مجلة الدراسات التربوية والعلمية - كلية التربية - الجامعة العراقية
العدد الرابع والعشرون - المجلد الخامس - علوم الحياة - كانون أول 2024 م
doi.org/10.52866/esj.2024.05.24.21

The Action of Phytosynthesied Copper Nanoparticles From Cinnamon Plant Extract as Antibacterial and Anticancer Agent

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Abstract :

Due to the high frequency and emergence of multi-antibiotic resistant bacteria, especially common wound infection bacteria, and because nanotechnology has attracted great interest around the world. The current study aimed to synthesize copper nanoparticles (Cu NPs) and investigate its antibacterial and anticancer activity. Initially, sample collected from wounds infection and diagnosed using biochemical and theVITEK technique. Nanoparticles were biologically prepared using cinnamon bark extract and copper(I) chloride dihydrate salts ($CuCl_2.2H_2O$) concentration of 1 mM.The charecteristics of the particles were studied using techniques such as "UV-Visible Spectrophotometric UV-Vis, Atomic Force Microscope AFM, X-Ray Diffraction XRD, Scanning Electron Microscope SEM" and their efficacy against both multi-resistant bacteria and cell lines was tested. The results showed first indicator of the success synthesis was the change in the color from blue to green within two hours of stirring, and the absorption beam was formed at a wavelength of (340 nm), which is evidence of the production of copper nanoparticles, it was found the average size of the particles was about (16.5nm). The atomic force microscope showed a three-dimensional and two-dimensional section of the surface topography of the copper particles and the average grain size(100 nm), while the X-ray diffraction analysis showed a number of clear peaks that correspond to the crystalline structure of copper. Nanoparticles also showed high efficiency in inhibiting *S.aureus* bacteria with an inhibition diameter of (22mm) compared to *P.aeruginosa* bacteria with an inhibition diameter of (20mm) and inhibiting the cancer cell line (A375) (47%) compared to the inhibition rate of the natural line (WRL-68) (28%).

Keywords: Green synthesis, Cu NPs, multidrug-resistant, Antibacterial, Anticancer activity.

فعالية جسيمات النحاس النانوية المصنّعة حيوياً من مستخلص نبات الدارسين كعامل مضاد للبكتريا والخلايا السرطانية

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مستخلص:

نظرًا لارتفاع نسبة تكرار وظهور البكتريا ذات المقاومة المتعددة للمضادات الحيوية، وخاصة بكتيريا عدوى الجروح شائعة الانتشار، ولأن تقنية النانو جذبت اهتمامًا كبيرًا في جميع أنحاء العالم، فقد هدفت الدراسة الحالية إلى تصنيع جسيمات النحاس النانوية (Cu NPs) والتحقق من نشاطها المضاد للبكتيريا و للخلايا السرطانية . في البداية، تم جمع البكتيريا من عدوى الجروح وتشخيصها على مستوى الأنواع باستخدام التقنيات التقليدية وتأكيد تشخيصها بااستخدام تقنية الفايتك. تم تحضير الجسيمات النانوية بيولوجيًا في المختبر باستخدام مستخلص لحاء القرفة و أملاح كلوريد النحاس الثنائية ($CuCl_2.2H_2O$) بتركيز 1 ملي مولاري، وتم دراسة خصائص الجسيمات باستخدام تقنيات محددة مثل "UV-Visible Spectrophotometric UV-Vis، Atomic Force Microscope AFM، X-Ray Diffraction XRD، Scanning Electron Microscope SEM" ومن ثم اختبار فعاليتها ضد كل من البكتيريا ذات المقاومة المتعددة والخطوط الخلوية . وأظهرت النتائج أن أول مؤشر على نجاح عملية التصنيع هو تغير اللون من الأزرق إلى الأخضر خلال ساعتين من التحريك، وتشكل حزمة الامتصاص عند طول موجي (340 نانومتر)، وهذا دليل على انتاج جسيمات نانوية من النحاس، و أن متوسط حجم الحبيبات كان حوالي 16.5 نانومتر. وأظهر المجهر الذري مقطع عرضي ثلاثي وثنائي الأبعاد لتضاريس سطح جسيمات النحاس ومعدل حجم الحبيبات (100nm)، بينما أظهر تحليل حيود الأشعة السينية عددًا من القمم الواضحة التي تتوافق مع البنية البلورية للنحاس، كما وأظهرت جسيمات النحاس النانوية كفاءة عالية في تثبيط بكتيريا S.aureus بقطر تثبيط (22mm) مقارنة ببكتريا P.aeruginosa بقطر تثبيط (20mm) وبلغت نسبة تثبيط خط الخلايا السرطانية (A 375) (47٪) مقارنة بنسبة تثبيط الخط الطبيعي (WRL-68)(28%).

الكلمات المفتاحية: التصنيع الأخضر، جسيمات النحاس النانوية ، المقاومة المتعددة للادوية ، مضاد للبكتيريا، مضاد للسرطان.

Introduction

Skin is the largest external organ in the body that is directly exposed to the external environment [1]. Wounds are skin damage caused by trauma, accident, surgery or burn which can lead to bacterial infection [2]. Wound injuries are public health concern due to their serious nature and complex healing process, which is influenced by various factors that can delay healing [3]. Wound infections can be caused by various microorganisms such as bacteria, the most common bacterial infectious agents are *Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella pneumoniae,* and *Acinetobacter baumannii* [4-5]. These species are naturally resistant to some antibiotics and antiseptics and can multiply on injured skin even when sufficient nutrients are absent [6]. Antibiotics used to prevent bacterial wound infections are now restricted by the widespread emergence of multidrug-resistant (MDR) pathogens that pose a major threat to human health [7-8].

Due to the increasing emergence of bacteria that are resistant to multiple antibiotics, many researchers have resorted to alternative and effective solutions such as the use of nanocomposites, which is the first step in combating the development of resistance and reducing the consumption of antibiotics [9].

Nanotechnology is a rapidly expanding interdisciplinary field that fill the gap between science and technology [10]. Nanoparticles, smaller than 100 nm, possess unique surface properties due to their large surface area compared to their size [11]. Nanoparticles are an alternative to antibiotics, and they play an effective role in ending the problem of the development of multiple drug resistance. One of the most common nanomanufacturing methods is the biomanufacturing method using plants or bacteria to produce nanoparticles [12]. Since ancient times, copper has been known to have antimicrobial activity and is used in wound healing, skin remodeling, and anti-inflammatory treatments [13]. It also enhances immunity by stimulating the production of interleukin 2 [14]. Copper nanoparticles (NPs) with plasmonic properties are being studied for their applications in cancer therapy, information technology, photonics and materials science [15]. Studies have shown that green synthesis of nanoparticles is more ben-

eficial than other synthesis methods because it is cost-effective, environmentally friendly, can be easily scaled up for large-scale synthesis, and does not require high pressure, temperature, and toxic chemicals [16]. Green synthesis of nanoparticles using plant extracts has gained great importance Plant extracts play a dual role as stabilizing and capping agents, for nanoparticle synthesis. Another advantage of biological methods is improved manipulation and control of crystal shape, size and stabilization [17].

The study synthesizes copper nanoparticles (Cu NPs) using *Cinnamomum zeylanicum* bark powder due to its high concentration of flavonoids [18]. Copper nanoparticles are formed through the biological reduction of copper ions due to plant metabolites [19]. When treated with copper nanoparticles, disruptions in signal transduction pathways and cell mechanisms can lead to cell damage and death and the production of reactive oxygen species that oxidize lipids, proteins, and damage DNA [20].

The current aims to synthesize copper nanoparticles by green synthesis and study their inhibitory activity against bacteria and human lines.

Material and Methods

1. Preparation of Cinnamon plant Extract: The hot aqueous solution was prepared by adding 10 g of commercial cinnamon powder obtained from (local market, Baghdad, Iraq) to a sterile glass vial containing 100 ml of deionized water and placed on a magnetic stirrer with a hot plate and measuring the temperature of the solution until the temperature of the solution reached 80 °C and then the solution was left to cool and filtered using Whatman No. 1 and stored in the refrigerator at 4 °C.

2. Green Synthesis of Copper Nanoparticles: Copper is prepared by adding 5 ml of hot aqueous cinnamon solution to copper(I) chloride dihydrate ($CuCl_2.2H_2O$) with continuous stirring using a magnetic stirrer for (5-30) minutes until the color of the solution changes from blue to green, indicating the formation of copper nanoparticles. The solution is stored in a dark glass bottle to prevent oxidation and left to cool and kept at room temperature for further experiments [21].

3. Characterization of Copper Nanoparticles: Many tests have been carried out using various modern techniques to Study the charecteristics

of of copper nanoparticles, including measuring absorption spectra using ultraviolet visible (Uv-vis) spectroscopy , study the surface and shape properties using scanning electron SEM the size and topography of nanoparticles using atomic force AFM and study the crystalline nature of NPs by X-ray diffraction XRD.

4. Bacterial collecting and identification: Samples were collected from wound infections of patients admitted to and referred to Salah Al-Din General Hospital, in the amount of 25 swabs, and were placed directly in sterile tubes containing a carrier medium, then transferred directly to the laboratory for the purpose of cultivation and diagnosis using biochemical and VITEK technique, and then study their sensitivity to antibiotics.

5. Study the antibacterial activity: The inhibitory activity of copper nanoparticles against *Pseudomonas aeruginosa* and *S.aureus* isolates was tested using focal diffusion method on Mueller Hinton medium with different concentrations of nanoparticles (1, 0.5, 0.25, 0.125) mM ,The inhibitory activity was evaluated by placing 100 µL of copper nanoparticle concentrations in the wells and incubating the plates for 24 h at 37 °C and then measuring the diameters of the inhibition zone in mm around the wells.

6. Study the anticancer Activity: The activity of copper nanoparticles against skin cancer cells (A 375) and normal liver cells (W R L 68) was tested after treating them with different concentrations of copper nanoparticles (25, 50, 100, 200, 400)µg/ml. These lines were obtained from the Biotechnology Research Center/Baghdad. The cancer cells lines were maintained and grown and tested at the Biotechnology Research Center/Baghdad. After preparing the samples, the absorbance was read using an ELISA reader at the wavelength (570nm).

Results and Discussion

1. Color change: After adding cinnamon extract to 1 mM copper(I) chloride dihydrate ($CuCl_2.H_2O$) gradually, the color of the mixture changed from blue to green as shown in (Fig.1) within 2 hours at 40-60 °C and continuous stirring, and the color change stopped completely after 24 hours, as a result of the interaction of many phytochemicals present in the plant extract with copper chloride to form copper nanoparticles, which indicates the possibility of man-

ufacturing copper nanoparticles from cinnamon bark extract, and the color change of the solution indicates the excitation of surface plasmon resonance as a result of the reduction of Cu^{+2} ions to copper nanoparticles Cu^{0} through the active molecules present in cinnamon extract, which play a major role in the reduction and stabilization of the nanoparticles. Many previous studies have proven the possibility of copper nanoparticles biosynthesis from cinnamon bark extract and reducing mineral salts, and similar changes in the visible color were observed during the manufacturing process in previous studies [22- 23].

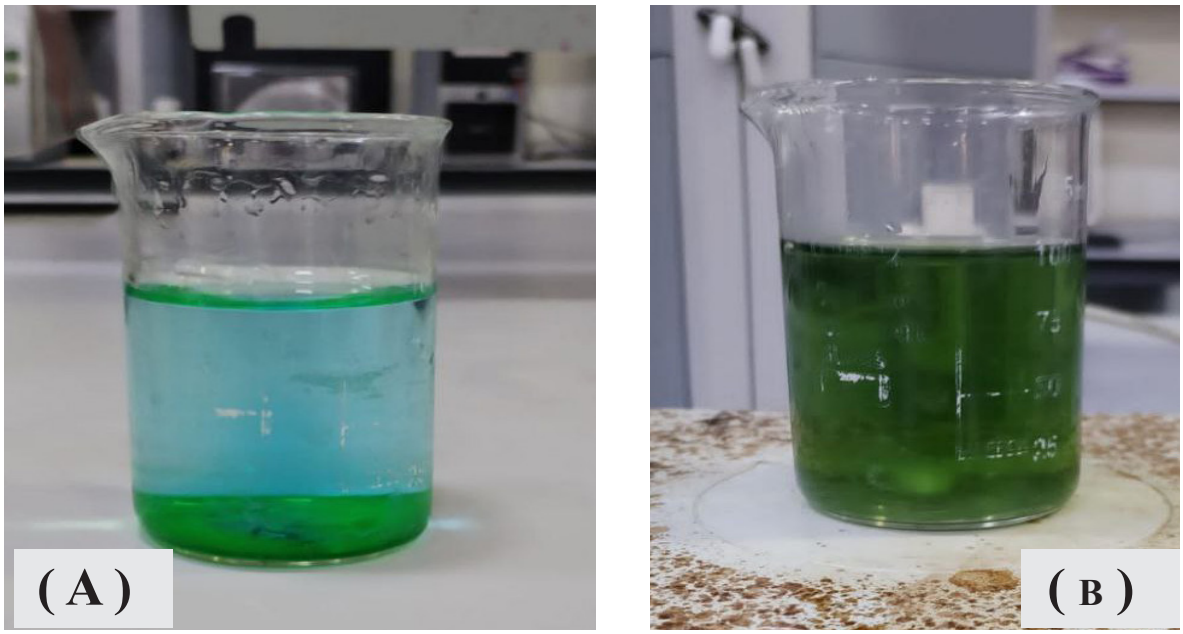

Fig. 1. Color change
A: Color copper(II) chloride solution,
B: Color of solution after adding extract

2. UV-Vis absorption analysis: Cu NPs were examined at wavelengths ranging from 190 nm to 1100 nm**,** which represents the absorption peak of copper nanoparticles.(Fig. 2) shows the surface plasmon peak of copper nanoparticles at wavelength (340 nm), indicating the production of copper nanoparticles, which is consistent with previous studies [24].

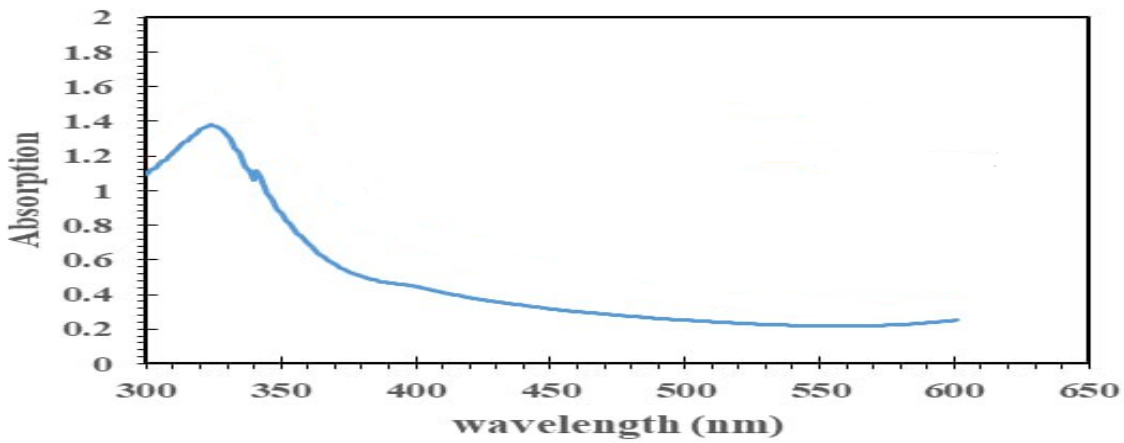

Fig. 2. UV-Vis absorption spectra of biosynthesized Cu NPs.

3. **XRD analysis:** The X-ray diffraction device (XRD) gives an accurate explanation of the crystalline structural qualities of nanoparticles, where the (Fig.3) shows the prominence of a set of clear peaks within an angular range (2 θ) of X-ray diffraction(43.48°), (50.50°), (°74.23) for atomic levels(111), (200), (220) respectively, where the values of the apparent peaks of the corresponded to Cu- Card Number (JCPDS 040836) of copper with a centrally-facing cubic shape depending on the peak areas and agreed with [25].

The extended high-density diffraction peaks clearly indicate that copper nanoparticles are highly crystalline, the X-ray diffraction pattern shows that the peak similar to The (111) plane is a dominant diffraction peak and more intense than other planes and the average crystallite size of the synthesized nanoparticles was calculated using the" Debye-Scherrer equation"

$$D=\frac{K\lambda}{\beta\cos\theta} \qquad (1)$$

Where D: crystal size in nanometers, K: constant of (0.9), λ: wavelength of X-rays incident on the target, β: Full Width at Half Maximum (FWHM), θ_B: diffraction angle.

The crystal size to be about (17.3 nm) for the high intensity of the direction of the level (111) agreed with the previous study [26].

The study reveals the presence of copper nano oxides (CuO) due to minor surface oxidation and environmental influences, influenced by high temperatures and partial oxygen pressure [27].

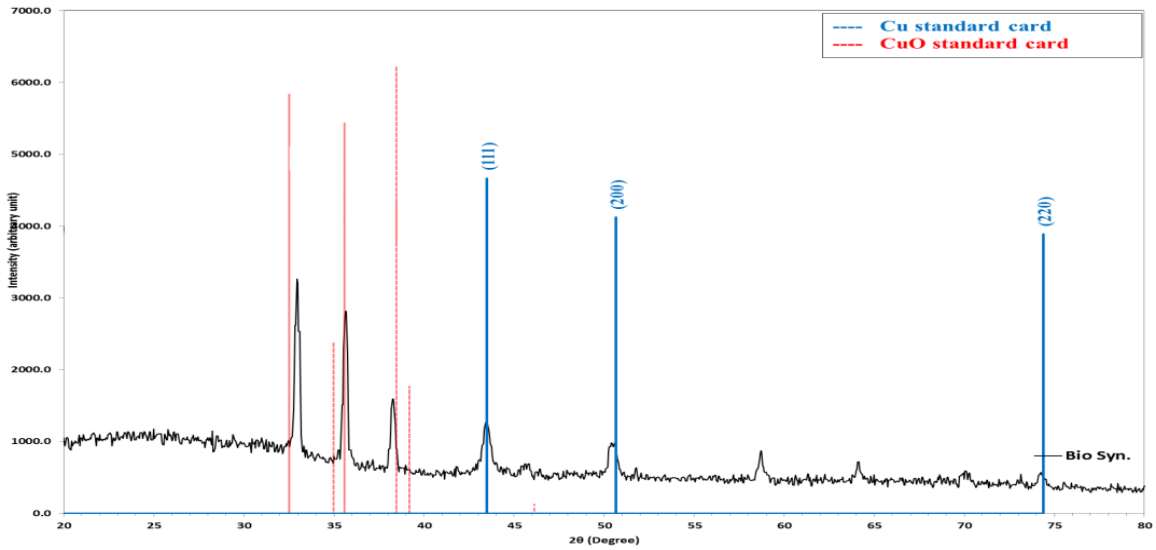

Fig. 3. XRD patterns of prepared Cu NPs

4. AFM analysis: The device was utilized to identify and photograph prepared nanoparticles, determine their size in three dimensions, general distribution, and study their topography [28].

By measuring the prepared sample, we obtained the (Fig.4) that shows 2D images showing the surface roughness and 3D images representing the surface topography and the morphological appearance of the grains formed on the surfaces. The figure also shows the arrangement of the nanoparticles in a vertical manner and heterogeneous in shape and size (average size about 100) nm. This may be due to the sedimentation of the samples, which was done manually. When comparing the average sizes of the nanoparticles for the results of the atomic microscope with the results of the XRD and SEM measurements, it can be noted that the results are almost similar, but the sizes determined by the atomic microscope AFM are larger than those determined by XRD. The reason for this difference is that the atomic microscope AFM measures the size of the grains on the surface (which are larger than the grains inside).

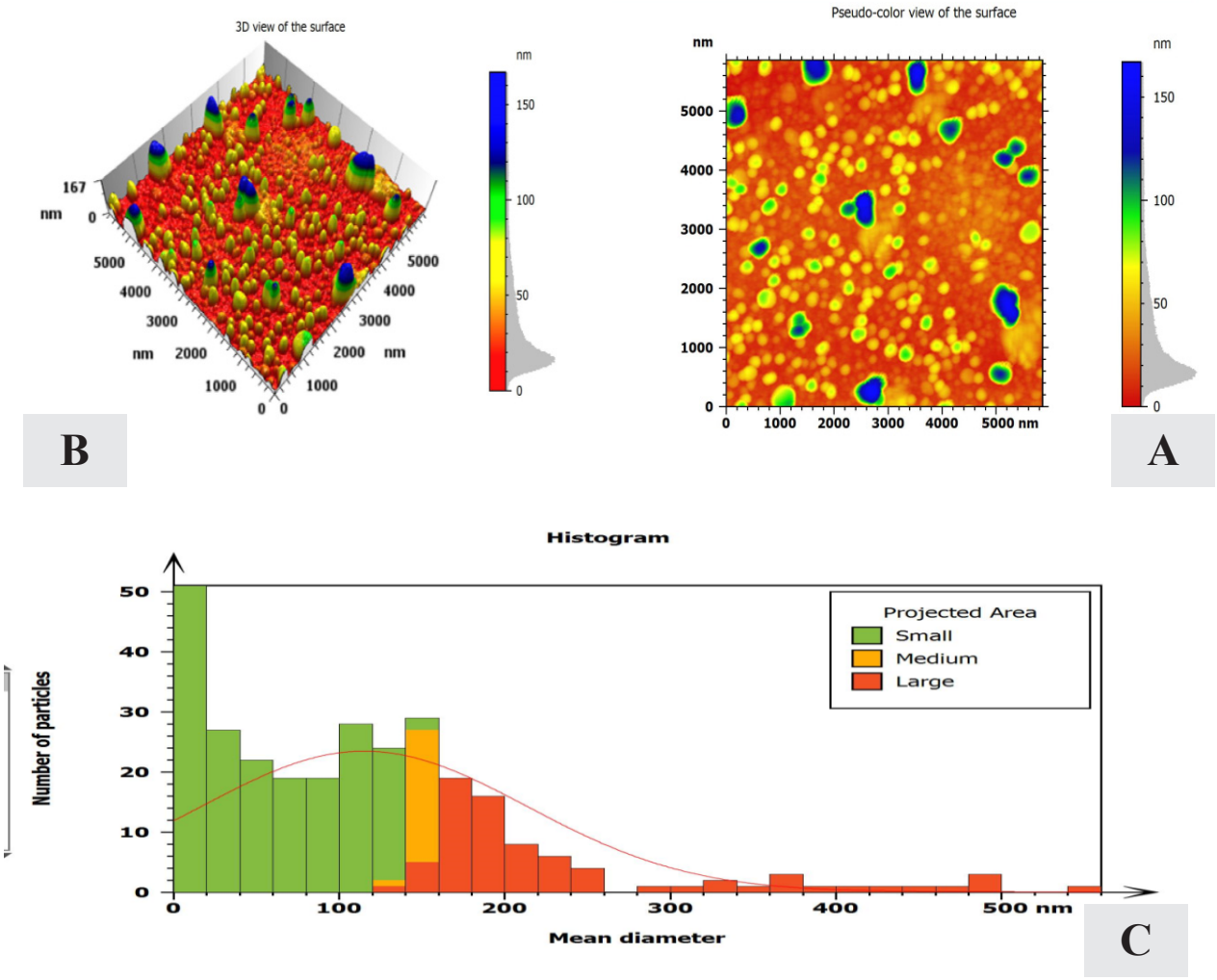

Fig.4. AFM images of Cu NPs
(A: 2D image, B: 3D image, C: General distribution of grains)

5. SEM analysis: Scanning electron microscopes provide high-resolution surface imaging for morphology, shapes, and sizes of nanoparticles, providing evidence of their formation. (Fig.5) shows a typical surface of copper nanoparticles, where various shapes are clearly visible, including irregular spherical shapes with different diameters in size ranging from(10-40) nm and average(16.5)nm, and these results are consistent with [29].

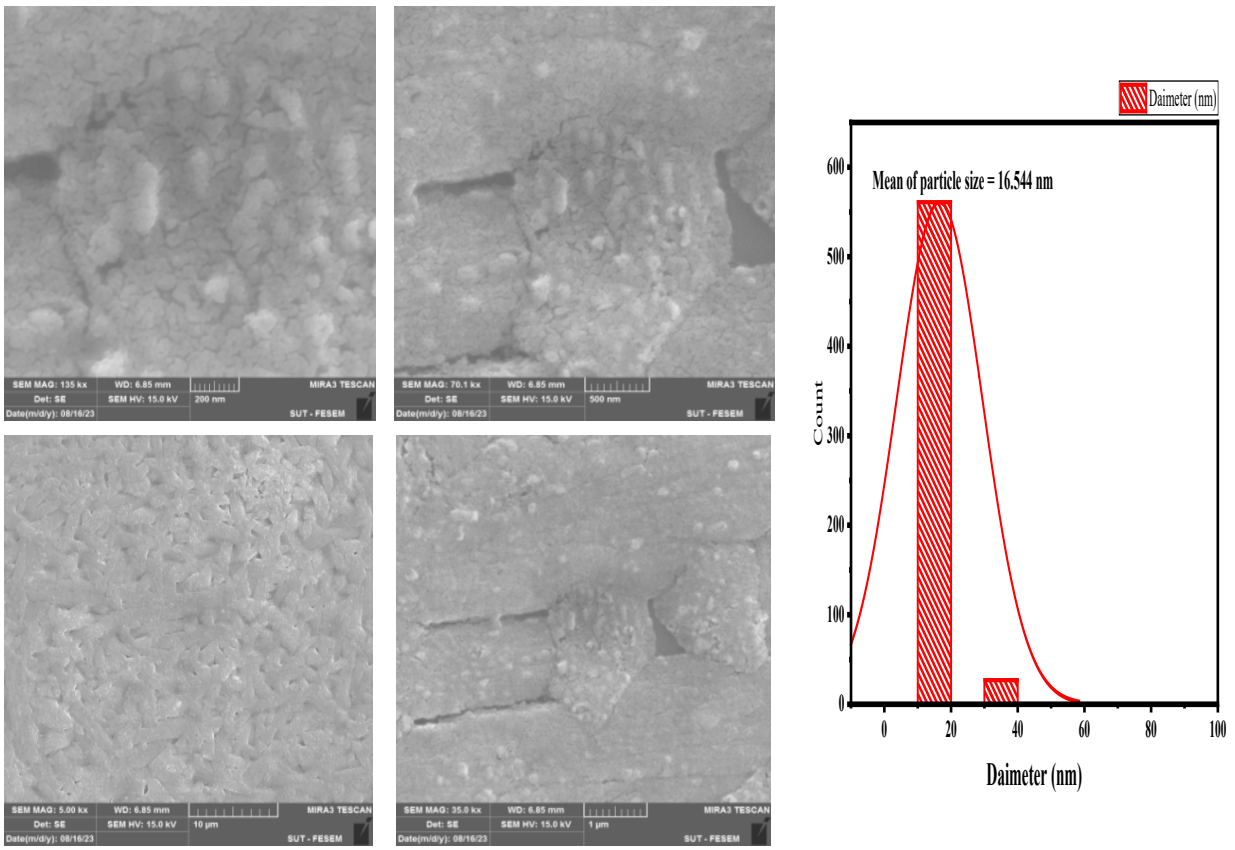

Fig.5. SEM images and particle size histograms of Cu NPs

Antibacterial ctivity of copper nanoparticles: The effectiveness of copper nanoparticles was tested for inhibiting the growth of *S. aureus* and *P. aeruginosa* where the concentrations (1, 0.5, 0.25, 0.125 mM) showed different inhibition diameters, and the highest inhibition zone was for the concentration (1mM) against *S. aureus* with a diameter of (22 mm), while it was with a diameter of (20 mm) against *P. aeruginosa* as (Fig.6).

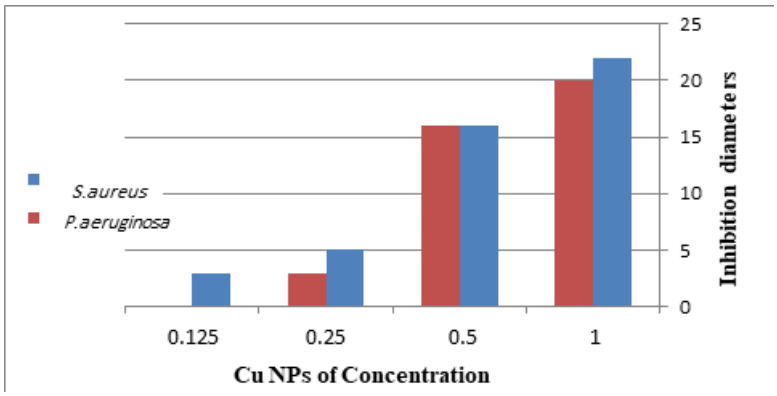

Fig.6. Activity of Cu NPs against the bacterial.

By measuring the diameters of inhibition, it was found find that Gram-positive bacteria are more sensitive to copper nanoparticles compared to Gram-negative bacteria, due to the difference in the chemical composition of bacterial cell walls [30].

Nanoparticle size affects their inhibitory ability, with smaller particles having greater penetration, better absorption, and increased toxic effects on bacterial [31].

The bactericidal property of copper nanoparticles is mainly due to the release of copper cations from copper nanoparticles, which react with the bacterial membrane and lead to severe changes in the membrane structure and increase its permeability. In general, copper cations bind to the bacterial cell wall due to electrostatic attraction and rupture it, leading to protein degradation, dissipation of the proton motive force and finally cell lysis [26].

The attraction between the positive charge of the nanoparticles and the negative charge of the surface of bacterial cells leads to the antibacterial activity of the nanoparticles[32].

The inhibition process can also be explained by the production of reactive oxygen species on the surface of the nanoparticles, which adhere to the bacterial wall and kill the bacteria by the force of attraction [33].

Another mechanism for the action of copper nanoparticles is through the binding of copper to S-H groups on the cell wall to form R-S-S-R bonds, thus preventing the respiration process and thus cell death [34].

7. **Anticancer action of copper nanoparticles:** The action of copper nanoparticles were tested on the normal hepatocyte cell line (WRL-68) and the skin cancer cell line (A375) using five different concentrations of copper nanoparticles (25, 50, 100, 200, 400) µg/ml.

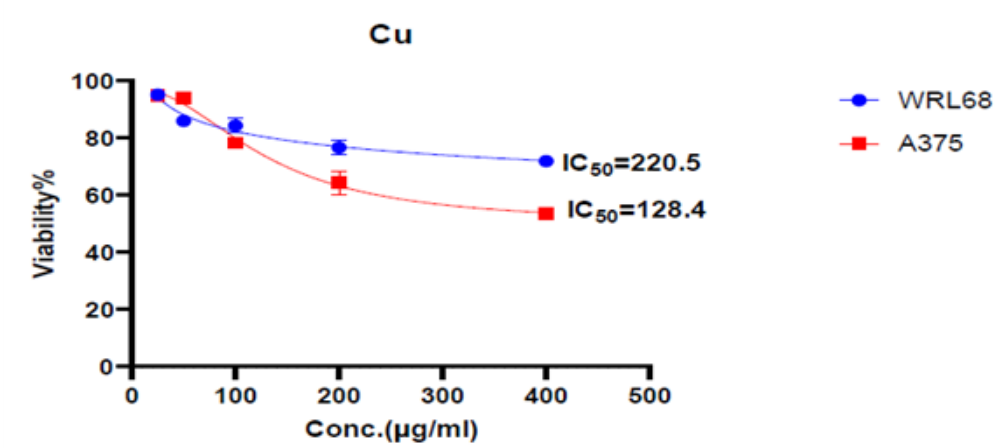

Fig.7. Effect of Cu NPs on (A 375) and (WRL68).

(Fig.7) shows the number of cells that have the ability to survive (live cells) after treatment with different concentrations of copper nanoparticles. From the figure, we notice that with increasing concentration, the number of live cells decreases and the number of inhibited cells increases as a result of treatment with copper nanoparticles

From the previous figure, which shows the number of viable cells after treatment with copper nanoparticles, the percentage of inhibited cells was calculated, as in (Fig. 8) where the results show the clear effect of copper nanoparticles, as the inhibition rate of the cancer cell line reached (47%) at a concentration of 400 µg/ml, while the inhibition rate of the normal line reached (28%) at a concentration of 400 µg/ml.

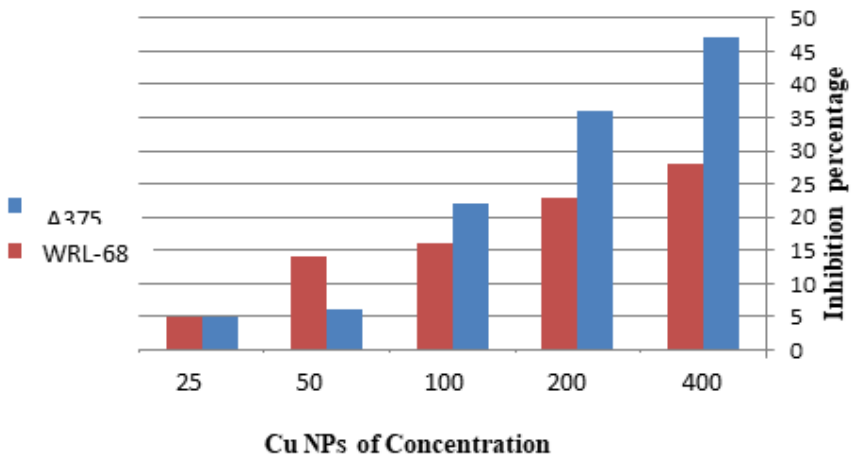

Fig.8.The percentage of growth inhibition of Cell lines

It could also note an increase in the percentage of cell inhibition with increasing concentration, consistent with [35]. Nanomaterials work to significantly reduce the survival time of cancer cells, as the small size, surface charge, and functional assemblies of nanoparticles play a role in penetrating cells [36].

The results showed that A375 cells were more inhibited than WRL-68 cells, because the level of lipids and peptides is clearly increased in the cancer cell wall, as these substances act as special receptors for copper nanoparticles, which can interfere with the genes responsible for the cell cycle and cause damage to the genetic material of the cancer cell through programmed death [37], as well Copper nanoparticles can disrupt the membrane of cancer cells, preventing the production of ATP and inhibiting the respiratory chain [38].

Nanoparticles impact on cancer cells is attributed to their metabolic selective properties, including the nature and shape of receptors on cancer cells and their ability to bind to various compounds [39].

Conclusions

Green nanotechnology is an emerging field that is attracting researchers towards the biosynthesis of environmentally friendly nanoparticles, which is an easy alternative to the conventional methods for the synthesis of copper nanoparticles, and has tremendous potential to bring benefits in the fields of bridging physical, chemical and biological sciences with a myriad of applications. Through this study, the results demonstrated that the green manufacturing method using cinnamon bark extract is highly effective in the synthesis of NPs. The initial evidence of copper nanoparticle synthesis was observed when the reaction mixture changed color within two hours of preparation. The results showed the efficiency of copper nanoparticles in inhibiting bacterial species associated with wound infections and inhibiting cancer cells, which indicates the possibility of using them as antibiotics in the future and in treating cancerous tumors.

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