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Antibacterial and Cytotoxic Effect of Synthesized CuoNPs from *Staphylococcus epidermidis*

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Abstract: The risky of major concern resistance antibiotics become for public health. The alternative treatment metallic nanoparticles such heavy metals effects on antibiotic resistance bacteria different type antibiotic- impossible to treat using novel eco-friendly synthesis technique nanoparticles. The aim of the study to search for alternative antimicrobial medicines has been prompted by growing worries about multidrug resistance bacteria by synthetize novel nanoparticles. This study was to explore whether green synthesized copper nanoparticles (INPs) by multi drug resistance bacteria *Staphylococcus epidermidis* isolation from wound and burn infection. The results showed remarkable antimicrobial activity against *Staphylococcus. aureus* Minimum inhibitory Concentration range (16, 32, 64,128, 256, 512) µg/ml via well diffusion method in *vitro*, discover those concentrations effected in those bacteria and the best concentration is 64 µg/ml, characterization CuO NPs to prove this included atomic force microscope, Ultraviolet-visible spectrophotometer, X-ray Diffraction and Transmitted electrons microscopy, field emission scanning electron microscopy and anticancer activity was tested against cell membrane A375. The cells viability was decreased with increasing the copper nanoparticles and displayed a dose-dependent sequence of progressive cytotoxicity beginning at lower concentration to its maximum inhibition, (22) % inhibition human dermal fibroblast neonatal (HdFn) cells and (66) % inhibition of A375cells. It was concluded CuONPs were successfully synthesized friendly environment, few cost, highly CuO NPs product showed exceptional activity against bacterial strains and activity against cell line A375cells *invivo*.

Keywords: CuoNps, Green synthesis, A375cell, *S. epidermidis*.

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Introduction

Nanomaterial have distinct physicochemical properties and serve as adaptable scaffolds for biomolecule functionalization. Furthermore, certain Nanomaterial's, such as gold and magnetic nanoparticles, as well as polymeric or hybrid nanomaterials, have been found to respond to external stimuli, resulting in spatiotemporal regulated macromolecule release. For these reasons, engineered nanomaterials have been effectively evaluated and deployed in medicine and pharmacology throughout the last two decades, particularly for diagnostic and therapeutic applications (1) Copper (Cu) nanoparticles are superior to all other metal nanoparticles due to their unique chemical and physical features, such as strong heat transfer, electrical conductivity, and bioactivity (2), its less toxic, nature eco-friendly and support Environmental Protection agency for

human (3). Different nanomaterials shaped Cu/ CuO green synthesis Cost-effective and effective agent against pathogens microorganism and complications environmental such toxicology field and potential risk (4). The metal nanoparticals synthesis by chemical was major use of hazardous environmental pollution concerns and expensive *in vivo* and *in vivo* toxicity. Natural processing agent's biology nanopartical completely eco-friendly the present that is less toxic and good antimicrobial agent (5) CuoNps green synthesis, on the other hand, is environmentally friendly and economically effective and it does not employ harmful chemicals, making CuoNps appealing in biological applications (6). Most *Staphylococcus spp.* is of coagulase-negative (CoNS) and might cause many infections: septicemia, peritonitis, otitis, urinary tract infections, and respiratory infections (7). The resistance of *Staphylococcus aureus* has a very high resistance to antibiotics, and this has raised a problem in treating many difficult infections. *Staphylococcus aureus* is associated with multi drug resistance infections and high levels of illness and the efflux pump has vital role in multi-drug resistance for antimicrobial agent in *Staphylococcus. aureus* (8).

Materials and methods

Isolation and identification of *Staphylococcus epidermidis* and *Staphylococcus aureus*

Bacterial Confirmation one hundred fifty (150) wound and burns swaps were taken from (Al-Imamain Al-Kadhimain Medical City in Baghdad) and cultured on mannitol salt agar and biochemical characteristics and confirmation by vitek 2 system.

Antibiotic sensitivity test

Antibiotic sensitivity test was done for all 60 isolates by Vitek 2-compact system by using card containing 16 different antibiotics Benzylpencillin, Oxacillin, Gentamicin, Levofloxacin, Moxifloxacin, Erythromycin, Clindamycin, Linezolid, Teicoplanin, Tetracycline, Vancomycin, Tigecycline, Nitrofurantoin, Fusidic acid, Rifampicin and Trimethoprim/Sulfamethoxazole (9). CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute; 2021 CLSI, performance Standards for Antimicrobial Susceptibility Testing.

Suspension of *Staphylococcus epidermidis*

Bacteria were cultured in Mueller Hinton Broth to prepare a suspension; it was incubated at 37° C for 18 hours. At 4° C, the bacteria were cooling centrifuged for 10 minutes. Remove bacteria cell and preservative suspension to synthesis copper nanoparticles.

Synthesis of CuoNps

Steps of CuoNps synthesis

Green synthesis of *Staphylococcus epidermidis*m was prepared by dissolving 0.1 g of copper acetate dissolved in 100 ml of deionized water. Making centrifugation for *Staphylococcus epidermidis* that cultured on muller henton broth, 15 ml of *Staphylococcus epidermidis* supernatant was transfer in 100 ml Erlenmeyer flasks. The flasks were incubated at 30°C and color changes were noted. Copper acetate only which depended negative control no color change was observed over time (10) (Figure 1).

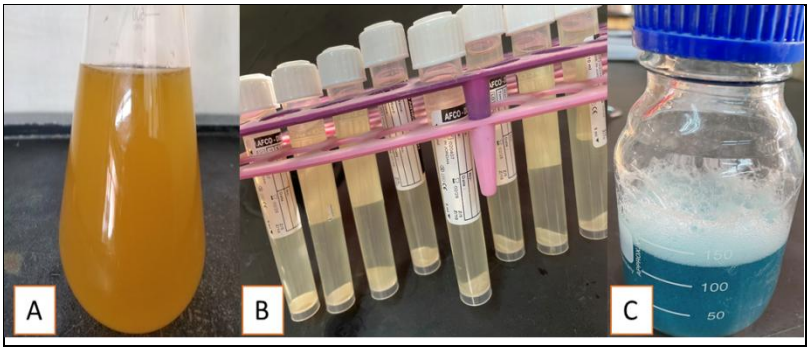

Figure (1): Synthesis CuoNps by biological method.
A-*Staphylococcus epidermidis* cultured on Muller Henton broth.
B- *Staphylococcus epidermidis* after centrifugation to collect the bacterial supernatant
C- The mixing of bacterial supernatant and copper acetate.

Minimal inhibitory concentration (MIC) determination

Using MHB, serial doubling dilutions of CuoNps were produced and added to each test culture bacterium in a tube incubator for 24 hours at 37° C. different concentrations between (16, 32, 64, 128, 256, 512) µg/ml (Figure2).

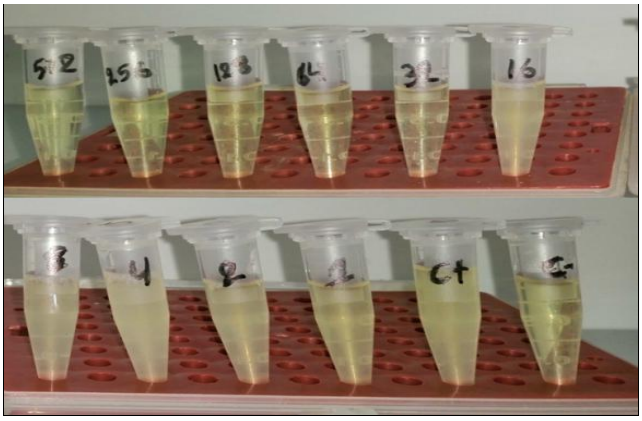

Figure (2): MIC CuoNps by biological method.

Characterizationtechniques included

- UV-vis Spectroscopy.
- (XRD)
- TEM
- FE SEM
- (AFM)

Activity of CuoNps in *vitro*

Copper oxide nanoparticles synthesis by biologically method assay Antimicrobial by Agar Diffusion Method. Against multidrug resistance (MRSA) on MHA was used for bacteria. using a sterilized cork borer an 5mm diameter agar well was made The several prepared concentrations of CuoNps were taken different concentrations between (16, 32, 64,128, 256, 512) µg/ml the (MIC) that dissolved with sterile deionized water, in addition to on Muller Hinton agar incubator for 48 hrs at a 37 ° C and then the growth inhibitory zone was examined.

Cytotoxicity of assay

Cell line maintenance

As the cells in the vessel established a confluent monolayer, the A375 cells and HdFn cells from the

human colon were tested using microtiter plates at concentrations ranging from (0.5 2.5) mL.

MTT protocol

The cytotoxic effect of CuoNps synthesis from *S. epidermis* performed by using MTT ready to use Kit contents.

Results and discussion

Bacterial isolates

Isolates and culture media

Isolation (wounds and burns) was identified as *S. epidermis.* Colonies on mannitol salt agar show (smaller than usual *S. aureus* yellow pin head colonies) and showed variable reaction on mannitol salt agar. Some strains showed yellow fermenter colonies (11), and some other strains showed whitish pink non fermenter ones (12), While the isolates to assay hemolysis On Blood agar, yellow-gray colonies (4-3) mm in diameter were observed on the zones of –hemolysis, as shown in (figure 3).

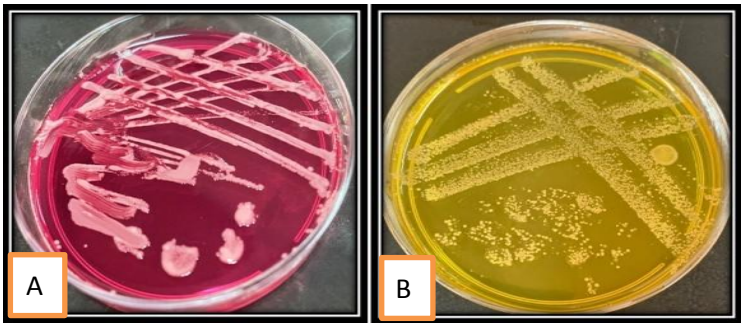

Figure (3): A) *S. epidermids* B) MRSA on MSA agar at 37c for 24 hrs.

Antibiotic susceptibility test

MDR strains screen

The results showed that all the 60 (100%) isolates It can be seen how high its resistance to oxacillin and benzylpencillin, respectively followed by Erythromycin (90%), Fusidic acid (86.6%) and Tetracycline (85%). Resistance to Gentamicin and Clindamycin were present in (71.6%) isolates. A moderate degree of resistance was observed among isolates to Moxifloxacin (58.3%) and Trimethoprim/ Sulfamethoxazole (43.3%). A lower degree of resistance was seen to Teicoplanin (28.3%), Levolfloxacin and Rifampicin (26.6%) followed by Vancomycin (20%) and Nitrofurantoin (3%). All (100%) isolates showed high level of sensitivity toward Tigecycline and Linzolid as clarified in (.figure4). The results are approximately parallel with a study by Naqid et al. (13) who demonstrated (100%) and (95%) of isolates were resistant to oxacillin and benzylpencillin respectively.

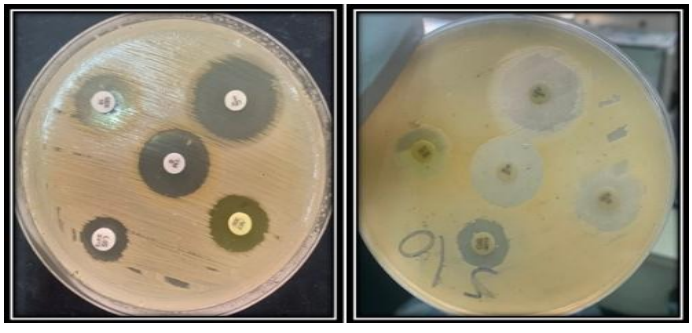

Figure (4): AST test of S. aureus on MHA

Biosynthesis of CuONPs synthesis and characterization of CuoNps:

Were successfully CuoNps synthesized using as reducing and stabilizing agents bacteria extracts, A rapid change from blue colour of the copper solution to a pale green the addition of the extracts indicated (Figure 5).

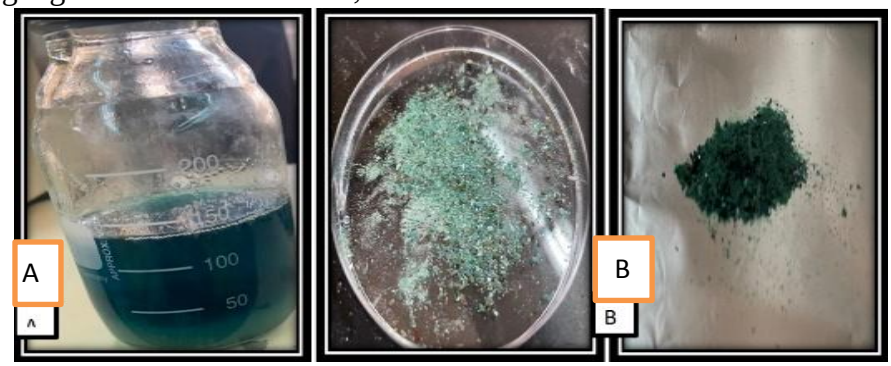

Figure (5): CuoNps solution biosynthesized by S. epidermids A: CuoNps with *S. epidermids* after 72 hrs at room temp B: Synthesis CuNPs as Precipitate green metallic particles like powder.

In recent years, an obvious coordination to use bacteria to synthesizes nanomaterials (mainly silver, zinc, gold, and nanoparticles) with remarkable properties have been observed for the development antimicrobials with *in vitro* activities against pathogenic bacteria (14).

UV–Vis spectral analysis

The findings revealed that biosynthesized CuoNps had a maximum peak at (absorption peaks at 275 and 280 nm were) nm as shown in (figure 6).

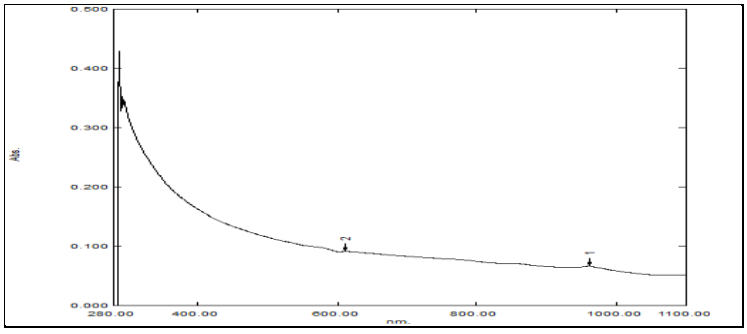

Figure (6): UV–Vis spectrophotometry of CuNPs.

Green synthesized CuO NPs reduced absorption by raising or decreasing in extract volume. The yellowish green edge is responsible for the depth flaws at 230 nm.

X-ray diffraction (XRD)

The XRD spectra of CuO NPs, nanoparticle powder is shown in Figure 7, validated the hexagonal (wurtzite) structure of CuNPs NPs by exhibiting significant peaks that correlated to diffraction peaks (100), (002). Peaks assured the detected the presence of CuO monoclinic phase as synchronized.

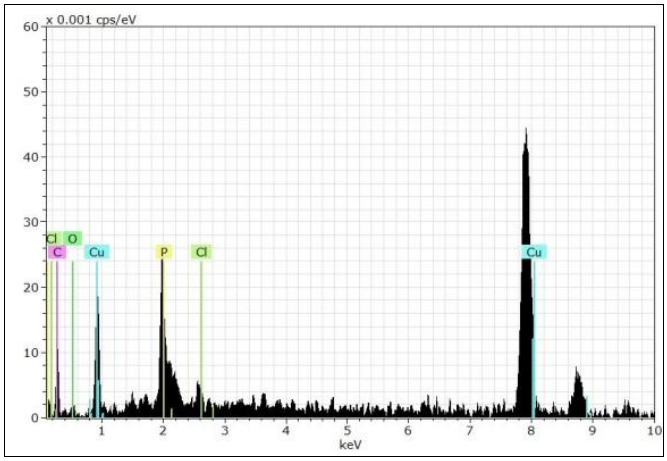

Figure (7): XRD analysis of synthesized CuoNps.

Atomic force microscopy (AFM) analysis

Characterize the production of CuoNps using biological methods, such as measuring their average diameter and shape in two and three dimensions. The results obtained in this study showed that the biosynthesized CuoNPs by *S. epidermidis* diameter of 36 nm as shown in figure 8 AFM analysis of CuO NPs was performed to identify and characterize distributions of nanoparticles (15).

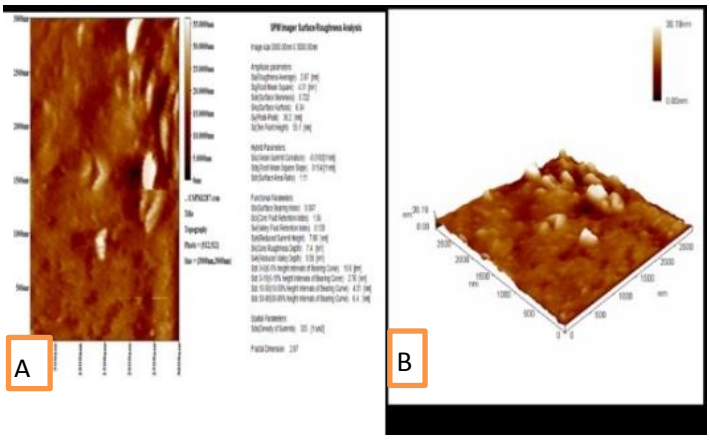

Figure (8): The biosynthesized CuoNps (A) 2D AFM of CuNPs (B) 3D AFM CuNPs

Transmission electron microscopy analysis of CuoNps

The figure 9 shows prepared CuoNps have a spherical shape and are crystalline, with diameters ranging from 5 to 40nm.. On other hand agree Morphological alternations in *E. coli* were observed using (FE SEM) (16).

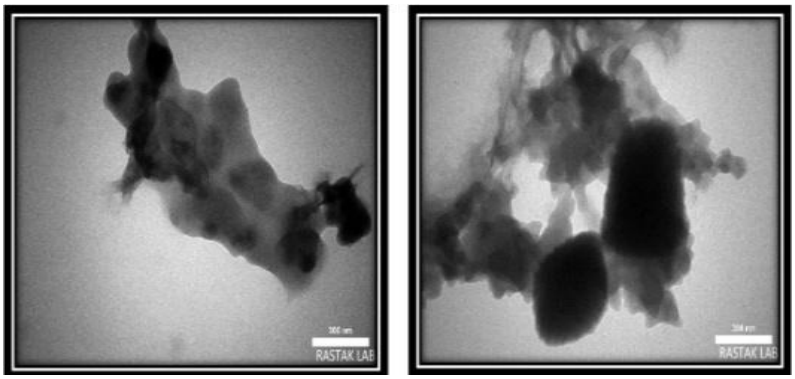

Figure (9): The transmission electron microscopic.

Field emission-scanning electron microscope (FE-SEM)

CuoNps with morphologies and sizes up to 20 nm were synthesized using the noval biosynthesis method of field emission scanning electron microscopy Figure10 shows The result according to (17), CuoNps spherical shapes exhibited clustered with, size distribution ranging from 22 nm SEM nanoparticles was clustered with a mean diameter of 6 nm.

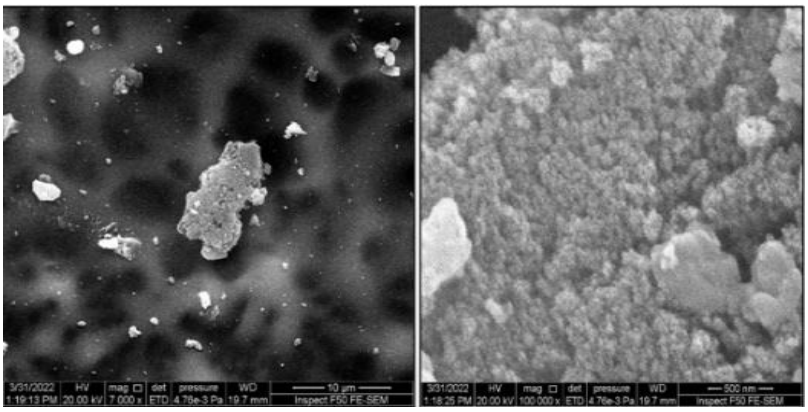

Figure (10): FE-SEM Image of CuoNps.

Minimum inhibitory concentration (MIC) of CuoNps

Well diffusion was used to detect the activity of CuoNps nanoparticles against *S. epidermids*. in different concentration (16,32,64,128,256,512) µg/ml showed highly activty anti-resistance bacteria and the zone of inhibition reached (14), shows figure11 The result agree, (18) synthesis CuoNps showen effection against microorganism such as bacterials the dimeter of inhabation bacterial growth inhibition reached more 17mm.

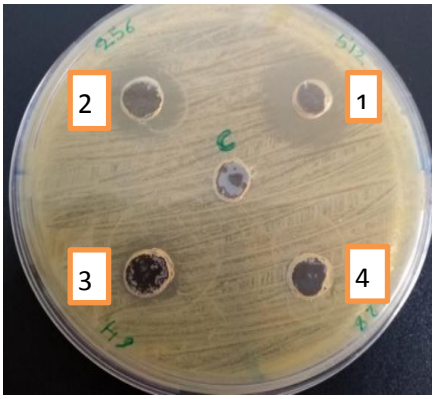

Figure (11): Minimum Inhibitory Concentration of CuoNps at different concertation on MHA at 37°C for 24 hrs.
1-512 , 2- 256 , 3- 64 , 4- 128

Toxicity testing

The MTT results (Figure 12) The concentration rang between (16, 32, 64, 128 , 256, 512) µg/ml of CuoNps resulted reduction of HdFn cells .The cells viability was decreased with increasing the concentration of the CuoNps and displayed adose-dependent sequence of progressive cytotoxicity beginning at lower concentration to its maximum inhibition, (22)% inhibition of HdFn cells and (66)% inhibition of A375 cells, CuO-NPs were found to cause significant cytotoxicity Toxicity is frequently associated with apoptosis, and decreases in cell viability result in cell death, such as cell membrane damage A375 (19). The findings demonstrated that CuoNps have strong cytotoxic effect on cancerous cells (BC3 cell line) (20).

	A375		HdFn	
Concen.	Mean	SD	Mean	SD
400.00	37.71	6.94	74.58	3.52
200.00	48.79	3.73	86.03	0.85
100.00	66.05	2.41	92.13	1.56
50.00	75.15	5.10	96.18	1.25
25.00	85.15	4.36	96.95	1.14
12.50	95.41	1.41	94.91	2.20
6.25	96.14	1.05	96.10	0.48

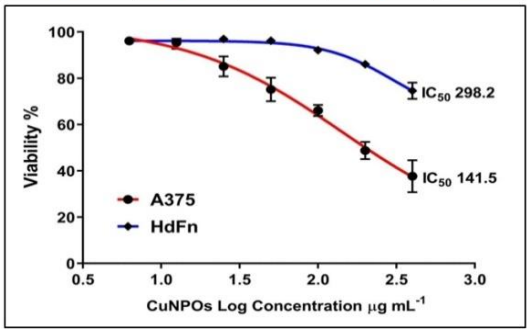

Figure (12): The MTT assay results of synthesized CuoNps on HdFn cells and normal A375

Conclusion

As bacteria and health problems have recently multiplied, alternatives must be found CuoNps were successfully synthesized following an in, direct, eco- friendly, low cost, high-

yield and green method, CuoNps showed exceptional antimicrobial activity against several bacterial strains, CuoNps shows significant activity against the viability of cancer cells decreased when the CuoNps concentration increased.

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