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Antimicrobial and antibiofilm activity of Biosynthesized Nickel Oxide Nanoparticles Using The culture supernatant of Staphylococcus aureus against Acenitobacter baumannii

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Abstract

The current study aimed to focus on the biosynthesis method of Nickel Oxide nanoparticles (NiONPs) using the cell- free culture supernatant (CFCS) from *Staphylococcus aureus* as a stabilizing and reducing agent, the nanoparticles of Nickel Oxide were synthesized using Nickel nitrate $Ni(NO_3)_2$, in addition to the therapeutic applications of Nickel Oxide nanoparticles that will be used against *A. baumannii* MDR bacteria, the Nickel Oxide nanoparticles were characterized using UV-VIS spectroscopy, atomic force microscopy (AFM), X-ray diffraction (XRD), field emission scanning electron microscopy (FE-SEM), energy-dispersive X-ray spectroscopy (EDX) and Fourier-transform infrared spectroscopy (FTIR). AFM analysis revealed that NiONPs have an average diameter of 63.81 nanometers and the FE-SEM image showed a spherical shape, Additionally, the most resistant bacterial isolate was identified, selected and tested for antibacterial activity against MDR-*A. baumannii* using different concentrations of Nickel Oxide nanoparticles (500µg/ml, 1mg/ml, 2mg/ml, and 4mg/ml), the results of the biogenic Nickel Oxide nanoparticles showed a promising activity against MDR-*A. baumannii*, with an inhibition zone diameter of 22 mm at a concentration of 4 mg/ml, while the inhibition zone diameter was 18 mm at 2 mg/ml, which is the minimum inhibitory concentration (MIC), the biogenic synthesis of NIONPs showed promising antibacterial activity against biofilm production.The results of the anti-biofilm activity using Nickel nanoparticles (NiONPs) against multidrug-resistant bacteria (MDR-*A. baumannii*) at different concentrations (500 µg/ml, 1 mg/ml, 2 mg/ml, 4 mg/ml and 4 mg/ml), respectively, against biofilm production showed that the best biofilm inhibitory concentration is 8 mg/ml of Nickel Oxide nanoparticles, which may have applications as a treatment against multidrug-resistant MDR-*A. baumannii* bacteria.

Introduction:

Multi-drug resistant bacteria *Acinetobacter baumannii* developed as a result of improperly used antibiotics, which are hard to treat and led to serious health problems all over the world therefore, it has become necessary to find some alternative ways to overcome

this problem [1], One of the most important opportunistic organisms causing many diseases is *A.baumannii*, as this pathogen is characterised by its high resistance to many antibiotics (multidrug resistance), which gives it the advantage of evading many antibiotics and this problem causes a global and health threat [2-4], numerous illnesses, including as meningitis, ventilator-associated pneumonia, bacteremia, infections of soft tissues, urinary tract infections, and infections resulting from prosthetic devices, have been linked to these bacteria [5], *A. baumannii* is widespread in many clinical settings and samples in people exposed to wounds and burns, as well as in urine, respiratory secretions and intravenous fluids. [6]

Biofilms are a collection of microorganisms that dwell on surfaces and coat them with an extracellular matrix [7], *A. baumannii's* ability to synthesis biofilms to a high degree is one of the most important characteristics of antibiotic resistance, and this factor (virulence factor) plays an important role in the development of the pathogenicity of this bacterium. [8]. Due to the widespread and noticeable spread of these antibiotic-resistant bacteria, which could pose a serious global health problem, health organizations and scientific research have invested millions of dollars to confront this threat, limit its spread, and find a safe and healthy alternative method to eliminate it [9], on the other hand the manufacture of nanomaterials through the use of a simple and inexpensive method known as the biological or green method is an important alternative and environmentally safe option to eliminate the high properties exhibited by antibiotic-resistant bacterial species. [10], Biosynthesis of NiO using bacteria extracts offers benefits over chemical and physical processes, including ease of processing, cost- effectiveness and scalability for mass production. [11, 12], interestingly, the synthesis of metal nanoparticles using biologically active agents such as plant materials, microbes, and various biological wastes has established a rapid and cost-effective biosynthetic methodology for the synthesis of stable metal nanoparticles [13,14], various techniques have been used for the synthesis of Nickel nanoparticles (NiONPs), including chemical precipitation, electrodeposition, microemulsion technique, photocatalytic reduction, co-precipitation methods, and microwave irradiation [15], Moreover, the antibacterial activity of biosynthesized Nickel Oxide nanoparticles was investigated in Gram-negative bacteria with promising results. [16-18] . This Study aims to Finding an alternative method to antibiotics to solve the problem of multidrug resistance and biofilm-producing in *Acinetobacter baumannii*, biogenic synthesis of Ni Oxide nanoparticles using a biological method using Cell- Free Culture Supernatant (CFCS) of *S. aureus* as a reducing and stabilizing agent, and Ni Oxide nanoparticles were used as an antibacterial and antibiofilm, studying of the efficiency of nanoparticles on the effectiveness of the strongest isolate in its resistance to antibiotics and its biofilm production from *A.baumannii* and determination of the Minimum Inhibitory Concentration (MIC) and minimum bactericidal concentration.

Materials and methods

Bacterial collection isolation and culture media

Collection of 122 clinical samples were collected, including samples of wounds, burns, urinary tract infections, and respiratory tract infections, From three locations in Medical City/Baghdad Burns Specialist Hospital, Martyr Ghazi Hariri Specialist Surgical Hospital and the National Centre for Educational Laboratories wabs (samples) were taken and transported to the laboratory for cultivation and planting on different planting media, including MacConkey agare, blood agar and HiCrome™ Acinetobacter Agar in the aseptic laboratory,

where they were subsequently incubated for 24 hr at 37°C, Many of them were diagnosed with the VITEK 2 compact System and collection and preparation of *Staphylococcus aureus* mannitol salt agar (MSA) and blood agar media were employed to culture *S. aureus* at 37°C for 24 hr each to establish the identification of the produced bacterial colonies, this media was prepared under the manufacturer's instructions depending on the results of morphological characteristics and biochemical tests identification tests, several biochemical tests were performed (Catalase, Oxidase and Coagulase Tests). [19]

Antibiotic Susceptibility of *Acinetobacter baumannii*

The antibiotic sensitivity test was performed using 12 antibiotic discs: Ampicillin-Sulbactam (10 mg), Ceftazidime (30 mg), Levofloxacin (5 mg), tobramycin (10 mg), imipenem (10 mg), meropenem (10 mg), Amikacin (30 mg), Piperacillin- Tazobactam (110 mg), Trimethoprim sulfamethaxazole (25 mg), Minocyclin (30 mg), doxycyclin (30 mg) and cefotaxime (30 mg), Based on their characteristic inhibitory zones, antibiotic isolates were categorized as either sensitive (S), intermediate (I), or resistant (R), this test was used to select multidrug-resistant *A. baumannii* for future study.

VITEK 2 System Identification

To confirm the highest susceptible antibiotic isolate of *Acinetobacter baumannii* was cultured overnight on a brain heart infusion agar plate to be identified using the VITEK 2 system (Biomérieux, France), the system's Gram-negative card used to automatically identifies fermenting and non-fermenting Gram-negative bacterial species. [20]

Biosynthesis of Nickel Oxide Nanoparticles

Nickel oxide NPs were prepared using of cellular extract derived from *Staphylococcus aureus* and this is the first time, with some modifications as follows: 2 g of Nickel (II) nitrate hexahydrate. Ni $(NO_3)_2$, $6H_2O$ was dissolved in 200 ml of deionized water, after complete dissolution, 50 ml cellular extract of *Staphylococcus aureus* bacteria filtrate the solution was then left at 37°C for 48 hr, after that, 10 ml of sodium hydroxide NaOH (30 mg/ml) was added drop wise to the precursor solution and then left for 24 hr until the solution became dark in color with the appearance of nano suspension, the separation process was carried out using a centrifuge, with repeated washing 5 times with deionized water and ethanol to ensure the purity of the precipitates, the drying process was carried out in an oven at 37°C for 24 hr, resulting in the formation of a fine black fine powder.

Characterization of Nickel Oxide NPs

The Nickel Oxide nanoparticles were characterized by several techniques like UV- visible, AFM, XRD, SEM, EDX and FTIR UVvisible was performed by using UV-1800- Vis Spectrophotomete , AFM was used to find out the average diameter of Nanoparticles, XRD analysis was also used to know the element identity.

Minimum Inhibition Concentration (MIC) method

Bacterial growth was prepared using nutrient broth, which was incubated for 24 hr, the bacterial culture was then diluted and compared to a McFarland standard of 0.5 to ensure uniform density, following this 100 microliters of the nutrient broth without bacterial growth were added to each well of the microtiter plate, followed by the addition of 100 microliters of the sample whose activity was to be measured, applying a series of dilutions, Additionally, 10 microliters of the bacterial growth equivalent to the McFarland standard were added to all wells, except for the control (-) well, which was kept containing only the broth.

The plates were incubated at 37°C for 24 hr, after this period, the plates were removed, and 20 microliters of the prepared resazurin dye were added to all wells, the plates were then returned to the incubator for an additional 2 hr optical density was measured at 492- 630 nm. [21]

Antibacterial activity assay

The antibacterial activity of NiO nanoparticles was investigated using gram-negative bacteria *Acinetobacter baumannii* that produce biofilm were isolated from hospitals, well diffusion agar method was used to determine the minimum inhibition concentration (MIC) of NiO nanoparticles for the test microorganism [22], muller-Hinton agar medium was sterilized, cooled, and then poured into sterilized Petri dishes and allowed to solidify at room temperature. Sterile cotton swabs were used to transfer and spread the overnight growth of test microorganisms onto the agar medium, and then wells were made. After that, various concentrations test (500 µg/ml, 1 mg/ml, 2 mg/ml, and 4 mg/ml) of NiO nanoparticles were added to the wells and then incubated for 24 hr at a temperature of 37**°C** the inhibition zone surrounding the well was measured after incubation at 24 hr. [23]

Antibiofilm activity assay

The experiment was performed by ELISA, by filling 96 wells with a concentration of 1* $I0^6$ and then treated with NiONPs for 24 hr, according to the method developed by Al Rugaie *et al* (2022) [24] with some modifications, where the samples were washed with PBS solution and crystal violet dye was used to stain the bacteria and the dye concentration was 0.1%, the process was repeated twice, then 0.2 ml of 95% ethanol was added to each well of the experimental plate in order to determine the growth of biofilms, rinsed twice with saline, then incubated with shaking for two hours, and finally the 595 nm wavelength was used to measure the optical density.

Results and Discussion

Isolation and Identification of *Acinetobacter baumannii*

The results of microscopic examination (Gram Staining), biochemical tests and VITEK 2 system were used to identify *A.baumannii* isolates showed that out of 122 clinical specimens of wounds, urine, burns and sputum, 41 isolates were identified as *A. baumannii* these positive isolates were obtained in high percentages, 70.73% (n=29) from wound and Burn specimens, sputum specimens constituted 19.51% (n=8) and the low percentage was obtained from urine specimens. Which achieved 9.75% (n=4), Number and percentage of *A. baumannii* isolates per specimens source were shown in Table 1.

Table 1: Number and percentage of *Acinetobacter baumannii* isolate in accordance to specimen source.

Source of specimens	No. of samples (%)	No. of *A. baumannii* (%)
Wound and Burn infection (swab)	58 (47.54%)	29 (70.73%)
Urinary tract infection (Urine)	30 (24.59%)	4 (9.75%)

Respiratory tract infection (Sputum)	34 (27.86%)	8 (19.51%)
Total	122 (100%)	41 (100%)

Antibiotic Susceptibility of *Acinetobacter baumannii*

This study concluded that a total of 41 *A. baumannii* clinical isolates had a high resistance to Ceftazidime, Levofloxacin, Imipenem, Amikacin, Piperacillin- Tazobactam and Cefotaxime (100%), Tobramycin (95.12%), Meropenem (85.36%), Ampicillin (80.48%) and Trimethoprim sulfamethaxazole (78.04%), Minocyclin (29.26%) showed moderate resistance, while Doxycyclin showed low-level resistance (7.31%), the differences in the susceptibility patterns may be due to the type of patients enlisted for the study and the source of collected specimens, this test was used to select a multi-drug resistance *A. baumannii* for further study.

VITEK 2 System Identification

The VITEK 2 compact system was used as a confirmatory test for the identification of the multi-drug resistance, *A. baumannii* and the characterization of the findings for *A. baumannii*.

Field Emission Scanning Electron Microscope

FE-FSM is often used to describe the morphological and microstructural properties of various materials (such as crystal structure, particle shape, and nanosize for NiO, the results of the examination of nanosized Nickel Oxide by FE-SEM examination under 200 nm magnification revealed that the Nickel Oxide was spherical in shape Figure 1, these results showed that Nickel Oxide can be synthesized with good nanosize but the size obtained did not agree with Jassim *et al* (2023) [25] who prepared Nickel Oxide with a size of 39.7 nm.

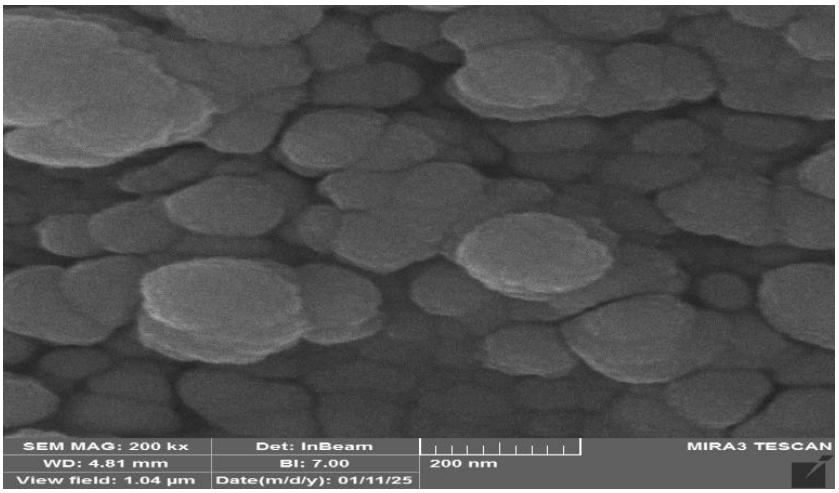


Fig. 1 *FE-SEM Images of NiONPs*

Atomic Force Microscope (AFM) of NiO Nanoparticales

Analysis used to confirm the morphology and particle size of Nickel Oxide NPs, and the results are displayed in Figure 2, Atomic Force Microscopy (AFM) was used to examine the surface shape development of Ni Oxide nanoparticles and show the 2D and 3D appearance of Ni Oxide nanoparticles, the average size of Ni oxide nanoparticles, which was found to be 63.81 nm, was also ascertained using AFM, and analysis revealed both spherical like Nickel Oxide nanoparticles in the size range of 43 to 85 nm.

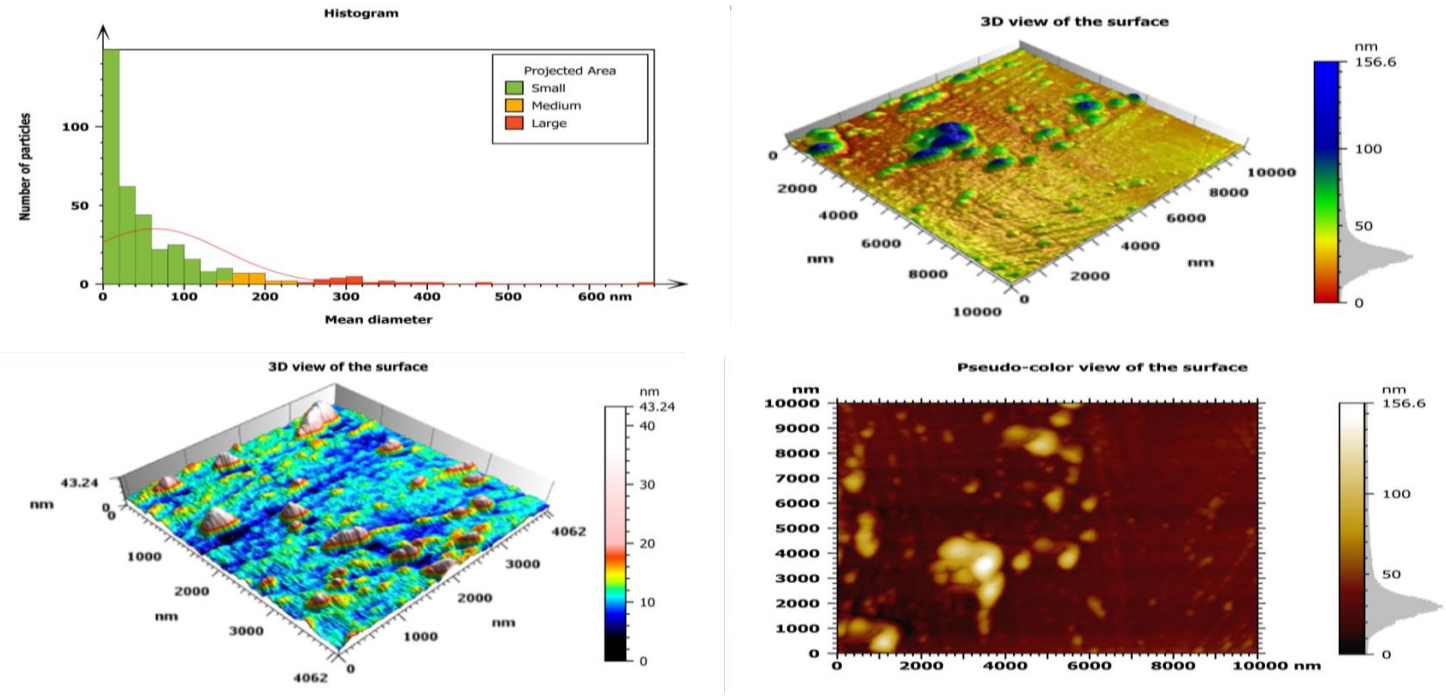


Fig. 2 *AFM scan results for NiONPs*

X-Ray Diffractometer

The (XRD) technique relies on the remarkable interaction between the crystalline sample and monochromatic X-rays, the interaction between the sample and the incident rays generates constructive interference and diffracted rays, which will produce an XRD pattern, the results of the biosynthesized NiONPs in XRD examination, Figure 3 shows the presence of several peaks of 37.250^0, 43.340^0, 62.950^0 and 75.650^0, which are equivalent to the peaks found in the standard model (111), (200), (220), (220), and (311), respectively [26], According to Debye-Scherrer formula the crystallite size can be calculated from the XRD pattern.

$$C.S = \frac{K\lambda}{\beta\, COS\theta}$$

C.S=size of crystallite (nm)
k=constant dependent on crystallite shape
λ=x-ray wavelength (mostly λ for cu)
β =FWHM(full width at half max)or integral breadth
θ=Brag Angle

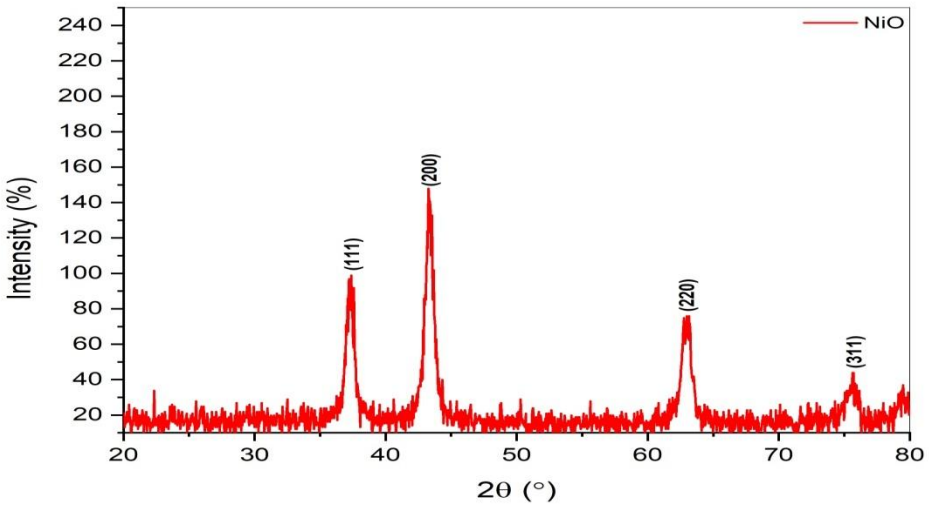


Fig. 3 *XRD images of NiONPs.*

Fourier Transform Infrared Spectroscopy (FTIR)

The FT-IR measurements were recorded to identify the major functional groups of Ni Oxide NPs, the range of the synthesized Ni Oxide NPs by using the infrared spectrum was (400-4000cm^{-1}) the spectrum of biosynthesized NiONPs from cellular extract derived *Staphylococcus aureus* is shown in Figure 4, the large absorption peak at 3494 cm^{-1} suggests hydroxyl group, commonly seen in carboxylic acids and phenolics , another signal 1632 cm^{-1} is linked to the bending mode (H-O-H) of water molecules, oxide groups have distinctive absorption peaks at 834cm^{-1}, 930cm^{-1}, 1047cm^{-1} and 1356cm^{-1}, respectively, the peak at 2161 cm^{-1} and 2027 cm^{-1} suggests the existence of an OH group, the peak at 402 cm^{-1} is created by Ni-O vibrations, which were consistent with what he found by Lingaraju *et al* (2020) [27] thus, it is obvious that the chemicals play a role in the biotransformation of nitrates into oxides, bacterial compounds function as a reducing agent during the creation of nanoparticles. [28]

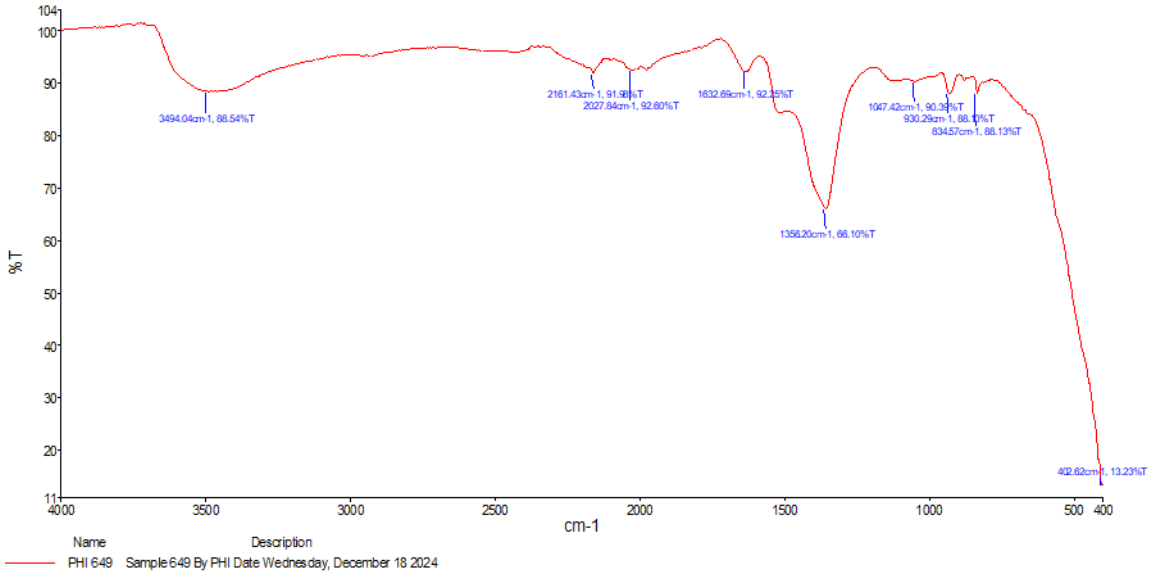


Fig. 4 *FTIR images of NiONPs*

Diagnosis of NiONPs through EDX screening

Figure 5 shows the results of NiONPs examination by EDX, the percentage of Nickel was very high, as it was found in peak 5 and peak 8, Table 2 also shows the percentages of each element present in the examined sample, the table in addition shows the presence of nickel at a rate of 55.93%.

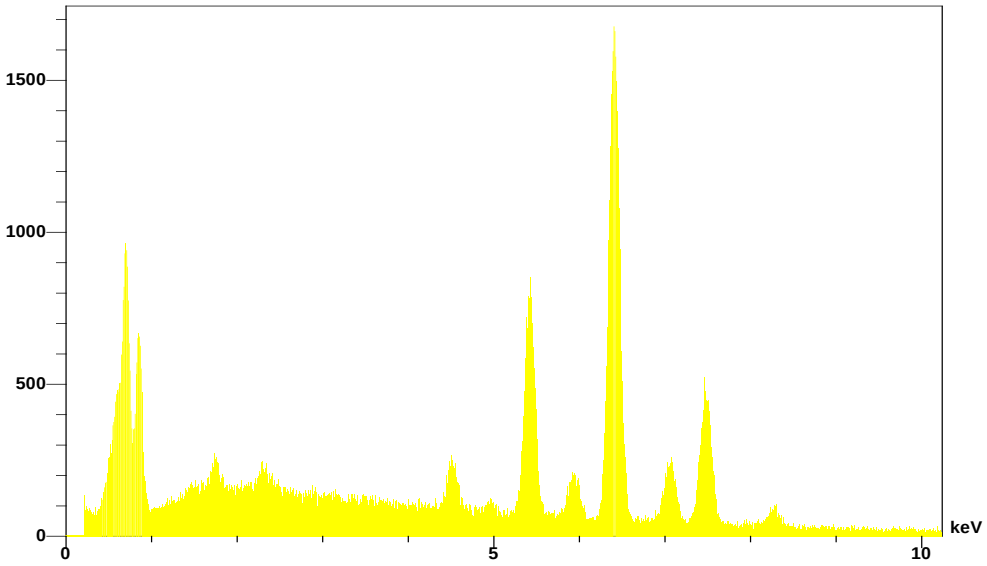


Fig. 5 *Diagnosis of NiO NPs through EDX screening*

Table 2: Percentages of NiO NPs through EDX screening

Elements	Weight (%)	Atomic (%)
O	44.07	74.30
Ni	55.93	25.70

UV–VIS spectral analysis Ni Oxide NPs

To detect the maximum absorption, figure 6 shows the results of UV-visual examination of Ni oxide NPs, an absorption peak appeared at 340 nm, indicating the presence of nanoparticles. [29]

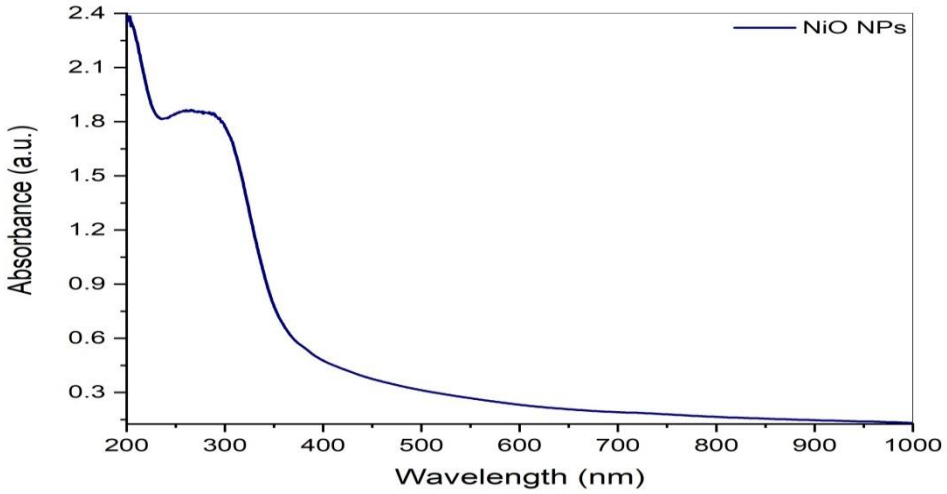


Fig. 6 *Absorption spectra of the purified Ni Oxide NPs*

Nanoparticle antibacterial susceptibility test:

The results of the evaluation experiment of NiO nanoparticles prepared by the bio-based method showed that they possess antibacterial activity against MDR-*A. baumannii* at values of 500 µg/ml, 1 mg/ml, 2 mg/ml and 4 mg/ml in Table 3 and NiO nanoparticles showed antimicrobial activity in Figure 7 and it is clear that the antibacterial activity of NiO nanoparticles is dependent on the concentrations of NiO used, with concentrations as low as 500 µg/ml producing an inhibition zone of 15 mm, while a NiO concentration of 4 mg/ml produces an inhibition zone of 22 mm, the extent of the inhibition zone may be determined by the sensitivity of the bacteria and the many ways in which NiO interacts with the selected bacteria, it was discovered that the maximum inhibition zone at a concentration of 4 mg/ml results in an inhibition zone of 22 mm, the difference in inhibition diameter may be due to the different interactions between NiONPs and microorganisms and due to the sensitivity of the bacteria used in the current study. [30]

The harmful effects of nickel oxide particles on bacteria are mostly caused by the formation of reactive oxygen species (ROS) that act on the membranes and walls of bacteria and thus affect the destruction of lipids, proteins and other biomolecules and because of this will cause oxidation of the bacteria and then their death [31] Also, Nickel Oxide particles of nanoscale size can produce free radicals (OH^-) that have an antibacterial role. [32]

Table 3: Explain the antibacterial activity of nanoparticles

Sample	A	B	C	D	E
NiONPs concentration	Control	500 µg/ml	1 mg/ml	2 mg/ml	4 mg/ml
A.baumannii	6	15	17	18	22

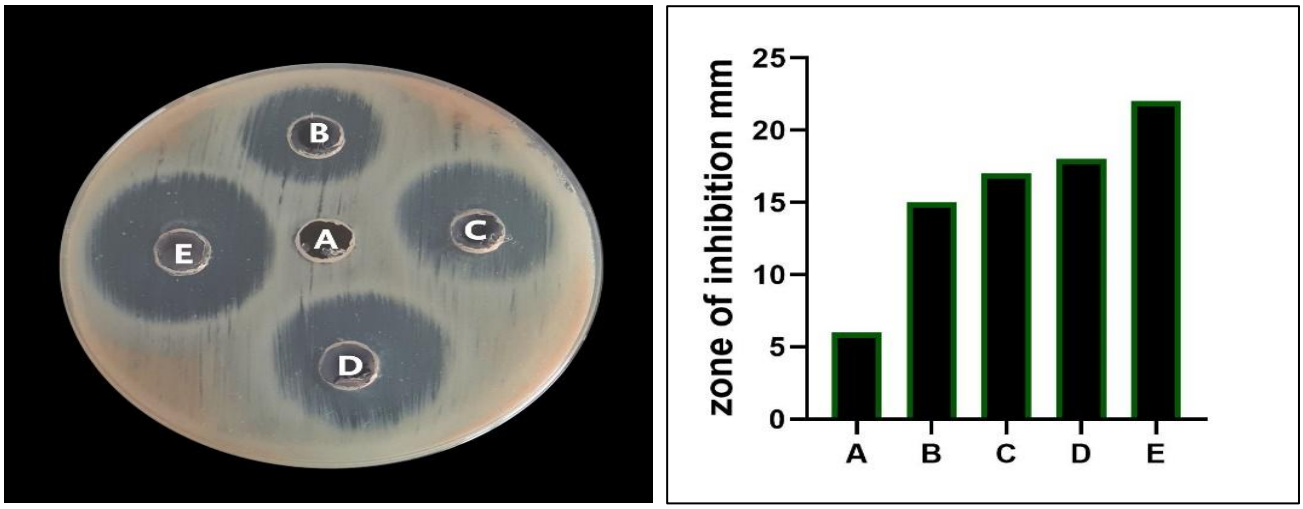


Fig. 7 *Inhibition zones Antibacterial activity of NiO nanoparticles against MDR- A.baumannii A= (Control), B= (500 µg/ml), C= (1 mg/ml), D= (2 mg/ml), E= (4 mg/ml).*

The MIC represents the lowest concentration that can be chosen with the best inhibition results this concentration and dose of the antigen completely stops the growth of the bacterial strain, and this is evaluated by preparing a series of in vitro dilutions with dye resazurin in staining the samples the range of different concentrations 7.8µg/ml- 4 mg/ml as in Table 4 and Figure 8, the concentration of 4 mg/ml showed the highest concentration diameter of inhibition of *A. baumannii* to kill bacteria and the results showed that the lowest concentration of inhibition of *A. baumannii* was 2 mg/ml. [33]

Table 4: Minimum Inhibitory Concentration (MIC) Explain result of sample by Elisa advice (spectrophotometer)

NiONPs Concentration		- ve	+ ve	4 mg	2 mg	1 mg	500 µg	250 µg	125 µg	62.5 µg	31.2 µg	15.6 µg	7.8 µg
Sample of *A.baumannii*	NiONPs	0.0	1.9819	0.0	0.0	0.200	0.256	0.476	0.481	0.494	0.520	0.539	0.547

Fig. 8 *Minimum Inhibitory Concentration (MIC) determination assay by resazurin in microtiter plate. A.baumannii using the (NiONPs).*

Antibiofilm of MDR- *A.baumannii* by synthesized NiO nanoparticles

The diffusion of nanosized materials into the biofilm is highly dependent on the presence of water channels and pore size as well as the charge of the nanomaterials as well as the

extracellular polymeric matrix (EPM) and when these particles migrate into the biofilm will be determined by the size and charge of the nanoparticles as well as the composition and structure of the EPM. [34]

In an experiment, biofilm formation from *A. baumannii* was a response to concentrations of NiO (a = control, b = 500 µg/ml, c = 1 mg/ml, d = 2 mg/ml, e = 4 mg/ml and f = 8 mg/ml) using the microtiter plate by ELISA took the dye crystal violet dye staining method Figure 9 and Table 5, the nanosized NiO particles showed a significant inhibition of biofilm development, and the prevention of biofilm formation was significantly reduced with the increase of Ni subconcentration as shown in Figure 8, NiO nanosized particles inhibit biofilm development by inhibiting the adhesion steps and processes in bacteria, the presence of Nickel leads to bacterial cell death upon exposure. [24]

Fig. 9 *Reduces biofilm formation by Sample in A.baumanii (NiONPs) in A.baumanii: A= (Control), B= (500 µg/ml), C= (1 mg/ml), D= (2 mg/ml), E= (4 mg/ml), F= (8 mg/ml).*

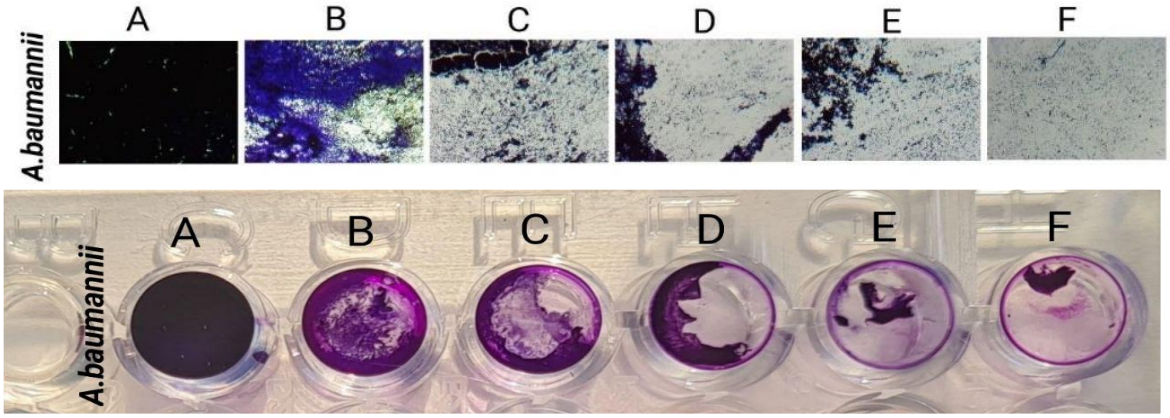
Table 5: The effect of NiONPs on *A.baumanii* Biofilm

Sample	A	B	C	D	E	F
NiONPs concentration	Control	500 µg	1 mg	2 mg	4 mg	8 mg
A.baumanii	0.9	0.6	0.5	0.35	0.2	0.15

Antibacterial activity and antibiofilm activity was done through the disc diffusion method and ELISA using gram-negative MDR-*A.baumanii* bacterial strains, the antibacterial and antibiofilm study results exhibited that the NiO nanoparticles have a significant impact on the Gram- negative MDR-*A.baumanii.* [35]

limitation of the study

The difficulty in obtaining samples, especially burn samples, is due to their unavailability and the need for specialized hospitals in addition to the challenge of convincing the patient and their relatives to take a sample, the challenges and difficulties include the lack of financial support from the ministry to complete the research requirements and related matters, as well as to finish the master's thesis Additionally, some equipment is not available in the college



laboratories, forcing us to work outside the province or in external laboratories, samples may also be damaged due to power outages, which lead to the shutdown of equipment and the destruction of samples.

Conclusion

The ability of Biosynthesized Nickel Oxide NPs from cell- free culture supernatant (CFCS) of *Staphylococcus aureus* as a reducing and stabilizing source, was an effective method to gave best properties for nanoparticles synthesized, Nickel Oxide NPs synthesized by biological method was characterized by UV–visible spectroscopy where a final band was at 345 nm, the crystallinity determined by X-ray Diffraction (XRD), the surface morphology of the NiONPs by Atomic Force Microscopy (AFM) to gave 3D topological for NiONPs and the size was estimated 63.81 nm, the FTIR measurements are recorded to identify the major functional groups for purified (CFCS) of *Staphylococcus aureus* and to identify the functional groups of (CFCS) act as reducing agent to synthesis NiONPs, the (FE-SEM) to determine the shape and size of the nanomaterial, and the NiONPs were characterized by spherical, semi-spherical and uniform shapes, biosynthesized Nickel Oxide NPs shows remarkable inhibition activity against the growth of *A. baumannii*, the results showed that the inhibition concentration was 4 mg/ml with a diameter of 22 mm it may be considered one of the most promising methods for treating these types of bacterial infections.

Recommendations

Studying the antibacterial activity effects of Nickel Oxide Nanoparticles the inhibitor for other bacteria, studying the synergistic and antagonistic effects of NiONPs with ZnONPs for the enhanced biofilm inhibitor for other bacteria, possibility of synthesized NiO nanoparticles by using other extracts (plant, bacteria, fungi and algae).

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فعالية أكسيد النيكل المخلق حيوياً من بكتريا المكورات العنقودية الذهبية ضد الميكروبات و أغشية الراكدة البومانية

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الخلاصة:

هدفت الدراسة الحالية للتركيز على طريقة التخليق الحيوي لجسيمات أكسيد النيكل النانوية (NiONPs) باستخدام المستخلص الخلوي الناتج من *Staphylococcus aureus* كعامل استقرار واختزال تم تصنيع الجسيمات النانوية من أكسيد النيكل باستخدام نترات النيكل $Ni(NO_3)_2$, بالإضافة إلى التطبيقات العلاجية لجزيئات اكسيد النيكل النانوية التي سوف تستخدم ضد بكتريا MDR-*A.baumannii* وتم توصيف جسيمات اكسيد النيكل النانوية باستخدام الأشعة فوق البنفسجية المرئية (UV-VIS), المجهر الذري (AFM)، مقياس حيود الأشعة السينية (XRD)، والمجهر الإلكتروني الماسح (FE-SEM) و تحليل الأشعة السينية المشتتة للطاقة (EDX) و التحليل الطيفي للأشعة تحت الحمراء (FTIR). كشف تحليل AFM أن NiONPS يبلغ متوسط حجم قطرها 63.81 نانوميتر وتعرض الصورة FE-SEM صورة كروية بالإضافة إلى ذلك , تم تحديد العزلة البكتيرية الاكثر مقاومة, تم اختيارها و إجراء اختبار النشاط المضاد للبكتيريا ضد MDR-*A.baumannii* عليها باستخدام تركيزات مختلفة من جزيئات أكسيد النيكل النانوية (500µg/ml, 1mg/ml, 2 mg/ml و 4 mg/ml), أظهرت نتائج جزيئات أكسيد النيكل المصنعة حيوياً نشاطاً واعداً ضد بكتريا MDR-A. *baumannii* وكان قطر منطقة التثبيط يبلغ 22 ملم عند تركيز 4 mg/ml بينما كان قطر منطقة التثبيط يبلغ 18 ملم عند 2 mg/ml و هو تركيز التثبيط الأدنى (MIC) أظهر التخليق الحيوي لـ NiONPs نشاطا واعدا كمضاد للبكتيريا ضد إنتاج الغشاء الحيوي. كما أظهرت النتائج المضادة للأغشية الحيوية باستخدام جسيمات النيكل النانوية NiONPs في البكتيريا المقاومة المتعددة للأدوية MDR-*A.baumannii* بتركيزات مختلفة (500µg/ml, 1mg/ml, 2 mg/ml و 4 mg/ml 8)، على التوالي، ضد إنتاج الأغشية الحيوية، أظهر أن افضل تركيز مثبط للأغشية الحيوية هو 8 mg/ml من جسيمات اكسيد النيكل النانوية، مما قد يكون له تطبيقات كعلاج ضد بكتيريا MDR-*A.baumannii* المقاومة المتعددة للأدوية.