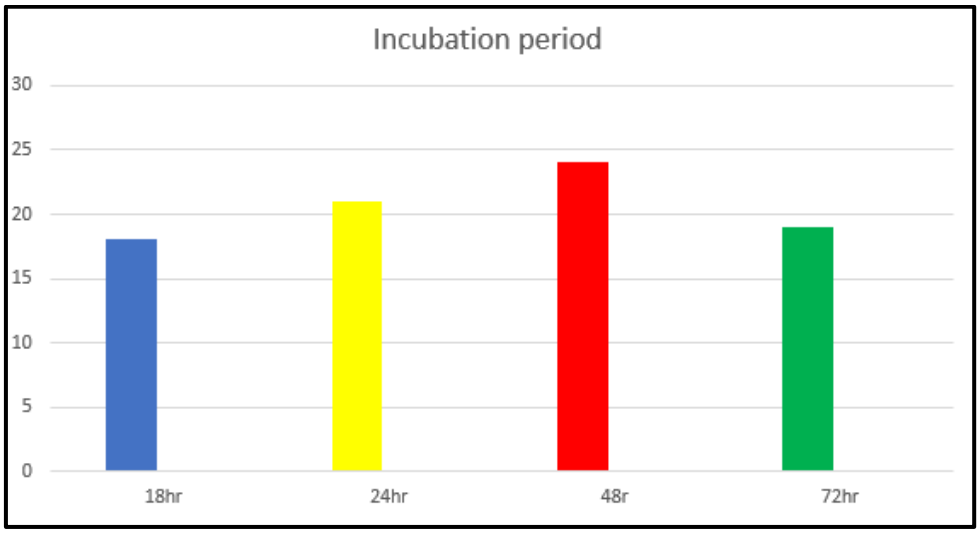


Figure 3: Relationship between diameter of the inhibition zone in mm with different incubation times.

Optimum nitrogen Source was peptone water reached 24 mm zone, best nitrogen source than yeast extract, Lactose, and glucose carbon source reached (22 and 21) mm [22]. As shown in Figure 4.

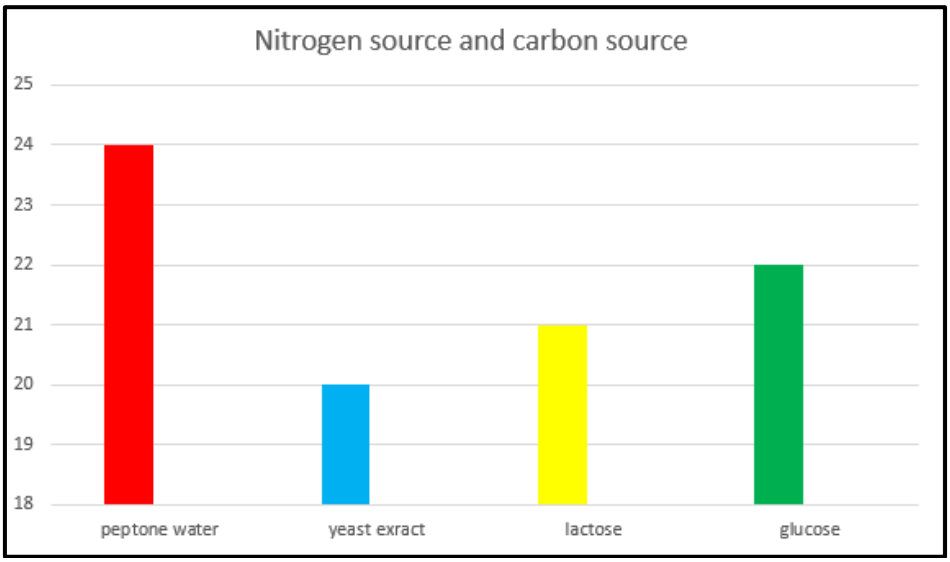


Figure 4: Relationship between diameter of the inhibition zone in mm with different Nitrogen & carbon source

Ammonium sulfate precipitation and dialysis

Gradually was added ammonium sulfate to the crude BLIS, stirring constantly on ice at 60% saturation. Then dialysis by dialysis membranes (wide flat 34MM – MwCO 8000-14000D) as shown in Figure (5). Concentration for all steps in Table 2.

Table 2: The steps of bacterocin purification from *A. baumannii*

Sample	Protein Conc.µg/ml
Culture filtrate Crud	**4.343**
(NH4)2SO4 precipitate	**6.524**
Dialysis	**10.65**

Biosynthesis of Selenium Nanoparticles

The biological reduction of selenite leads to the formation of elemental red selenium, which is considered insoluble and non-toxic (8). In the process, sodium selenite salt (Na2SeO3) was added to the volume of BLIS solution (baumanniicin), and the resulting reaction mixture was incubated at 37 °C for duration of 96 hours. After this incubation, the color of the reaction mixture changes completely to red. This change was the indication for the synthesis of nanoparticles [23]. As shown in figure (8). Therefore, the development of a bacteriocin like inhibitor with selenium nanoparticles (SeNP) products was planned through a biosynthetic method using bacteriocin (MF19) from A.baumannii in a cost effective and facile manner. This study also analyzed the properties of nanoparticles and their therapeutic applications. A progressive increase in selenium reduction and production of selenium nanoparticles (SeNPs)from selenium-enriched media as well as reduction of soluble, toxic, colorless eleniumions to red, insoluble, nontoxic elemental SeNPs was demonstrated. According to [24], the reduction phase (SeO) of *Bacillus subtilis* BSN313 occurred during the log phase. Similar observations have been made in the same genre.

Characterization of SeNPs

1- UV-Vis Spectral Analysis

Based on the findings, biosynthesized SeNPs displayed a highest peak (absorption peak) at 200 nm to 800 nm. As shown in Figure (5), smaller particle size was indicated previously at sharp peak observation at 270 nm. Using UV–Vis spectrophotometry, morphological changes (nanoparticle size and agglomeration) were noted for the dispersion of SeNPs. The absorption bands, where the maxima are situated at 300 and 800 nm. The characteristic spectrum and the previously published spectra of even SeNPs made by Bacillus species did not match up well [25].

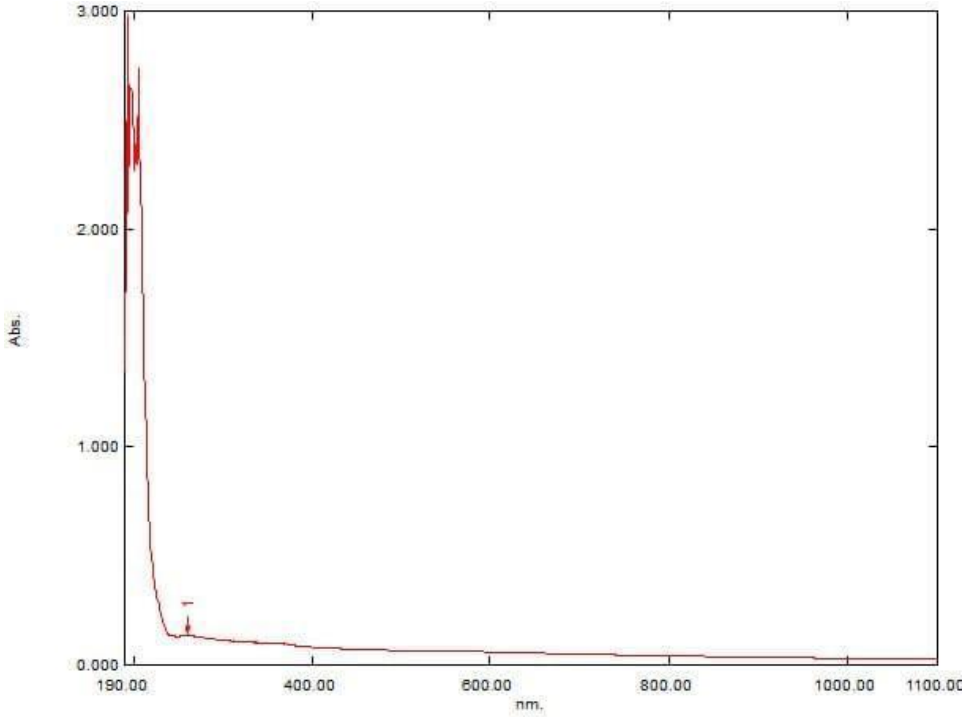
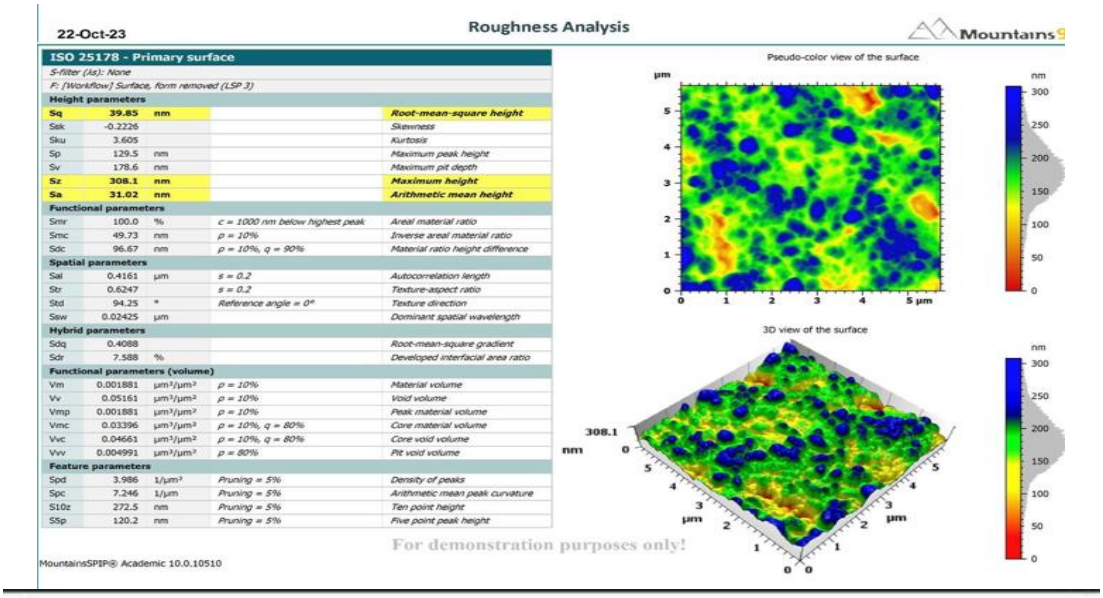


Figure 5: UV–Vis spectrum of Synergistic SeNPs.

2- Atomic Force Microscopy (AFM) analysis:

AFM was employed to identify and characterize the size distribution of selenium nanoparticles (SeNPs), which exhibited an average diameter of 81.23 nm. The two-dimensional and three-dimensional AFM images, as depicted in Figure 6, revealed that all the SeNPs possessed a uniform size and shape. However, green synthesis zinc oxide nanoparticles exhibit an average size lower than that of selenium based on the weight of the molecules and the chemical structural influence of their characterization. [26].

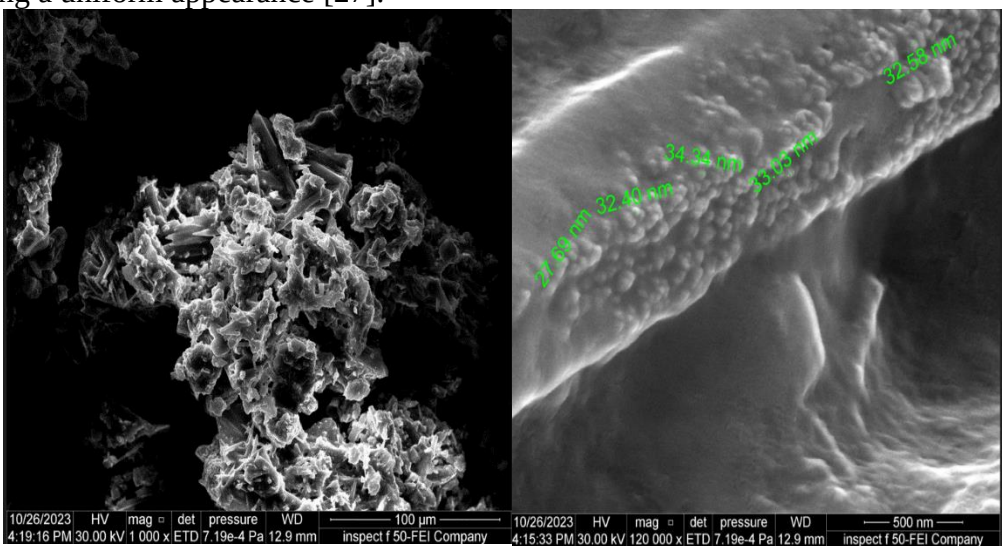


Global statistics				
Mean	*****	11587	81.23	1028
Min	*****	141.1	8.299	919.6
Max	*****	118265	358.0	1714

Figure 6: AFM images of SeNPs and average diameter of SeNPs and Two-dimensional image (2D) and 3D image.

3- Field Emission Scanning Electron Microscope (FESEM)

The elemental and structural composition of NPs samples, as well as their morphology and size, were all examined using SEM analysis. Figure (7) demonstrates the spherical shape of SeNPs at 40000 magnification power and 3000 kv. SEM images revealed that the particles were predominantly spherical in shape with a diameter ranging from 34 to nanometers, exhibiting a uniform appearance [27].



(a) **(b)**

Figure 7A: Field Emission Scanning Electron Microscope (FESEM). **Figure 7B:** Field Emission of SeNPs

4- Energy Dispersive X-ray (EDX):

In comparison with EDX-ray, FESEM allows to determine the presence of different components in the examined model. The SeNPs components were examined using EDX. The results showed the EDX spectra along with a prominent elemental peak around 22 keV that is uniquely characteristic of selenium metal, as well as the corresponding percentage composition. Other Additional minor peaks for *A. baumannii* biomolecules were detected, as demonstrated in Figure 8.

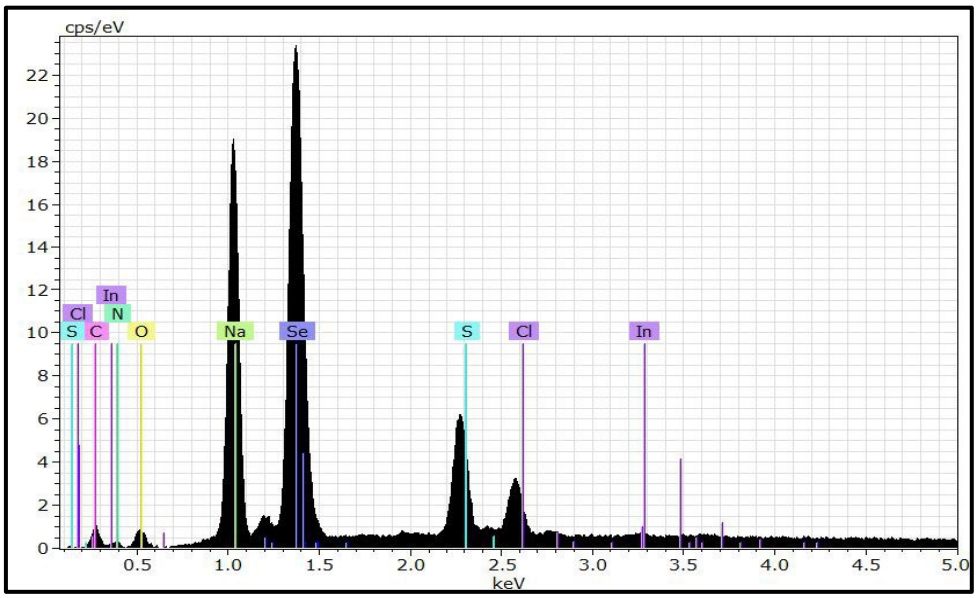


Figure 8: Shows the EDX image of SeNPs

5- Fourier transform infrared (FTIR) spectroscopy

The FTIR analysis of the prepared SeNPs was conducted by scanning the chemical bonds within the wavelength range of 400–4000 cm-1, as illustrated in Figure 9. The FTIR analysis revealed the presence of negatively charged surface functional groups on the SeNPs. Specifically, the SeNPs synthesized in this study exhibited amino groups (NH). The NH group is electron-rich due to the one available electron, which keeps the net charge negative. The SeNPs' appearance in a dispersed form is favored by the presence of reducing agents (NH).The outcome agrees with that of another study [28].

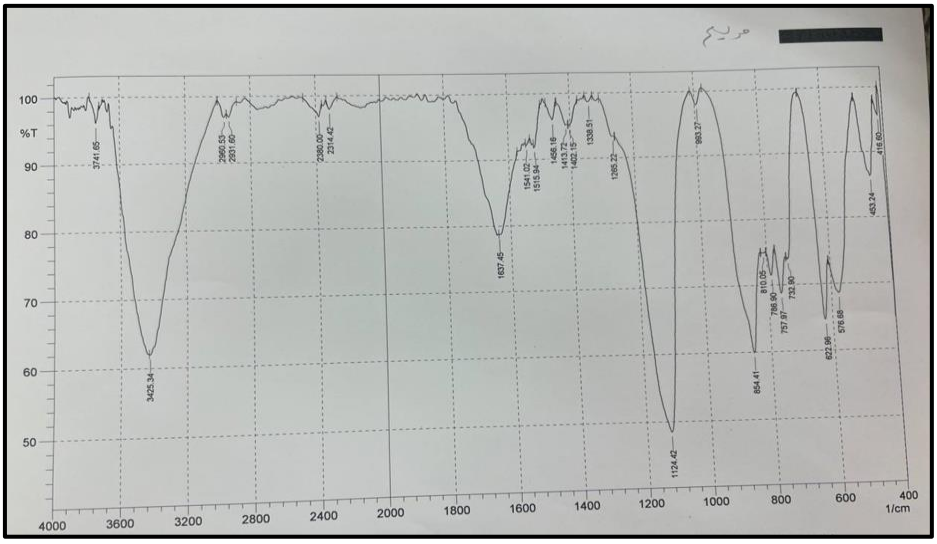


Figure 9: Fourier transform infrared (FTIR) spectroscopy of SeNPs

Antibacterial activity of SeNPs

Biogenic SeNP produced by Anabaena sp. PCC. By7120 has been shown to be successful in combating gram-positive Gram-negative and *Staphylococcus aureus* bacteria Pathogenic strains of *Escherichia coli* of bacteria. [29]. The increasing use of nanoparticles in medicine has led to an increasing number of studies investigating the potential antimicrobial mechanisms of nanoparticles. For example, metal nanoparticles can alter the metabolic activity of bacteria.[30]. The minimum inhibitory concentration (MIC) against Gram positive and Gram negative bacterial strains that evaluated using the agar well diffusion method and SeNPs solution with 5 concentrations (1000,500,250,125and 62.5) µg/ml. Evaluated for their antibacterial activity SeNPs against gram positive bacteria, as indicated in Figure 10-A. It was discovered that the most effective inhibitory concentration range was between 125 and 250 micrograms per milliliter, and this demonstrated the greatest efficacy against resistant strains of Gram-positive bacteria. Using the well diffusion assay method, the inhibition zone between 5–25 mm was measured after 24 hours of incubation at 37 °C. In the dissemination mean zone of bacterial inhibition after at dfferent concentrations of SeNPs, statistical analysis revealed a significant difference ($p < 0.05$) against *Staphylococcus haemolyticus* and *Staphylococcus sciuri* compared to *Staphylococcus epidermis* and *Kocuria kristinae* that was not as significant.

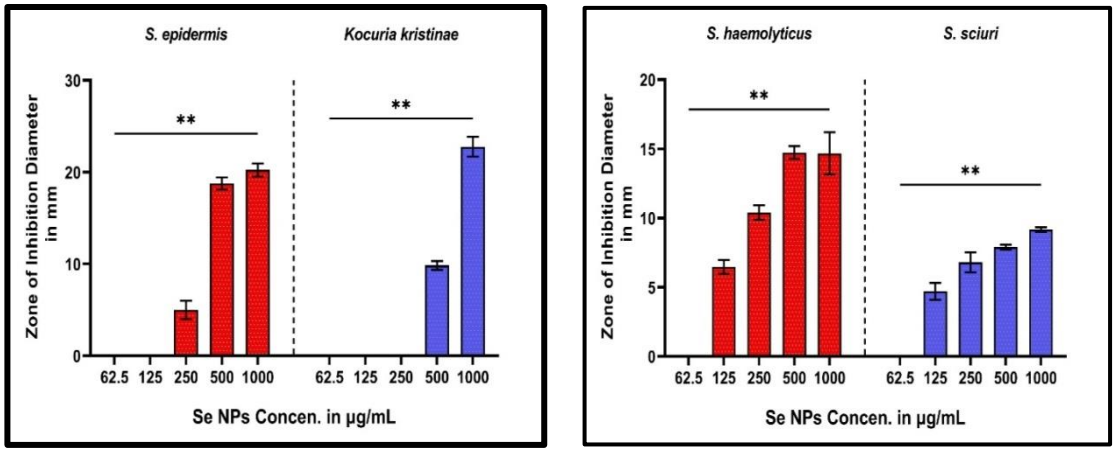


Figure (10-A) : Mean (± SD) zone of bacterial inhibition in mm treated with different concentrations (in µg/mL) of SeNPs against gram positive standard deviation, (n = 3).

The statistical analysis emphasized a significant difference ($p < 0.05$) in the dissemination mean zone of bacterial inhibition after different concentrations of SeNPs (Figure10-B). Where it is possible to notice significant differences against Gram-negative *Klebsiella pneumoniae*, *Pseudomonas fluorescens,* and *Pseudomonas aeruginosa* compared to other species, *Enterobacter cloacae complex,* and *Escherichia coli,* while the less significant differences at different concentrations show *Pantoea spp. is* only 1000 µg/ml concentration active.

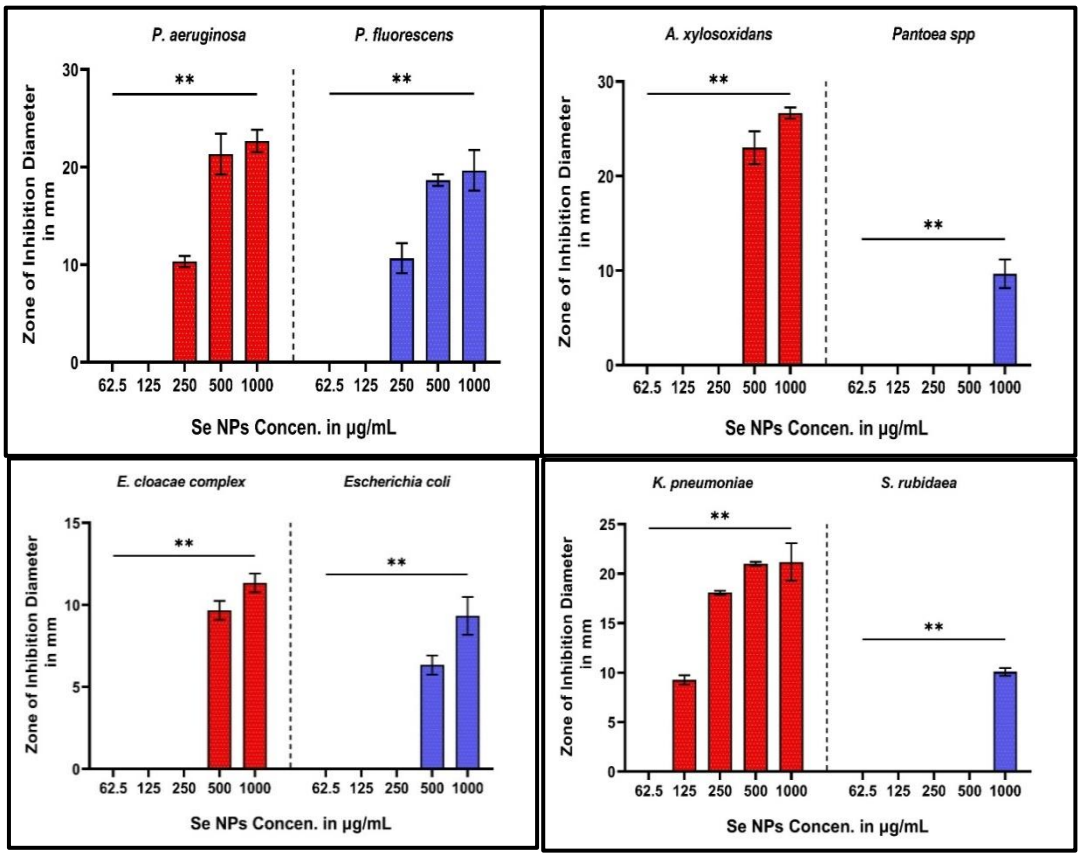


Figure (10-B) : Mean (± SD) zone of bacterial inhibition in mm treated with different concentrations (in µg/mL) of SeNPs against gram negative bacteria *Klebsiella spp* , *P. aeruginosa,* E. *coli* and *Salmonella spp* without magnetic feld standard deviation, (*n* = 3). **: $p < 0.05$, NS: Non- Significant, SD: Standard Deviation

The study reported other results showing that at a concentration of 150 µg/mL, SeNPs exhibited no discernible variations ($p > 0.05$). Additionally, at a concentration of 200 µg/mL, SeNPs demonstrated antibacterial activity against Pseudomonas aeruginosa, Staphylococcus aureus ATCC 9027, and Escherichia coli. Using probiotic *Bacillus subtilis* as biofactories, this work will be useful in the future to produce biogenic SeNPs that may be safely used in nutritional and biomedical applications [31].

On other hand this investigation builds on our earlier work, which isolated bacteria resistant to selenium (*Bacillus subtilis* BSN313) from a medium enriched in the element. This probiotic strain is better able to demonstrate resistance in a medium that is enhanced with selenium. The BSN313 strain could be utilized in a safe manner as a probiotic to produce SeNPs for dietary and therapeutic purposes [32].

Nanoparticles on lymphocytes

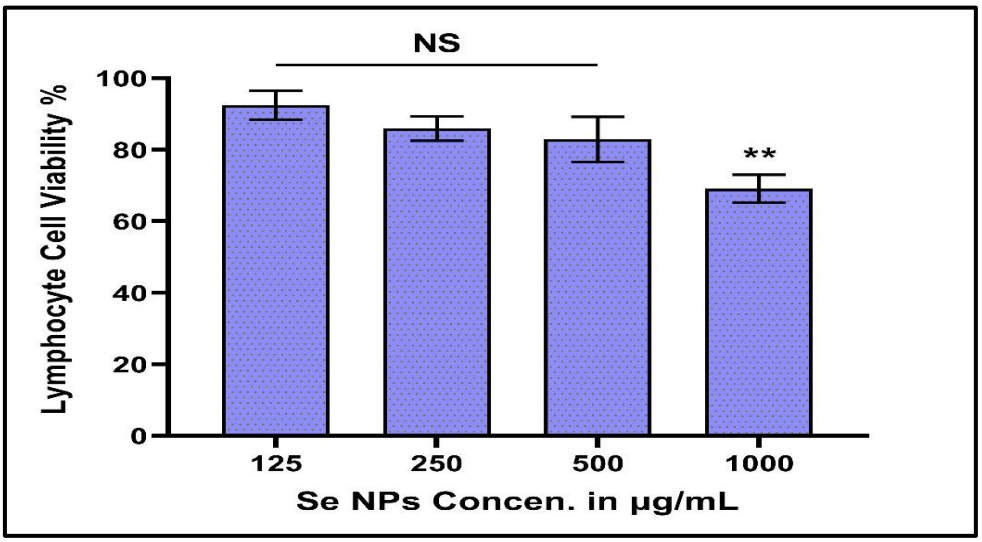


Figure (11): Cell survival curve (mean±SD%) of A375 and Cellular viability in human lymphocytes after treatment with SeNPs using MTT *in vitro* assay **: $p \leq 0.01$, NS: Non-Significant, SD: Standard Deviation, $n = 3$

The cytotoxicity assay results showed that the biologically synthesized SeNPs nanoparticles did not significantly reduce the viability of human lymphocytes. These results suggested that the biologically synthesized SeNPs nanoparticles could be a good substitute for the physical methods currently used to assess environmental toxicity. By using MTT assays, the cytotoxicity was detected in human lymphocytes 24 hours after SeNPs interaction. MTT assay results showed no appreciable decrease in cell viability when compared with control in a dose-dependent manner. MTT assays have been widely used to assess the in vitro cytotoxicity of NPs in cell culture experiments. 125, 250, 500, and 1000 µg/ml. The Selenium NPs exhibit the least reduction in cellular viability and a comparable substantial decrease(Figure 11). SeNPs treatment of human lymphocytes measured cellular viability using the MTT assay. After the cell cultures were exposed to SeNPs for approximately 24 hours, the cytotoxic effect of SeNPs on lymphocyte viability was assessed. The MTT assay results revealed that SeNPs outcome concurs with the concentration of AgNPs used determines how cytotoxic they are to cells. [33]. When IONPs were tested for cytotoxicity at different concentrations, the viability of cell lines was significantly reduced at 1000 µg/mL but did not significantly change at 125 µg/mL.

Conclusion

The extensive application of these nanoparticles can be attributed to their large surface area and positive charge, enabling them to interact with the negatively charged bacterial cell surfaces. SeNPs are the most researched materials among bacteriocin–metallic nanocomposites, demonstrating synergistic benefits in biological applications. Antimicrobial properties have been widely documented. Consequently, the rationale for combining selenium nanoparticles (SeNPs) and bacteriocins becomes evident. This combination will lessen the toxicity of nanoparticles while simultaneously broadening their antibacterial spectrum.

References

[1] C-R. Lee, J. H. Lee, M. Park, K. S. Park, I. K. Bae, Y. B. Kim, C. J. Cha, B. C. Jeong, and S. H. Lee, "Biology of *Acinetobacter baumannii*: Pathogenesis, Antibiotic Resistance Mechanisms, and Prospective Treatment Options", *Frontiers in cellular and infection microbiology*, vol. 55, no. 7, 2017.

[2] I. Sugrue, P. M. O'Connor, C. Hill, C. Stanton, and R. P. Ross, "*Actinomyces* Produces Defensin-Like Bacteriocins (Actifensins) with a Highly Degenerate Structure and Broad Antimicrobial Activity", *Journal of Bacteriology*, vol. 202, no. 4, 2020.

[3] E. S. Bindiya, and S. G. Bhat, "Marine bacteriocins: A review", *Journal of Bacteriology & Mycology: Open Access*, vol. 2, 2016.

[4] A. J. Shnoudeh, I. Hamad, R. W. Abdo, L. Qadumii, A. Y. Jaber, H. S. Surchi, and S. Z. Alkelany, "Synthesis, characterization, and applications of metal nanoparticles", *In: Advances in Pharmaceutical Product Development and Research, Biomaterials and Bionanotechnology*, pp. 527-612, 2019.

[5] D. Moulick, A. Mukherjee, A. Das, A. Roy, A. Majumdar, A. Dhar, B. K. Pattanaik, B. Chowardhara, D. Ghosh, M. K. Upadhyay, P. Yadav, S. Hazra, S. Sarkar, S. Mahanta, S. C. Santra, S. Choudhury, S. Maitra, U. N. Mishra, K. L. Bhutia, M. Skalicky, O. Obročník, V. Bárek, M. Brestic, and A. Hossain, "Selenium – An environmentally friendly micronutrient in agroecosystem in the modern era: An overview of 50-year findings", *Ecotoxicology and Environmental Safety*, vol. 270, pp. 115832, 2024.

[6] Heidari, Z., F. Ghasemi, M.,and Modiri, L. "The synergistic antibacterial effect of bacteriocin produced by *Lactobacillus casei* ATCC 39392 and iron oxide nanoparticles (IONPs) on selected foodborne pathogens", *International Journal of Molecular and Clinical Microbiology*, vol.10.1 , pp. 1301-1311,2020.

[7] Fernando, D. M., Khan, I. U., Patidar, R., Lapen, D. R., Talbot, G., Topp, E., & Kumar, A. "Isolation and characterization of Acinetobacter baumannii recovered from Campylobacter selective medium", *Frontiers in microbiology*, vol.*7*, pp. 229309, 2016.

[8] V. Namiganda, Y. Mina, A. Meklat, D. Touati, N. Bouras, M. Barakate and N. Sabaou, "Antibiotic Resistance Pattern of *Acinetobacter baumannii* strains isolated from different clinical specimens and their sensibility against bioactive molecules produced by Actinobacteria", *Arabian Journal for Science and Engineering*, vol. 44, pp. 6267-6275, 2019.

[9] Matijašić, B. B., and Rogelj, I. "Bacteriocinogenic activity of lactobacilli isolated from cheese and baby faeces ". *Food Technol Biotechnol*, vol.*37*, pp. 93-100, 1999.

[10] M. M. Bradford, "A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding", *Analytical biochemistry*, vol. 72, pp. 248-254, 1976.

[11] F.E. Ettadili, S. Aghris, F. Laghrib, A. Farahi, S. Saqrane, M. Bakasse, S. Lahrich, and M. A. El Mhammedi, "Recent advances in the nanoparticles synthesis using plant extract: Applications and future recommendations", *Journal of Molecular Structure*, vol. 1248, pp. 131538, 2022.

[12] N. H. Faiq, M. E. Ahmed, "Effect of Biosynthesized Zinc oxide Nanoparticles on Phenotypic and Genotypic Biofilm Formation of *Proteus mirabilis*", *Baghdad Science Journal*, vol. 21, no. 3, pp. 0894-0894, 2024.

[13] Yilmaz, M. T., İspirli, H., Taylan, O., and Dertli, E. "A green nano-biosynthesis of selenium nanoparticles with Tarragon extract: Structural, thermal, and antimicrobial characterization", *Lwt*, vol.141, pp. 110969, 2021.

[14] P. J. Bouvet and P. A. Grimont, "Identification and biotyping of clinical isolates of *Acinetobacter*", *Annales de l'Institut Pasteur / Microbiologie*, vol. 138, no. 5, pp. 569–578, 1987.

[15] A. Jawad, P. M. Hawkey, J. Heritage, and A. M. Snelling, "Description of Leeds *Acinetobacter* Medium, a new selective and differential medium for isolation of clinically important *Acinetobacter* spp., and comparison with Herellea agar and Holton's agar", *Journal of clinical microbiology*, vol. 32, no. 10, pp. 2353–2358, 1994.

[16] Z. Weng, N. Yang, S. Shi, Z. Xu, Z. Chen, C. Liang, and X. Du, "Outer Membrane Vesicles from *Acinetobacter baumannii*: Biogenesis, Functions, and Vaccine Application", *Vaccines*, vol. 12, no. 1, pp. 49, 2024.

[17] C. A. Moubareck, and D. H. Halat, "Insights into *Acinetobacter baumannii*: a review of microbiological, virulence, and resistance traits in a threatening nosocomial pathogen", *Antibiotics*, vol. 9, no. 3, pp. 119, 2020.

[18] J. S. Glover, T. D. Ticer, M. A. Engevik, "Profiling Antibiotic Resistance in *Acinetobacter calcoaceticus*", *Antibiotics*, vol. 11, no. 7, pp. 978, 2022.

[19] R. Vázquez-López, S. G. Solano-Gálvez, J. J. J. Vignon-Whaley, J. A. A. Vaamonde, L. A. P. Alonzo, A. R. Reséndiz, M. M. Álvarez, E. N. V. López, G. Franyuti-Kelly, D. A. Álvarez-Hernández, V. M. Guzmán, J. E. J. Bañuelos, J. M. Felix, J. A. G. Barrios, and T. B. Fortes, "*Acinetobacter baumannii* Resistance: A Real Challenge for Clinicians", *Antibiotics* (*Basel*), vol. 9, no. 4, pp. 205, 2020.

[20] G. Chen, K. Xu, F. Sun, Y. Sun, Z. Kong, and B. Fang, "Risk factors of multidrug-resistant bacteria in lower respiratory tract infections: a systematic review and meta-analysis", *Canadian Journal of Infectious Diseases and Medical Microbiology*, vol. 2020, 2020.

[21] M. E. Ahmed, and A. Q. Al-Awadi, "*Enterococcus Faecium* Bacteriocin Efflux Pump Mexa Gene And Promote Skin Wound Healing in Mice", *Journal of Microbiology, Biotechnology and Food Sciences*, vol. 13, no. 5, pp. 10711, 2024.

[22] N. O. Tymoshok, M. S. Kharchuk, V. G. Kaplunenko, V. S. Bityutskyy, S. I. Tsekhmistrenko, O. S. Tsekhmistrenko, M. Y. Spivak, and O. M. Melnichenko, "Evaluation of effects of selenium nanoparticles on *Bacillus subtilis*", *Regulatory Mechanisms in Biosystems*, vol. 10, no. 4, pp. 544–552, 2019.

[23] N. H. Faiq, and M. E. Ahmed, "Inhibitory Effects of Biosynthesized Copper Nanoparticles on Biofilm Formation of *Proteus mirabilis*", *Iraqi Journal of Science*, vol. 65, no.1, pp. 65-78, 2024.

[24] M. Ashengroph, and S.-R. Hosseini, "A newly isolated *Bacillus amyloliquefaciens* SRB04 for the synthesis of selenium nanoparticles with potential antibacterial properties", *International Microbiology*, vol. 24, no. 1, pp. 103–114, 2021.

[25] S. H. Seddiq, A. M. Zyara, and M. E. Ahmed, "Evaluation the Antimicrobial Action of Kiwifruit Zinc Oxide Nanoparticles against *Staphylococcus aureus* Isolated from Cosmetics Tools", *BioNanoScience*, vol. 13, no. 3, pp. 1140–1149, 2023.

[26] L. Gunti, R. S. Dass, and N. K. Kalagatur, "Phytofabrication of selenium nanoparticles from *Emblica officinalis* fruit extract and exploring its biopotential applications: antioxidant, antimicrobial, and biocompatibility", *Frontiers in microbiology*, vol. 10, pp. 451408, 2019.

[27] S. Pandey, N. Awasthee, A. Shekher, L. C. Rai, S. C. Gupta, and S. K. Dubey, "Biogenic synthesis and characterization of selenium nanoparticles and their applications with special reference to antibacterial, antioxidant, anticancer and photocatalytic activity", *Bioprocess and Biosystems Engineering*, vol. 44. pp. 2679-2696, 2021.

[28] T. G. Chatzimitakos, and C. D. Stalikas, "Qualitative alterations of bacterial metabolome after exposure to metal nanoparticles with bactericidal properties: a comprehensive workflow based on 1 H NMR, UHPLC-HRMS and metabolic databases", *Journal of proteome research*, vol. 15, no. 9, pp. 3322–3330, 2016.

[29] L. Zhao, and M. A. Ashraf, "Influence of silver-hydroxyapatite nanocomposite coating on biofilm formation of joint prosthesis and its mechanism", *The West Indian Medical Journal*, vol. 64, no. 5, pp. 506-513, 2015.

[30] A. Ullah, X. Yin, F. Wang, B. Xu, Z. A. Mirani, B. Xu, M. W. H. Chan, A. Ali, M. Usman, N. Ali, M. Naveed, "Biosynthesis of Selenium Nanoparticles (via Bacillus subtilis BSN313), and Their Isolation, Characterization, and Bioactivities", *Molecules*, vol. 26, no. 18, pp. 5559, 2021.

[31] F. Shakeri, F. Zaboli, E. Fattahi, and H. Babavalian, "Biosynthesis of selenium nanoparticles and evaluation of its antibacterial activity against *Pseudomonas aeruginosa*", *Advances in Materials Science and Engineering*, vol. 2022, pp. 1-10, 2022.

[32] N. Chen, P. Yao, W. Zhang, Y. Zhang, N. Xin, H. Wei, and C. Zhao, "Selenium nanoparticles: Enhanced nutrition and beyond", *Critical reviews in food science and nutrition*, vol. 63, no. 33, pp. 12360-12371, 2023.

[33] M. Prasathkumar, C. Sakthivel, R. Becky, C. Dhrisya, I. Prabha, and S. Sadhasivam, "Phytofabrication of cost-effective selenium nanoparticles from edible and non-edible plant materials of Senna auriculata: characterization, antioxidant, antidiabetic, antimicrobial, biocompatibility, and wound healing", *Journal of Molecular Liquids*, vol. 367, pp. 120337, 2022.