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Biosynthesis, Characterization, and Evaluation of the Therapeutic Potential of Zinc Oxide Nanoparticles

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Abstract

This research aimed to produce Zinc Oxide nanoparticles using bacterial isolates and examine their potent antibacterial properties and toxic effects on breast cancer cell. A clinical isolate of *Pseudomonas aeruginosa* was selected from 170 clinical bacterial isolates for the biosynthesis of nanoparticles. ZnO NPs were biosynthesized from the bacterial filtrate. The physiochemical properties of the synthesized ZnO NPs were then characterized using various techniques including UV–vis spectroscopy, Atomic Force Microscope (AFM), Fourier Transform Infrared (FTIR), Energy Dispersive Spectroscopy (EDS) and Field Emission Scanning Electron Microscopy (FESEM). The antibacterial tests were conducted using the agar well diffusion technique, employing ZnO NPs at concentrations ranging from 3.125 to 100 µg/ml. Additionally, the anticancer effects on MCF-7 breast cancer cells were examined using the MTT assay. The study also included an assessment of apoptotic activity using Annexin V, analyzed through flow cytometry. From FESEM analysis, ZnO NPs were found to have an average size of 32.5 nm. and the other characteristic analysis confirmed the production of nanoparticles. The activity of produces ZnO NPs was tested against three pathogens (*Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*) in solid media. ZnO NPs showed potential antibacterial activity against tested bacteria, at concentration 100 µg/ml of ZnO NPs which showed the highest inhibition zones were (28, 27 and 23mm) for *S. aureus* , *E. coli* and *P. aeuroginosa* respectively , Whereas the lowest inhibition zones were observed at 3.125 µg/ml for the *S. auereus* and *E.coli* by diameter (9 and 10 mm) respectively and with no effect on *P. aeuroginosa*. These nanoparticles also showed a dose response anticancer activity against MCF-7 cells with IC_{50} 72.87µg ml^{-1} while on normal HEF cell line showed less effective. Annexin-V / Propidium Iodide (PI) flow cytometry analysis confirmed that ZnO NPs induce apoptosis in MCF-7 cells.

Keywords: Biosynthesis , zinc oxide nanoparticles, antibacterial, anticancer, MTT assay , flow cytometry , apoptosis.

تخليق جسيمات أكسيد الزنك النانوية بواسطة التخليق الحيوي وتوصيفها وتقييم إمكانياتها العلاجية

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الخلاصة

يهدف هذا البحث إلى إنتاج جزيئات أكسيد الزنك النانوية باستخدام العزلات البكتيرية ودراسة خصائصها المضادة للبكتيريا وتأثيراتها السامة على خلايا سرطان الثدي. اختيرت العزلة السريرية لبكتريا الزائفة الزنجارية من بين 170 عزلة بكتيرية سريرية لغرض التصنيع الحيوي للدقائق النانوية. تم تصنيع ZnO NPs حيويا من

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الراشح البكتيري. تم بعد ذلك توصيف الخواص الفيزيائية والكيميائية لأكسيد الزنك NPs المُصنَّع باستخدام تقنيات مختلفة بما في ذلك التحليل الطيفي للأشعة فوق البنفسجية، AFM، EDX، FTIR، EDS و FESEM. تم إجراء الاختبارات المضادة للبكتيريا باستخدام تقنية الانتشار خلال الوسط الزرعي، باستخدام ZnO NPs بتركيزات تتراوح من 3.125 إلى 100 ميكروجرام/مل. بالإضافة إلى ذلك، تم فحص التأثيرات المضادة للسرطان على خلايا سرطان الثدي MCF-7 باستخدام اختبار MTT. وتضمنت الدراسة أيضًا تقييمًا لنشاط موت الخلايا المبرمج باستخدام Annexin V، وتم تحليله من خلال قياس التدفق الخلوي. من تحليل FESEM، وجد أن الحجم النموذجي لحجم ZnO NPs هو 32.5 نانومتر وأكد التحليل المميز الآخر إنتاج الجسيمات النانوية. تم اختبار نشاط منتجات ZnO NPs ضد ثلاثة مسببات الأمراض (المكورات العنقودية الذهبية، الإشريكية القولونية والزائفة الزنجارية) في الوسائط الصلبة. أظهرت ZnO NPs نشاطًا مضادًا للبكتيريا محتملًا ضد البكتيريا التي تم اختبارها. عند التركيز 100 ميكروجرام/مل من NPs التي أظهرت أعلى مناطق تثبيط كانت (28، 27 و 23 ملم) لكل من S. aureus و E. coli و P. aeuruginosa على التوالي، في حين كانت أدنى مناطق التثبيط هي (28، 27 و 23 ملم). ولوحظت مناطق التثبيط عند 3.125 ميكروجرام/مل لكل من S. auereus و E.coli بقطر (9 و10 ملم) على التوالي، ولم يكن لها أي تأثير على P. aeuruginosa. أظهرت هذه الجسيمات النانوية أيضًا نشاطًا مضادًا للسرطان استجابة للجرعة ضد خلايا MCF-7 مع IC50 72.87 ميكروجرام مل-1 بينما أظهر خط خلايا HEF الطبيعي أقل فعالية. أكد تحليل قياس التدفق الخلوي (Annexin-V / Propidium Iodide PI) أن ZnO NPs يحفز موت الخلايا المبرمج في خلايا MCF-7.

Introduction

Nanomaterials have garnered considerable attention in recent years for their potential applications across a range of scientific and technological domains [1]. Among these nanomaterials, zinc oxide (ZnO) nanoparticles have demonstrated potential for use in antibacterial and anticancer therapies, among other biological applications [2-4]. The remarkable physiochemical properties of ZnO NPs make them highly attractive for use in biomedical application. These properties include a high surface area to volume ratio, excellent biocompatibility, and adjustable optical properties [5, 6].

ZnO NPs have become increasingly important as eco-friendly and sustainable manufacturing techniques have emerged [7]. Traditional methods for generating nanoparticles include the use of hazardous substances and extreme reaction conditions, which pose risks to both the environment and public health [8, 9]. Due to this consequence, researchers are now focusing on the use of eco-friendly methods for synthesis nanoparticles, such as biosynthesis. Biosynthesis involves the use of biological agents and natural resources [10].

The synthesis of ZnO NPs used a variety of laborious, risky and costly physical and chemical procedures. Bacterial-mediated synthesis of ZnO NPs offers several advantages over chemical and physical methods, such as being environmentally friendly, non-toxic, biodegradable, and biocompatible. The use of bacterial sources for the synthesis of ZnO NPs is a sustainable alternative to conventional methods. Several bacteria, including *E. coli, P. aeruginosa, B. subtilis*, and others, have been shown to produce ZnO NPs extracellularly through enzymatic activity [11]. As part of the biosynthetic process, enzymes produced by bacteria reduce the amount of zinc ions present in the growing medium [12].

In addition, ZnO NPs have piqued strong interest from the biomedicine field due to their potent antibacterial and anticancer effects [7]. Multiple studies have demonstrated that ZnO NPs possess good antibacterial properties against wide range of pathogenic bacteria, including both Gram-positive and Gram-negative strains [13]. ZnO NPs possess unique physiochemical

characteristics, including a large surface area and the ability to generate reactive oxygen species (ROS). These features damage bacterial cell membrane, prevent bacterial growth, and trigger oxidative stress [14, 15].

Nanoparticles (NPs) offer an adaptable mechanisms for therapeutic applications relying on their physical traits, enabling them to conduct numerous new bactericidal pathways that induce antimicrobial actions, which is especially valuable in view of increasing the problem of antibiotic resistance [16]. In this context, the size, scattering, shape, structure, and surface features of NPs contribute to their being known as antibacterial factors [17]. The biosynthesized ZnO NPs typically exhibit a relatively uniform size distribution, with an average diameter of approximately 20 nm. The high surface-to-volume ratio and small size provide more reliable antibacterial effects [18]. Bacterial cells are destroyed when ZnO NPs get attached with them as they generate reactive oxygen species involving hydroxyl radicals and hydrogen peroxide, which impact the lipids and proteins in the cell walls and membranes [17].

In addition to antimicrobial effects, ZnO NPs also exhibit high anticancer properties. Various cytotoxic mechanisms against cancer cells include induction of oxidative stress, DNA damage, cell cycle arrest, and ultimately apoptosis [7] . In addition to antibacterial properties, ZnO NPs also show great potential in anticancer properties. These substances exhibit selective cytotoxicity against cancer cells, specifically avoiding normal cells, making them attractive options for targeted cancer therapy [19]. The anticancer effects of ZnO NPs are attributed to multiple mechanisms, including induction of apoptosis, DNA damage, reduction of cell growth, disruption of cell signaling pathways, and changes in the tumor microenvironment [20]. Given ZnO NPs' promising biomedical applications, we aimed to biosynthesize them using *Pseudomonas aeruginosa* and evaluate their antibacterial and anticancer potential.

Materials and Methods

Microorganisms

A clinical strain of *Pseudomonas aeruginosa*, originating from a wound, was obtained from a collection of 170 samples gathered from various hospitals across Baghdad. Biochemical and molecular (*phzM* gene detection by PCR technique) identification of *p. auerogino*sa selection were done for the highly producing isolates of pyocyanin.

Preparation of the bacterial extract

P. aeruginosa cultured in 1L of nutrient broth incubated in shaker incubator 120 rpm for 96 hr at 37°C. Centrifugation was used to collect the supernatants for 10 minutes at 10,000 rpm. Then passes through Millipore filter 0.22um which be ready for nanoparticles synthesis [21].

Synthesis of ZnO NPs

According to the provided information [22], the bacterial filtrate (10 ml) was diluted with 10 ml of 1.0 mM zinc acetate salt solution. The pH of the mixture was adjusted to 6.5, and it was then incubated for 72 hrs. at 32°C while shaking at 150 rpm in an orbital shaker. The White precipitate was obtained by centrifugation at 10,000 rpm for ten minutes, after which it was lyophilized.

Characterization of biosynthesized ZnO NPs

Several characterization techniques, including atomic force microscopy (AFM), Spectroscopy using Fourier transform infrared (FTIR), ultraviolet-visual (UV-Vis) spectroscopy, and Field emission scanning electron microscopy (FESEM) were used to

characterize the physical and chemical properties of the materials. The next subsections provide a detailed description of the above-mentioned techniques:

Ultraviolet-Visible (UV-Vis) Spectral Analysis

To monitor the interaction between bacterial extract and Zinc ions, a UV-visible spectrophotometer was used to get the UV-Vis spectrum analysis, which was completed within the 200–1200 nm range at a resolution of 0.86 nm (JASCO 670-UV).

Fourier Transform Infrared (FT-IR) Analysis

The FTIR analysis was conducted to investigate the chemical bonding between the atoms of the prepared materials and the bacterial extract used in the biosynthesis process. The particular technique was performed using the Shimadzu-IR Affinity-I spectrophotometer. In this procedure, the samples under test are homogenization with KBr and then between 4000 and 400 cm-1, the FTIR spectrum was obtained under. Before the recording procedure, potassium bromide (ARgrade) was dried and mixed KBr (100mg) as well as ZnO (1 mg) and Bacterial extract separately whereby the KBr pellet was obtained under a vacuum with the temperature of 100 °C and a period of 48hrs [23].

Atomic Force Microscope (AFM)

This technique was introduced to investigate the surface shape of the prepared ZnO NPs using Inc. SPM-AA300. (U.S.) with AFM communication mode. In particular, 5 drops of Bacterial extract -ZnO NPs solution were dropped on a specific laboratory slide and kept for 30 min at 110 °C in an oven. Continuously, the 3D morphology/topography of the sample u under the test surface can be screened via the interaction of the probe force on the sample surface. This procedure is attained via a raster scan of the material under test-taking into consideration the distance and the force between the tip as well as the sample tested. To obtain clear sample topography, the force between the sample and the tip should be considered carefully [24].

(FESEM) Field Emission Scanning Electron Microscopy

The SEM technique was employed to investigate the surface morphology and shape of the synthesized ZnO NPs. The samples were collected using a centrifuge for 30 min and later dried at 100°C for 30min. In the FESEM technique, the beam of electrons is focused on the material/s under test for image generation through surface scanning. The atoms of the material/s interact with the electron beam which results in a singles generation which leads to obtaining some composition information and surface morphology [25].

Energy Dispersive X Ray Spectroscopy (EDX)

To gain a deeper understanding of the surface characteristics of the ZnO NPs, an EDS analysis of the sample was conducted to verify the existence of zinc oxide nanoparticles produced using the biosynthesized technique. The same area of dried sample previously used in FESEM was analyzed for EDX.

Antibacterial activity of synthesized ZnO NPs

The antibiotic resistant bacteria (*S. aureus, E. coli, and P. aeruginosa*) were selected for the estimation of antibacterial activity of ZnO NPs. Helpfully, samples of these bacterial strains were generously provided by Biotechnology Department/ college of Sciences, University of Baghdad. By agar well diffusion method, overnight cultured strains of 50 µl were streaked on Muller Hinton agar plates. Using a gel puncher, a 5 mm diameter well was created in the plates. Different concentrations of ZnO NPs (100, 50, 25, 12.5, 6.25, and 3.125 mg/ml) and zinc acetate as a negative control were added to the wells. For each of the three bacterial strains, the

procedure was repeated. The plates were placed in an incubator set at 37ºC for two days. The antibacterial activity of ZnO NPs was identified by examining the zone of clearance [26].

Anticancer activity of ZnO nanoparticles on Human breast cancer and Human foreskin fibroblasts cell lines

Cell Culture

MCF7 (breast cancer) and HFF (Human foreskin fibroblasts cell) cancer cell lines were obtained from Biotechnology Research Center, Al-Nahrain University , Iraq. These cell lines were maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 U/mL streptomycin (all were purchased from HiMedia). Both cell lines incubated at 37 °C in a humidified atmosphere with 5% CO_2 (Thermo Fisher Scientific, Waltham, MA, USA.

Cell viability evaluation

Based on the findings reported in [27] with minor modifications, the cytotoxic effect of the synthesized ZnO NPs was evaluated using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. In a brief, 96-well plates were seeded with 2.5 x 10^3 cell/ml of MCF7 and HFF cell lines separately , and to promote adhesion, the plates were incubated for 24 hours at 37 C. Treatment of the monolayer cells was carried on the following day using varying doses of ZnO NPs (100, 50, 25, 12.5, 6.25, and 3.125 µg/ml). For each concentration of ZnO NPs, three different sets of experiments were conducted. After a 72-hour incubation period, the culture media was removed, and an MTT solution containing 0.5 mg/mL was added to the cell. The freshly formed formazan crystals were incubated for 4 hours at 37 degrees Celsius before being dissolved in DMSO (170 µL/well) and mechanically agitated for 10 minutes. At the end of the process, a microplate reader (Bio-rad, Germany) was used to measure the absorbance at 570 nm in order to determine the viability of cells and the IC50 according to the following formula:

$$\text{Cell viability} = \frac{\text{Optical density of treated cell with ZnO NPs}}{\text{Optical density of untreated cell (control)}} \text{ X } 100$$

Cell Cycle Study

The annexin V-FITC apoptosis detection kit, provided by BD Biosciences in San Diego, USA, was utilized to quantify the apoptosis levels of the MCF7 cells. To summarize, three sub-lethal concentrations of bacterially produces ZnO NPs (25µg/ml , 50µg/ml, and 75µg/ml) were given to the developing cells within a 24-hours timeframe. Next the cell suspension was combined with 100 µl of 1 X binding buffer at a concentration of $1x10^8$ cells/ml. After mixing the cell suspension with 5 µL of annexin V-FITC and PI, it was left undisturbed at room temperature in the absence of light for 20 minutes. Lastly, the samples were examined using a BD FACS Calibur flow cytometer (BD, Miami, FL, USA). With the proper DNA analyzer, Histograms were analyzed after the sample was processed at a minimum absorption rate of 60 cycles per second.

Statistical Analysis

To perform the statistical analysis on the data, Version 6.0 of GraphPad was utilized (USA). The means multiplied by the standard deviation are used to present the data. (average ± standard deviation). There were discernible differences among the groups. If required, either a one-way or two-way student test was employed to assess the data ANOVA. A *p- value* of 0.05 or less was regarded as significant.

Results and discussion

Preparation of Zinc Oxide Nanoparticles

Bacterial extract of *P. aeruginosa* was employed for synthesis of ZnO NPs. The blue- green extract due to pyocyanin production was challenged with zinc acetate salts resulting in the alteration of color such indicated for synthesis of nanoparticles (Figure 3-1). This occurs as a result of zinc ions excitation via surface plasmon resonance [28]. Each batch of ZnO NPs synthesis yielded approximately 5 g of the final product, which was in the form of white crystalline material.

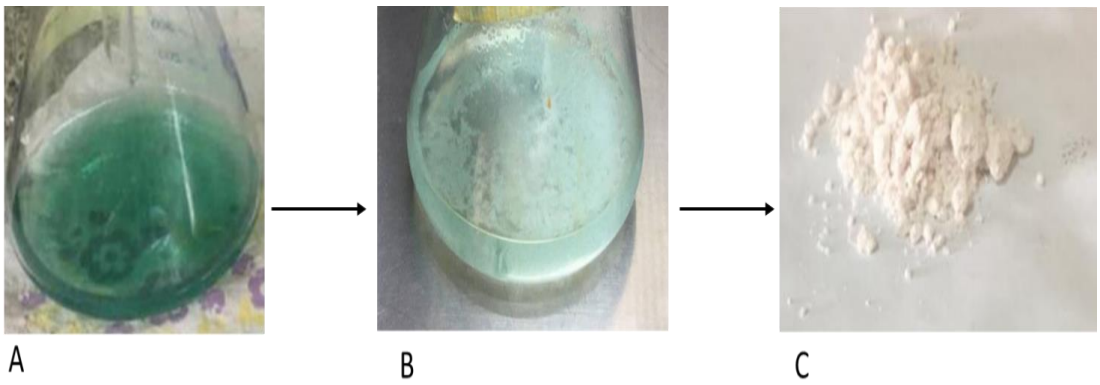

Figure (3-1): preparation of ZnO NPs steps which started by preparation of *P. aeruginosa* bacterial extract as shown in A. B shows the presence of nanoparticles. Dried nanoparticles as a white powder, C.

UV-visible analysis

Using an ultraviolet–visible spectrometer, the optical characteristics of the nanoparticles were investigated. Because of their high excitation binding energy, biosynthesized ZnO NPs exhibit absorption spectra in the Nano range at room temperature (Figure 3-2). It displayed a strong peak at a wavelength of 257 nm., which in a good agreement with [29]. High crystalline quality of the produced nanostructures is indicated by the appearance of the sharp peak [30].

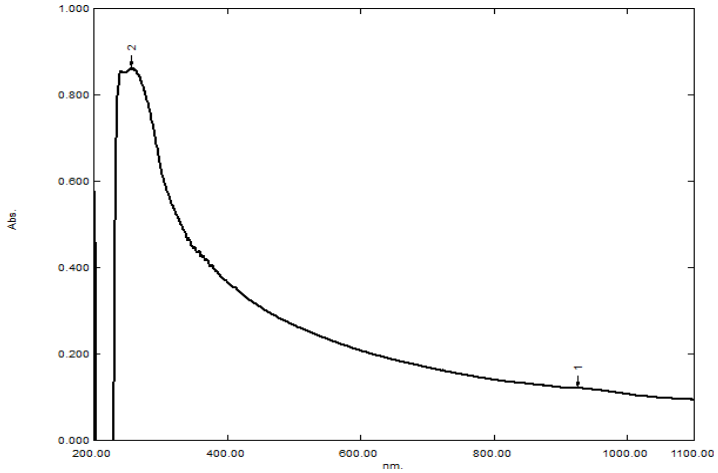

Figure (3-2): the UV-VIS of Zinc Oxide NPs s Synthesized Using Bacterial extract.

Energy Dispersive X Ray Analysis (EDX)

To further confirm the composition of the obtained products, an EDX examination was performed. The results presented in Figure 3-3 indicate that the ZnO NPs subjected to calcination at 800°C for 4 hours are composed primarily of zinc. The labeling includes the element names and percentages for the ZNO sample. Clearly , Zn and O represent the majority

of the sample's contents, and no traces of contaminants were found within EDX's detection limit. , these results agreed with [31].

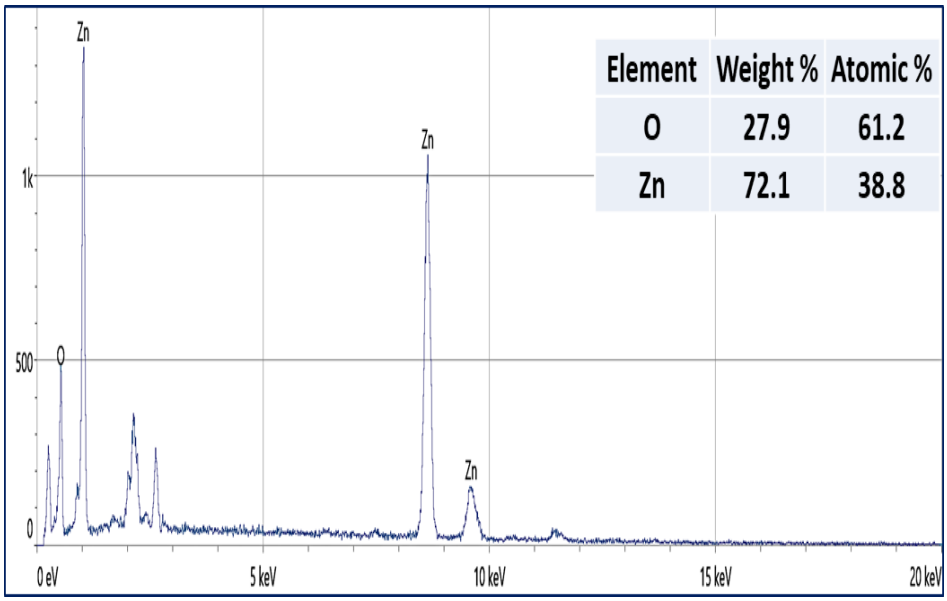

Figure (3-3): The EDX analysis of Zinc Oxide NPs s Synthesized Using Bacterial extract.

Field Emission Scanning Electron Microscopy (FESEM)

Through applying FE-SEM, images were taken of the sample at a magnification of 110kx. Focused on (Figure 3-4) the entire sample possesses homogeneous shape and soft planes in the form of ZnO nanocluster centers, measuring approximately 32.5 nm. an agglomeration is clearly visible in accordance with previous studies [32]. The observation of some larger nanoparticles in FESEM image is attributed to agglomeration [33].

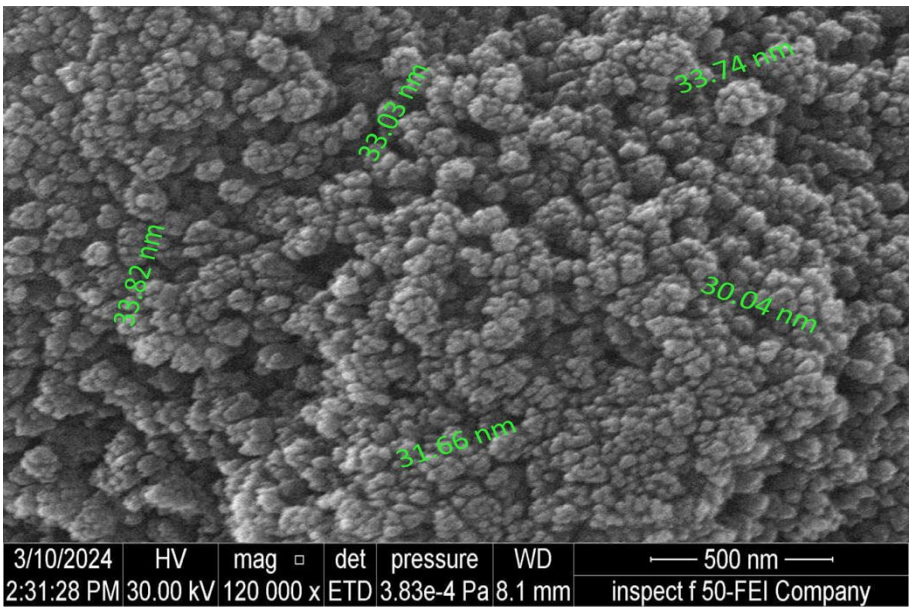

Figure (3-4): FE-SEM Image of ZnO Nanoparticles using bacterial extract as reducing agent.

Fourier Transform Infrared Spectroscopy (FT-IR) analysis

The FT-IR analysis was performed in order to identify the functional groups present in the bacterial extract used as a reducing agent and to investigate the potential impact of these functional groups on the synthesis of ZnO NPs. FTIR analysis of the biosynthesized ZnO

nanoparticles was conducted to identify the functional groups and chemical bonds present in the synthesized compound. FTIR spectroscopy was performed over a wavelength range of 400-4000 cm-1. Figure (3-5) presents the FT-IR spectrum of the utilized Bacterial extract, and bacterial extract-ZnO NPs. Generally, the FTIR spectra of the bacterial extract revealed more than one main region. The first of which (1402.15 cm -1) is corresponded to the bend vibrations of and as well as the O-H bases. Contiguously, the subsequent region (1652.88) resulted mainly from Alkenyl group. Particularly, broadband around 3409.91-3307.69 cm–1 is attributed to the stretching mode of the O-H hydroxyl group. Furthermore, the band located at 694.33 cm-1 is attributed to the Alphatic ether. Therefore, the FT-IR spectra revealed the occurrence of red pigment which are OH-H bonded, metal Oxgyn, and pyrrole [34]. In 2015, Elumalai and others found similar FTIR peaks regarding the prepared ZnO NPs using A.indica leaf extract [35].

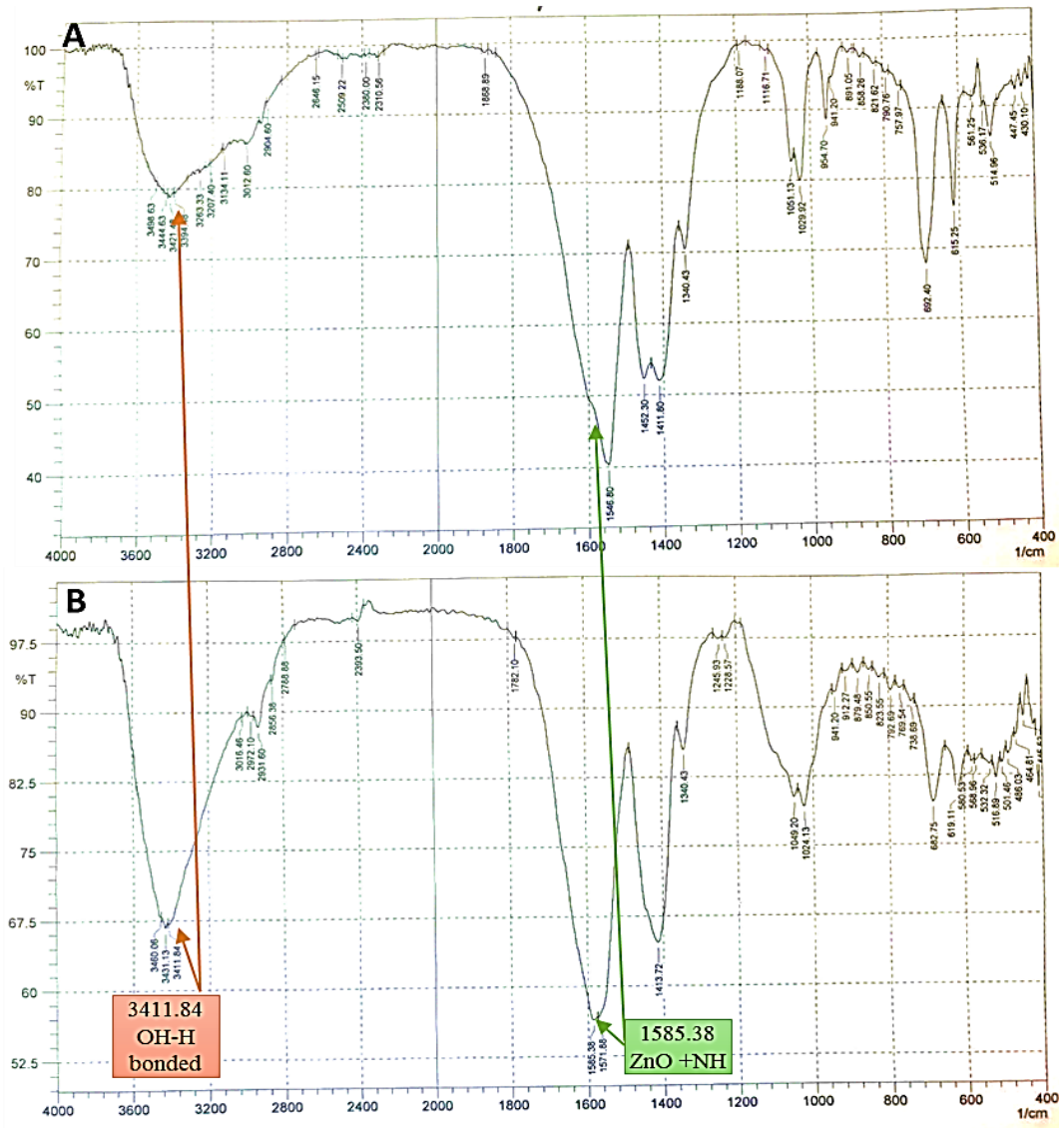

Figure (3-5): FTIR images of Zinc Oxide NPs synthesized using bacterial extract, A: Bacterial extract, B: Zinc Oxide NPs.

Table (3-1): FTIR of Zinc Oxide NPs.

Type of compound	Frequencyof Absorption(cm-1)	bonds	Compound class of functional groups
Bacterial Extract	3409.91-3307.69	O-H stretch	Alcohol and hydroxy compound
	1652.88	C=C Stretch	Alkenyl
	1402.15	O-H bend	Tertiary alcohol
Zinc Oxide NPs	3411.84	OH-H bonded	
	1585.38	ZnO+NH	of pyrol group
	516.89	Metal oxgyn	

AFM analysis

An Atomic Force Microscope (AFM) approach was used in this work to analyze the surface roughness, topography, and morphology. This specific method yields atomic-level two- and three-dimensional images of the targeted nanoparticles [36] as shown in Table (3-2) and Figure (3-6). It should be noted that the average size diameter of the scanned nanoparticles was determined at the nanoscale, and that the AFM technique was used to analyze the biosynthesized ZnO NPs using bacterial extract., Figure (3-7). This specific surface examination requires careful consideration because a variety of factors, including pollution, may have an impact on the results. The change in color from crystal white to milky white indicated the formation of manufactured ZnO NPs. Notable among these was the nearly uniform alignment and spherical morphologies of the produced nanoparticles.

Table (3-2): Estimation of ZnO Nanoparticles Size.

Avg. Diameter:59.61 nm	**<=10% Diameter:25.00 nm**
<=50% Diameter:50.00 nm	**<=90% Diameter:95.00 nm**

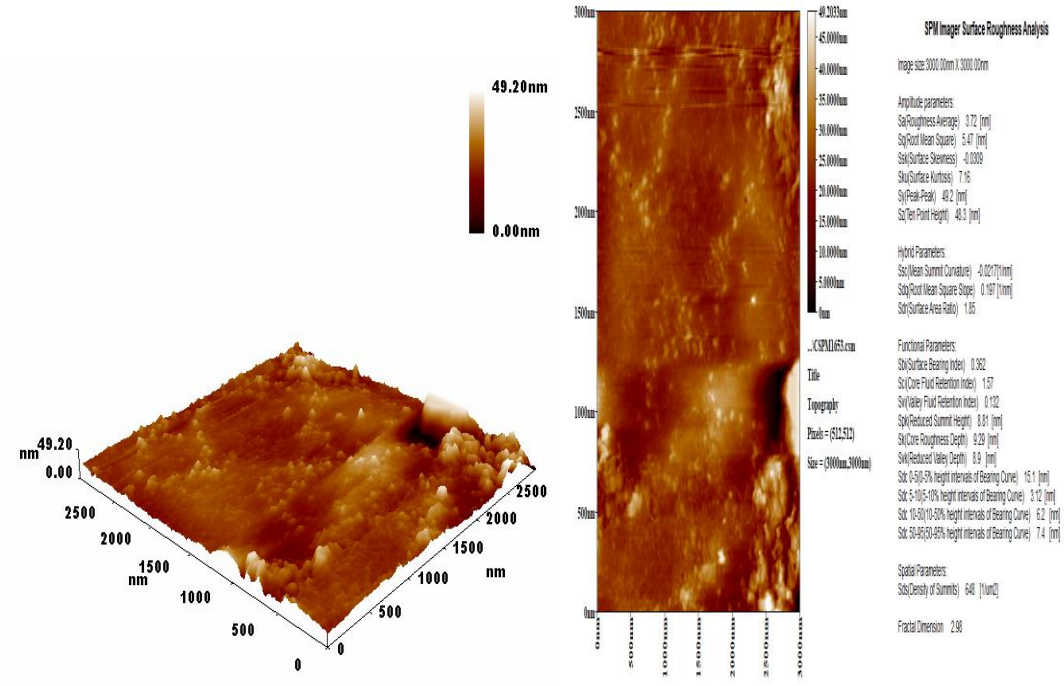

Figure (3-6): Atomic force microscopy showed the 2D and 3D nanoparticle ZnO.

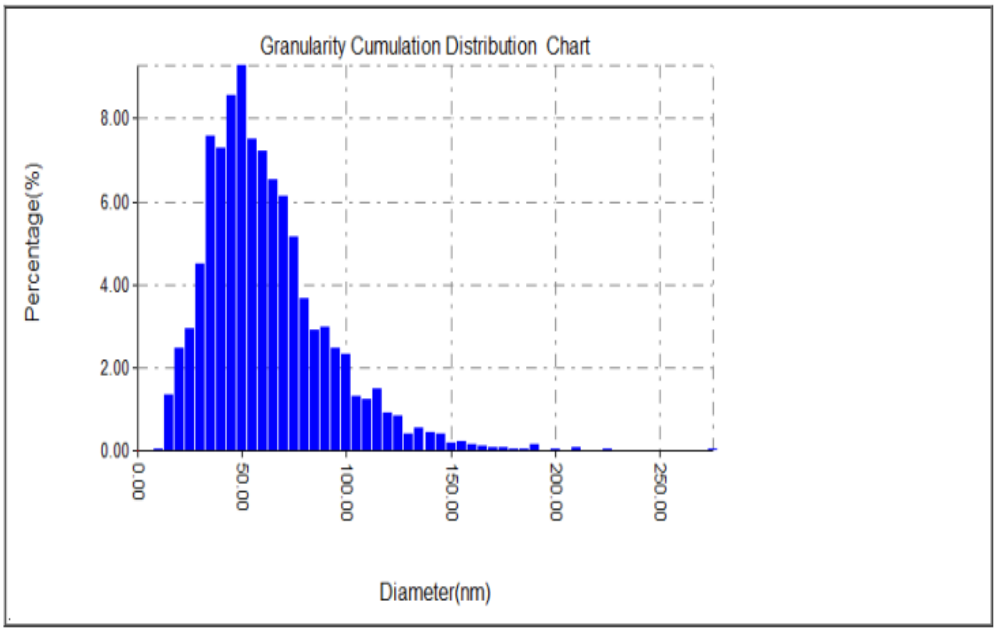

Figure (3-7): ZnO nanoparticles average size synthesized using bacterial extract.

Antibacterial activity ZnO NPs Against multidrug resistant Bacteria

In this study, the inhibition zone was measured in Mueller Hinton agar plates to assess the relative antibacterial activity of ZnO NPs suspensions against pathogenic isolates, including *S. aureus, P. aeuroginosa, and E. coli*. The results of testing six ZnO NPs suspensions at concentrations of 100, 50, 25, 12.5, 6.25, and 3.125 µg/ml are shown in Table (3-3). The findings showed that among the gram-negative bacteria tested, *E. coli* exhibited the largest inhibition zones. Specifically, *E. coli* displayed a maximum inhibition zone of 27 mm when exposed to ZnO NPs at a concentration of 100 µg/ml, Whereas the minimum inhibition zones were located at 3.125 mg/ml and for *P. aeuroginosa* were 23 mm at concentration 100 µg/ml of zinc oxide nanoparticles, Whereas the minimum inhibition zones were located at 12.5 mg/ml and no inhibition at the concentrations of (6.25 and 3.125 µg ml^{-1}) of ZnO NPs concentrations, for gram positive bacteria (*S. aureus*) that the maximum inhibition zones were 28 mm at concentration 100 µg/ml of zinc oxide nanoparticles, Whereas the minimum inhibition zones were located at 3.125 µg ml^{-1} of ZnO NPs concentration, the inhibition zone depended on the concentration of zinc oxide nanoparticles. It has been previously observed that the antibacterial effect of nanoparticles against both Gram-positive and Gram-negative bacteria is dependent on the structural composition of the cell membrane, specifically the thickness of the peptidoglycan layer [37]. While the cell wall of Gram-negative bacteria has a thinner peptidoglycan layer, they exhibited a significant bactericidal effect, Gram-positive bacteria have a thick peptidoglycan layer made of linear polysaccharide chain cross-linked with small peptides, forming a rigid cell membrane to penetrate ZnO NPs and preventing the formation of an inhibition zone. These results are the same as the results [38] that the nanoparticles have bactericidal activity in addition, they concluded from their study that when ZnO NPs produce hydrogen peroxide, the nanoparticles stay in touch with the deadly bacteria in order to stop them from acting further and to keep producing and releasing hydrogen peroxide into the medium.

Table (3-3): Antibacterial activity Test of Zinc oxide nanoparticles on some multi-drug resistant bacteria.

NO	Zinc oxide NPS con. (mg/ml)	Inhibition zone (Diameter, mm)		
		S. aureus	P. aeruginosa	E. coli
1	100	28	23	27
2	50	25	17	23
3	25	24	14	19
4	12.5	23	12	16
5	6.25	17	5	13
6	3.125	9	5	10

The Cytotoxicity Assessment

The study evaluated the toxic effects of ZnO NPs on cells by assessing cell survival rates and determining the IC50 value. This was done using the MTT assay on two cell types: a breast cancer cell line (MCF7) and a normal human foreskin fibroblast cell line (HFF). Various concentrations of ZnO NPs ranging from (25-500) µg/ml for 24h used to measure cell viability and IC50 on tumor MCF7 cells and HFF as a normal cells. It has been shown that ZnO NPs has an inhibitory effect on cells increased with dose augmentation which confirm that the ZnO NPs has dose dependent effect on MCF7 and HFF cell lines , as illustrated in figure (3-8). The viability significantly reduced to 4.5% and 40% at the highest concentration of 500 µg/ml for MCF7 and HFF respectively.

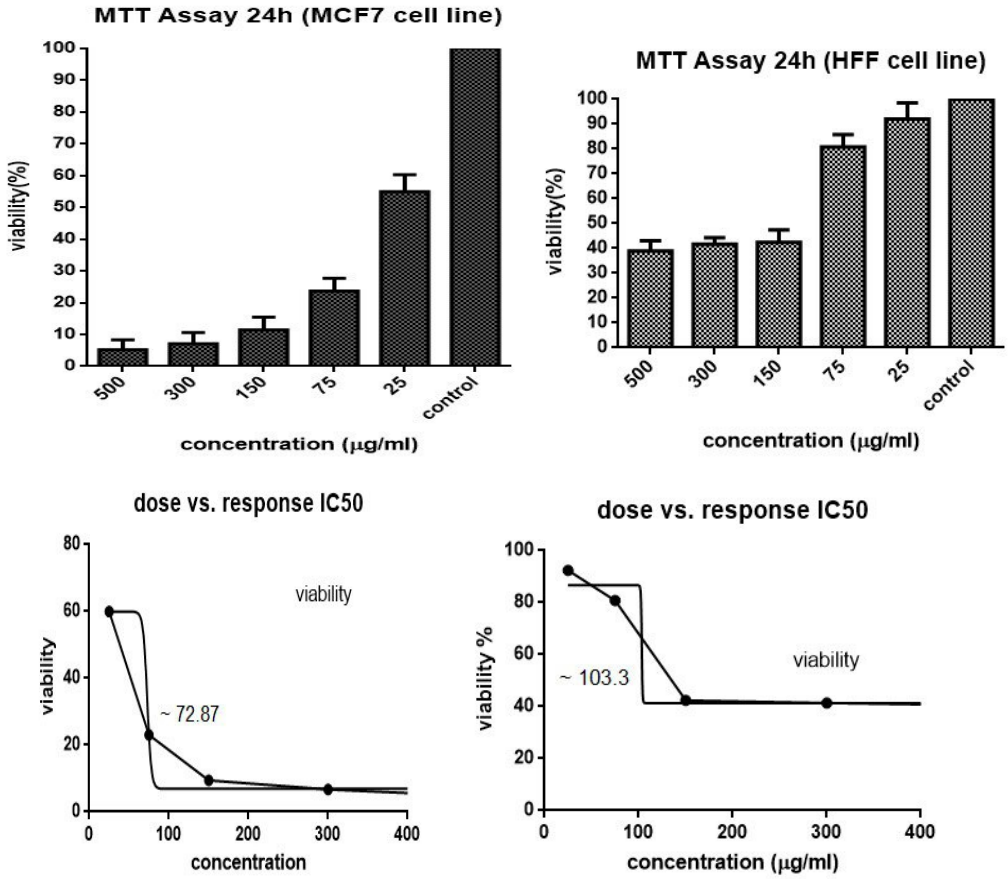

Figure (3-8): Cytotoxic effect of Zinc oxide against MCF7 (Human breast cancer) and HFF (Human foreskin fibroblasts) cell lines after incubation at 37ºC for 72 hours. Using *: p-value ≤ 0.05- significant.

It was seriously observed that the viability in MCF7 was less than 50 % in the concentrations 75 – 500 µg/ml while in the HFF the viability increased up to 90% at 25 µg/ml of ZnO NPs. The IC50 value for ZnO NPs on MCF7 and HFF cell lines were about 72.87 and 103.3 µg mL-

1, respectively. Zinc oxide nanoparticles showed anticancer activities towards MCF7 and HFF cell line with less cytotoxic effect on the normal cell line. However, the proliferation rate was less cytotoxic on the normal cell line treated with ZnO NPs than on the MCF7. These results are in agreement with study of [39] cytotoxicity of ZnO NPs against A431 cancer cell line.

Induced Cell Death in MCF-7 Cells by ZnO Nanoparticles

After staining the cells with propidium iodide, the DNA content was detected using a flow cytometer [40], this allowed for the analysis of the cell cycle phase distribution. An investigation was conducted to see if conjugated ZnO NPs' reduction of MCF7 cell viability was related to cell cycle arrest. A summary of the flow cytometry-derived results from the induction of apoptosis/necrosis is shown in (Figure 3-9). ZnONPs clearly caused cell death in MCF7 cells, according to the flow cytometry results. With values of 2.15%, 3.26%, and 1.35% of cells, which are typical for cells developing in cultures, Annexin V-PE/7-ADD staining revealed that 93.2% of MCF7 control cells were confirmed to be alive. When compared to untreated control cells, the ZnONP-exposed MCF7 cells showed a substantial increase in late apoptotic and necrotic cells. At 50 µg/ml ZnONPs concentration, there was a 95.6% rise in the percentage of necrotic and apoptotic cells. Our results findings are consistent with recent studies that have demonstrated that exposure to nanoparticles causes apoptosis in cells [41], including the exposure of Bacterial-synthesized Zinc oxide nanoparticles [42]. ZnO NPs have demonstrated dose-dependent cytotoxicity towards a number of cancer cell lines, including MCF-7, HeLa, A549, and others, with IC50 values between 10 and 100 µg/mL.

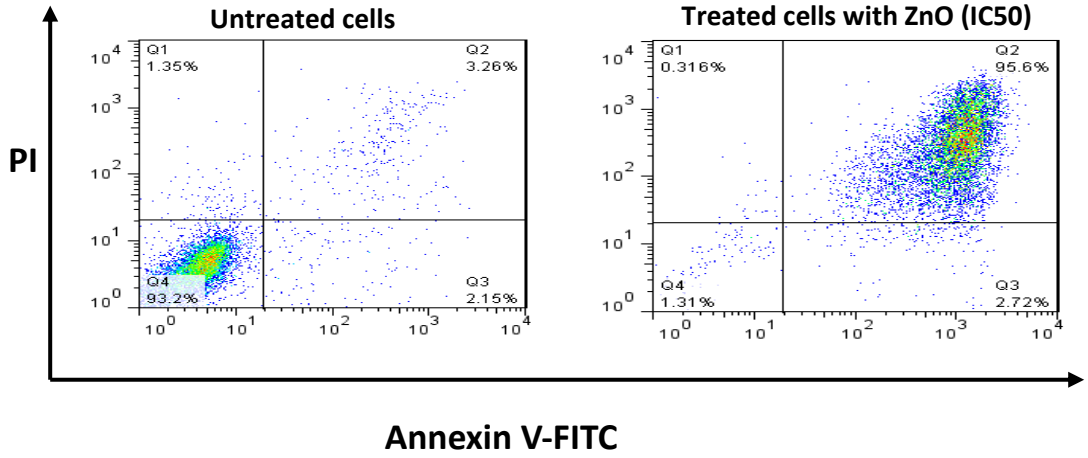

Figure (3-9) : The flow cytometer instruments was used to assess the percentages of Q4: viable cells , Q3: early apoptotic , Q2: late apoptotic , Q1: early necrotic and late necrotic cells in negative control (untreated cells) and MCF-7 treated cells after 24h of ZnO NP IC50 exposure.

Conclusion

The varied dosage of ZnO NPs demonstrated their antibacterial effect against drug resistant bacteria of gram positive and negative bacteria . As well as an anticancer effects on MCF-7 cells. The impact was achieved by inhibiting the proliferation of cancer cells. The underlying mechanisms involve the activation of cell-specific C0/G1, S and G2/M cell cycle arrest, as well as the initiation of apoptosis through apoptotic pathways. This research provides support for the concept that the bacterial isolates of *P. aeruginosa* can be utilized in specific anti-cancer therapies through the green synthesis of ZnO NPs. This study may have limited information about their possible toxicity in vivo and biocompatibility. To confirm the safety of ZnO NPs for therapeutic use, future research should concentrate on extensive toxicity evaluations. In the future, ZnO nanoparticles (ZnO NPs) may be used in combination with other therapeutic agents, such as antibodies or chemotherapeutic medication, to increase their effectiveness and

minimize their negative impacts. Future research may look into the synergistic effects of ZnO NPs with already available therapies.

Ethical clearance

This study was approved by the ethical committee of the College of Science, University of Baghdad, according to the reference number (CSEC/0623/0049).

Conflict of interest:

The authors declare that they have no conflict of interest.

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