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Activity of Biosynthetic Copper Oxide Nanoparticles by *Lactobacillus Plantarum* against Pathogenic Bacteria as Antibacterial Antibioflim and Antioxidant

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Abstract :. Copper oxide nanoparticles are the most common metal oxides. (CuONPs) The good capability of copper-tolerant probiotic of *Lactobacillus ssp* tolerating, Because of its unique properties and applications, these bacteria have gained more interest as a natural microbial cell nano-factory for a more effective and environmentally friendly method of biosynthesis of these nanoparticles. We examined the antibacterial activity of the Copper Oxide Nanoparticles on some pathogenic bacteria challenge of multidrug resistance (MDR) .It is clear in this the study that Copper Oxide has stronger inhibitory effect on pathogenic bacteriathe largest inhibition region of Cuo NPs from *L.plantarium* in *E coli* was 28 mm at 500 g/ml MIC followed by *E. faecalis* 25mm in the same concentration. The antibiofilm activity of the Copper Oxide Nanoparticles on some pathogenic bacteria formation biofilm was quantified in well plate assay , congo red method and Tube method ,The efect of copper oxide nanoparticles on strong ,modrate biofilm to weak or non formation. Antioxidant activity was determined using 2,2-diphenyl-2-picrylhydrazyl (DPPH), the Copper Oxide Nanoparticles from *Lactobacillus plantarum* had the highest antioxidant activity (73.60 %,67.11%,58.97 at 250,200,150 µg/ml) in concentrations (250,200,150,100,50) µg/ml.

words: *CuONPs, Lactobacillus plantarum, antibacterial efficacy ,Antibiofilm activity ,Antioxidant activity*

Introduction

Nanotechnology is the creation,Materials that are produced, exploited, and synthesized on a scale smaller than one µm [1]. For thousands of years, Lactobacillus has been exploited for the development of fermented produce desirable improvements in taste, flavor, and texture. [2]. Copper nanoparticles have gained a lot of interest among non-metal and metal particles because of their catalytic, optical, and electrical conducting properties. Several methods for the preparation of copper nanoparticles have been developed; however, mechanical chemically synthesized copper nanoparticles. [3]. Since numerous microb has flourished for thousands of years through their ability to become accustomed to antimicrobial agents, the action of antibacterial agents is the basis for their rational and safe use in the treatment of bacterial infectious diseases. This mechanism allows certain bacteria to resist the action of antibiotics, making them ineffective [4].In order to achieve multi-drug resistance, these microorganisms use a variety of pathways. After the invention of antibacterial compounds, The most important pharmacodynamics parameter in antibacterial regimen formulation has been (MIC). In vitro experiments, on the other hand, only assess the effectiveness of predetermined antibiotic concentrations over time. [5] Antibacterial agents have a distinct effect on Gram negative bacteria than on Gram positive bacteria due to the

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different composition of the wall of Gram-negative bacteria, which is mostly made up of the outer membrane.[6]. The antioxidants assay procedure is based on DPPH (Diphenylpicrylhydrazine), a free radical with a peculiar electron and a maximum absorption wavelength of 517 nm, is reduced (purple colour). In comparison to the DPPH-H form, decolorization (yellow color) happens when the amount of electrons caught grows when antioxidants react with DPPH, a stable free radical. [7] "Any material that slows, avoids, or eliminates oxidative damage to a target molecule" is what an antioxidant is. Free radicals are generated during oxidation reactions, which can set off a chain reaction that damages or kills the cell. Antioxidants destroy these free-radical intermediates by oxidizing themselves, as well as inhibiting other oxidation reactions, halting the dangerous chain reactions[8].

The Aim of the Study : According to the evidence, The rising threat of multidrug-resistant bacteria and biofilm-associated illnesses necessitates the development of new bactericidal methods. As a result, new and developing nanoparticle-based materials in the field of antimicrobial chemotherapy have received a lot of interest. so, the present study was aimed to identify the role of biosynthesized copper oxide nanoparticles in reduction the biofilm formation among multi- drug resistant pathogens according to phenotypic . The presnet study aim Antibacterial activity of cuo nanoparticales (CuONPs) biosynthesized by Lactobacillus sp .

Methodology

Bacterial Isolate:. Lactobacillus ssp was preferred as a biological model for CuO NP synthesis because it is more effective. This isolate was collected from dairy products stored at the University of Babylon's Advanced Microbiology Lab. We cultured the isolate for 24 hours on MRS agar at 38°C, and it was diagnosed using the Vitek2 system

Copper oxide Nanoparticles were made by biological method from *Lactobacillus plantarium* , measured by FTIR,XRD FOR Copper oxide Nanoparticles is 18nm ,SEM and TEM (20-30nm) means and mentioned in other research Biosynthesis by [9].

Antibacterial efficacy of CuONPs Disc Diffusion Method. This method was accomplish on Muller Hinton media as follows:To give the optimal concentration for each bacterial isolate, concentrations were taken from each and compared to McFarland solution. Each bacterial isolate was introduced to plates containing Muller Hinton agar, which was distributed on the dish's surface with a spreader at the appropriate concentration of 0.1 ml, and the dishes were left for an hour. each 9 mm diameter cork borers. it was equal distance between the film and well with 100 µl from chemical synthesis Cuo NPs solution at different concentrations (500,250,125,62.5 AND 31.52µg/ml) to each well [10] .After incubation time, each plate was examined by measured the diameter of complete inhibition zone to the nearest whole millimeter, using a ruler then record the results value.

Minimum inhibitory concentration (MIC): the test was done by using macrodilution method:Serial dilution of CuO NPs was made to distribution on 5 test tubes at 3ml with 1ml bacterial inoculum after turbidity checker with control tube (only bacterial inoculum without CuO NPs solution). CuO NPs concentrations were(500,250,125,62.5 and 31.52µg/ml) incubated all these tubes at 37°C for 24 hrs. The micro well dilution technique was used to calculate the (MIC)

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with minor modifications. This experiment was carried out on sterile 96-well micro test plates with a flat bottom. Each well was filled with 15 l of Mueller Hinton broth, 2 varied concentrations of CuO NPs nanoparticles, and 3 µl the test organism suspension . Each well had an ultimate volume of 20µl. Each test batch had three control wells: positive control (medium and test organism), negative control (media and nanoparticles), and plates were incubated at 37 °C for 24 hours. After incubation, the MIC was measured using spectrophotometry at 600 nm. [11].

Antibiofilm Activity of Copper Oxide Nanoparticals

Modified TCP method was considered as gold standard (Bose *et al*., 2009): The microtiter plate (also known as the 96-well plate) test is used to investigate antibiofilm using tissue plate techniques. [12] Bacterial adhesion to an abiotic surface can be observed using this approach. Isolates bacteria were inoculated in TSB (trypticase soya broth) with 1% glucose and cultured at 37°C for 72 hours before being diluted 1:100 with new TSB. Only broth was used as a control in individual wells of sterile polystyrene 96 well flat bottom tissue culture plat wells filled with 150 Ml aliquots of diluted culture. Each isolate was injected three times and incubated for 24 hours at 37°C. The supernatant from the microplate wells was then carefully removed by washing four times with (PBS pH=7.2) to eliminate free floating and unattached bacteria. Both wells were stained with 100 ml of a 0.1 percent (w/v) crystal violat solution after the biofilm created by adherent'sessile' species in the plate was fixed in the oven for 30 minutes at 37 °CFor 15 minutes at room temperature. The dish was cleaned four times with PBS before being allowed to dry to eliminate any remaining discoloration. To get rid of the bound crystal thief,150 Ml of a 95 percent ethanol: 95 percent aceton [8: 2 (v/v)] mixture was applied. The optical density at 630 nm was measured and the results were used to create a table..Classification of bacterial biofilm formation by tissue culture plate method in to three categories according to the formula described by [13].weak (BF < 0.120), moderat (0.120 >BF<_ 0.240), and strong (BF >0.240) .

Congo red agar method:

Brain- Heart- Infusion- Broth, agar agar supplemented, with 50gm/l sucrose, and 8gm/l Congo red [14].To obtain Congo, red agar we prepared a Congo red stain as stock solution, autoclaved at 121°C for 20 min then added to autoclaved brain heart infusion broth with agar agar and 5% sucrose at 55°C.(Hassan et al., 2011). The bacterial strains were inoculated and incubated at 37 °C for 24 to 48 hrs. then read the result as following: if the bacteria formed black colonies with a dry crystalline consistency that was mean it biofilm producer isolates while if it formed red colonies that was mean the non-biofilm producer isolates[15].

Tube method : Brian Heart Infusion (BHI) was prepared and sterilized in an autoclave before being inoculated with a loopful of each pathogenic microorganism and incubated at 37°C for 24 hours. 60 l of new BHI was spread in sterilized tubes after sterilization with 2 percent sucrose by filtration (0.45um Millipore), After that, 30µl of each overnight microorganism suspension and 30 µl of CuO NPs with a 500 g/ml concentration were applied to each tube separately. The control tube was incubated at 37°C for 24 hours with only bacterial suspension and no CuONPs[16]. The tube was removed from the bacterial suspension after incubation and washed with phosphate buffer saline (PBS) pH 7.4 before drying at room temperature. The tubes were stained with 1% crystal violet for 10 minutes at room temperature, then washed with deionized distill water to

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eliminate any remaining dyeTo discharge, the tubes were turned upside down. When,transparent film lined the wall, and bottom, of pipe, it was considered a good sign of biofilm formation.[13].

Antioxidant Activity

CuO NPs synthesized from *Lactobacillus Plantarum* were tested for antioxidant activity using the DPPH (1,1-Diphenyl-2-picryl-hydrazyl) radical scavenging assay identified by [17] , as follows: CuO NPs two-fold serial dilution aliquots (0.5 ml) andIn the test tubes, ascorbic acid (12.5, 25, 50, and 100 µg/ml) was added to the process. At the same time, 3ml of methanol-DMSO combination and 0.3ml of DPPH solution were administered to each concentration. At 37°C, samples were incubated for one hour. An ELISA reader was used to assess the spectrophotometric behavior of samples against the stable DPPH radical The colorimetric modified DPPH reduction was investigated at 517 nm (from deep violet to bright yellow) The following formula was used to calculate the percentage of radical inhibition by the samples:. ve power absorbance vs. sample absorbance 100 percent inhibition = control ve ¯ of Absorbance, Methanol-DMSO mixture and DPPH solution served as negative controls, while ascorbic acid served as a reference.DPPH•DPPH-H

Results and Discussion

Antibacterial Activity test of CuO NPs against MDR Bacteria
Minimal Inhibitory Concentration (MIC) Test

Three multidrug Gram-negative bacteria and three multidrug Gram-positive bacteria were examined for antibacterial activityby used Biosynthesis Cuo NPs. The use of varied quantities of copper oxide nanoparticals against MRD bacteria was a key step in this investigation. Using the broth micro dilution technique and spectrophotometer at wavelength 600 nm ., to determined the MIC concentration for nanoparticles during incubation.

Micro titer plate

Using the broth, micro dilution technique, the minimum inhibitory concentration (MIC) of nanoparticles was estimated. A 0.5 McFarland's Standard suspension of each bacterium was pipetted onto a microtiter plate. The bacterial isolates were cultured for 24 hours at 37°C on a micro titer plate., and colloidal Cuo NPs (500, 250, 125, 62.5, 31.52, and 15.76 µg/ml) were added to each well containing MDR bacteria, before the incubation time, measure the OD 600 nm after 24 hours of incubation under shaking, whereas bacterial growth without CuO NPs is used as a monitor in each MIC (120 rpm).Minimum inhibitory concentration (MIC) is the lowest concentration which prevents visible growth of bac Muller Hinton agar plates were used to subculture the incubation. Cuo NPs from *L.plantarium* have different MIC values depending on the tested isolates. terium are determined by take samples from wells used in broth micro dilution assay that did not show any observable growing after the 24 hr. 500 µg/ml bioconcentration of the MIC Cuo NPssynthesis from *L.plantarium* This concentration was shown to be selective on all six MDR bacteria in the same way, showing that it is a typical dose for bacterial inhibition. Copper oxide nanoparticals, on the other hand, have a substantial inhibitory impact on bacteria strains, according to the findings of this study.because the capacity of Cuo NPsto inter acted with the

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bacterial cell wall and ruptured him, disturbing the metabolism of bacteria by effecting on bacterial DNA and interaction with mitochondria, and ,other organelles, of bacteria[18,19,20].

Antibacterial activity of Cuo NPsbio-synthesized by *L.plantarium* . respectively were used to evaluate their ability for inhibition growth of some clinical bacteria characterized as a MDR bacteria (Table 1). Now after

selecting a MIC This entailed the use of a test that was carried out in sterile Petri dishes with a diameter of 90 mm containing sterile Mueller Hinton agar medium and was carried out according to the protocol described in the references (15 ml). Following a 24-hour bacterial culture recovery period, swab the freshly prepared bacterial inoculums over the entire surface of the medium three times, To ensure the spread of bacteria on the plates' surface, rotate the plate for each treatment with a sterile cotton swab. With the aid of a sterile cork-borer, a well of 9 mm diameter,was poured in the medium for each plate, and the tested Cuo NPs were filled with a micropipette, while water was used as a control. Type of microdilution every bacterial suspension was pipetted into a microtiter plate with 0.5 McFarland's Standard suspension ($1.5\times10_8$ cells/ml concentration). Plates were left at room temperature for 45 minutes to enable proper Cuo NP diffusion in the medium. Both plates were incubated for 24 hours at 37°C. Following that, the diameters of inhibition zones were measured. The average value of all observations was used to calculate the inhibition, of bacterial development, This was determined as 3-equidistant point diameters (mm) obtained from the center of the inhibition zone, the largest inhibition region of Cuo NPs from *L.plantarium* in Gram negative bacteria was 28 mm at 500 g/ml MIC. Because of the growing challenge of multidrug resistance (MDR), the research community has made it a priority to find an effective solution to replace existing antibiotics that have become resistant.

Table(1):Antimicrobial-Activity of (MIC Values in µg/ml) CuO NPs against of MDR Bacteria

Concentration CuO NPs	**500 µg/ml**	**250µg/ml**	**125µg/ml**	**62.5µg/ml**
Bacterial strain				
E.Coli	28	26	15	Non
E. faecalis	25	23	20	Non
K.pneumoni	18	15	10	Non
S.aureas	14	10	Non	Non
P. aerugenosa	10	Non	Non	Non
Streptococcus ssp	Non	Non	Non	Non

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Cuo NPs from *L.plantarium*, on the other hand, had the highest inhibition zone on Gram negative bacteria, measuring 30mm in E.coli with a MIC of 500g/ml. The effects of CuoNPs synthesis by *L.plantarium* on bacterial inhibition were discovered [21] According to the study, CuNPs at a concentration of 100 g/mL exhibited no antibacterial impact on K. pneumoniae, P. aeruginosa, or S. aureus. At a dosage of 1000 g/mL, CuNPs may kill 76 percent of Klebsiella bacteria, 69 percent of Staphylococcus bacteria, and 83 percent of P.seudomonas bacteria. CuNPs are bactericidal at a concentration of 500 g/mL by 91, 72, and 94 percent for bacteria, respectively, *K. pneumonia*, *S. aureus and P. aeruginosa*, respectively. CuNPs demonstrated a stronger antibacterial impact against pathogenic Gram positive and negative bacteria than silver NPs. Bacteria, yeasts, and viruses can be killed by "contact killing" on copper surfaces (contact-mediated killing). Copper contact kills logs at a rate of seven to eight each hour, according to research, Especially after a long period of incubation. This has to do with the fact that copper is a self-sanitizing material. To aid the CuNP toxicity process, several factors were employed, including temperature, pH, bacterial and NP concentrations, as well as aeration. [22.] includes Gram negative bacterial cell wall adhesion owing to electrostatic contact, altering cell protein structure Nano-scale Cu particles have been found to have antibacterial effects on bacterial cell activity in a variety of ways, including electrostatic interaction with Gram negative bacterial cell walls, affecting protein structure in the cell membrane, and denaturation of proteins intracellular, and interaction with phos- phorous and sulphur-containing compounds such as DNA [23,24].

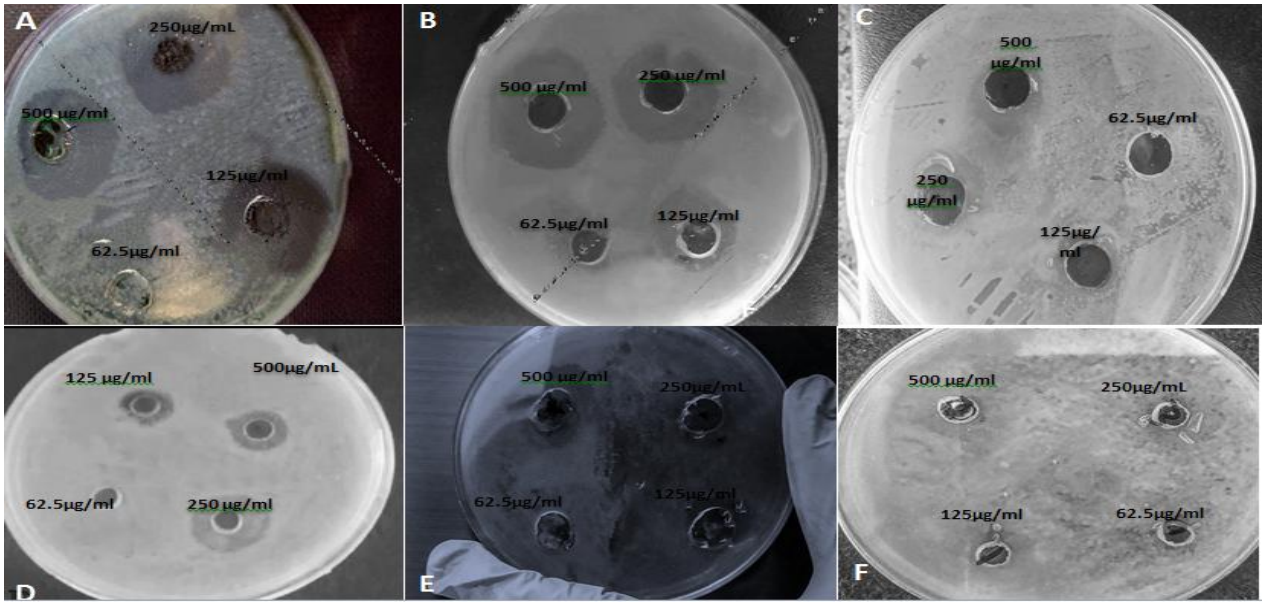

Figure (1): Antibacterial activity of colloidal Cuo NPs(synthesis by *L.plantarium*) against A- *E.Coli* , *B- E. faecalis* ,*C- P. aerugenosa* , *D- K.pneumoni* , *E -S.aureas* , *F- streptococcus* on the Mullar Hinton agar for 24 hr. at 37°C

4.7 Oxidative Damage and Anti-Oxidants, CuO NPs as Anti-Oxidants

Using a stable DPPH free radical scavenging assay, the scavenging activity of CuO NPs synthesized by Latobacillus sp bacteria was assessed. The results in Fig. (2) show that the higher the concentration of CUONPs synthesized from L. plantarium, the higher the proportion of free radicals scavenged. The concentrations that were used were as follows: (250,200,150,100,50)

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µg/ml, showed the CuO NPs significantly highest antioxidant activity (73.60 %,67.11%,58.97 at 250,200,150 µg/ml)while. the CuO NPs in concentration, (100 and 50 µg/ml) showed antioxidant activity (38.95 and 27.20 %) respectively.

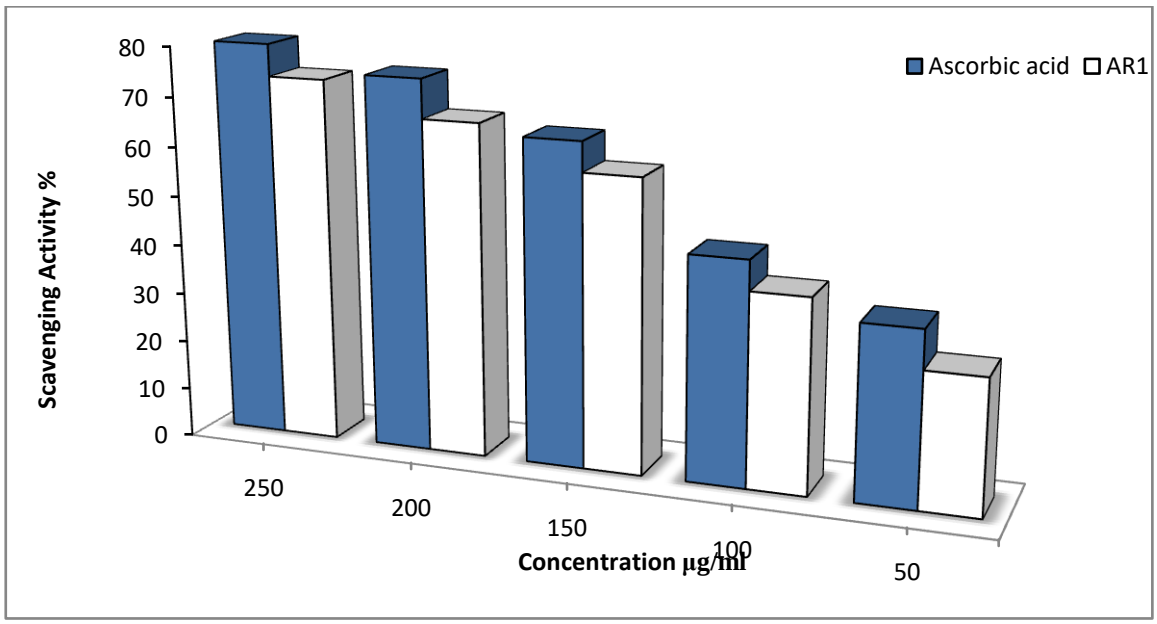

Figure 2: CuO NPs production from L. plantarium DPPH free radical scavenging activity compared to ascorbic acid as a control sample.

The above findings were compared to ascorbic acid's free radical scavenging function as a positive antioxidant, which, according to Fig (2) The CuO NPs synthesised from L.plantarium displayed the same pattern of ascorbic acid free radical scavenging action except at doses of 50g/ml. The DPPH free radical scavenging method appeared to be successful, based on these data. The antioxidant activity of CuO NPs was dose dependent, with greater CuO NP concentrations resulting in increased antioxidant activity... are adsorbed onto the CuO NPs' surface. Given their large surface area to volume ratio, these CUO NPs appear to have a high proclivity for interacting with and reducing DPPH accepts an electron from an antioxidant molecule on the CuO NPs solution's surface [25] The CuO NPs synthesised from L.plantarium displayed the same pattern of ascorbic acid free radical scavenging action except at doses of 50g/ml. The DPPH free radical scavenging method appeared to be successful, based on these data. The antioxidant activity of CuO NPs was dose dependent, with greater CuO NP concentrations resulting in increased antioxidant activity.

Antibiofilm Activity of CUO Nanoparticals by

1 - Multi-well Plate Count Method

Biofilms are bacterial communities that are resistant to antibiotics and the immune system of humans. Antibiotics that are selective against biofilms are in short supply. Nanoparticles have been used to tackle this issue, with Cuo NPs showing antibiofilm activity as one theoretically effective candidate treatment. This approach was also used to determine antibiofilm production qualitatively. the result recorded as purple ring in the wall and bottom of the well. Biofilm is the

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most important feature of some bacteria that enhance attachment of bacteria to the surfaces surgical tools and prosthetics. Therefore, it is targeted by bionanotechnology researchers In this study the same 40 isolates was used all of them are MDR bacteria, three of them are Gram negative bacteria (*K. pneumoniae* and *P. aeruginosa*) and other three are Gram positive bacteria *(E.faecalis*) and tested to producing biofilm, In the multiwell plate process, all of the microorganisms tested were able to form biofilm on the walls and bottoms of the wells. The findings appeared to be a solid biofilm that had been formed by *P. aeruginosa* and *Staph. aureus* . The Cuo NPs from *L.plantarium*. were prevented the formation of biofilm in(*E faecalis,K. pneumoniae and P. aeruginosa*) some bacterial isolates Although some isolates inhibit but do not preclude biofilm development in a dose-dependent way, others do not. Since metallic nanoparticles have been shown to disrupt exopolysaccharide synthesis, which restricts biofilm production, the anti-biofilm action of Cuo NPs may be due to inhibition of exopolysaccharide, synthesis[26]. Exopolysaccharide and cell surface hydrophobicity are essential in bacterial-host cell interactions and microbe biofilm architectures. Previous research has shown that cell surface hydrophobicity reduces biofilm formation in a variety of microorganisms, including *Candida* sp [27].The results of this analysis show that treatment with Cuo NPs decreases the hydrophobicity index of the bacteria studied, which inhibits biofilm formation. The presence of Copper oxide nanoparticles had an effect on bacterial biofilm growth in an in vitro experiment. Differences were seen in terms of bacteria composition, nanoparticle concentrations, and nanoparticle form. the formation of biofilm in *S.aureas*

Table(2): Antibiofilm Activity of (MIC Value in µg/ml) of CuONPs from *L.plantarium*

Bacterial strain	**With out CuONPs**	**CuoNPsfrom *L.plantarium* .**
1- P. aeruginosa	weak	None
	Moderate	None
	Strong	Weak
2 E. faecalis,	weak	None
	Moderate	None
	Strong	None
3- K. pneumoniae	weak	None
	Moderate	None
	Strong	Weak

Table (2) showed that Cuo NPssynthesis from *L.plantarium* showed reduction in (*E. faecalis, K.pneumoniae and P. aeruginosa)* biofilm growth completely in MIC 500 µg/ml in all three strains of bacteria, strain number one and three respectively compared to the control . Similarly,

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another study demonstrated that copper oxide nanoparticles showed excellent antibiofim potential against Gram-negative and Gram-positive bacteria biofilm formation is dependent on cell to cell signaling mediated, most anti-biofilm strategies target and interfere with this signaling mechanism. Cuo NPs can be used to suppress pathogenic bacteria as well as microorganisms, not only by regulating cell growth but also by interfering with biofilm formation and invasion [28]. As a result, it's possible that CuNPs could be used in antimicrobial and anti-cancer medications at higher concentrations. Copper has been linked to a variety of cell functions, including catalytic, structural, and regulatory functions, according to [29].

congo red method

The method used for qualitative assessment of biofilm formation the result recorded as change the color of colony on the congo red agar Table(4-5), Figure (4-18) the experiment was tested 40 isolates two of them was not producing biofilm (*E. faecalis, K.pneumoniae and P. aeruginosa*) , The Cuo NPs from *L.plantarium*. were prevented the formation of biofilm in(*E faecalis,K. pneumoniae and P. aeruginosa*) some bacterial isolates.Against *E. faecalis* Cuo NPs from *L.plantarium* showed significant antibiofilm activity with decreasing concentration activity at concentration of (500mg/ml). Against *P. aeruginosa* the largest inhibition zone of Cuo NPsfrom *L.plantarium* .on Gv- bacteria was 30mm in *P.aeruginosa* with MIC concentration 500µg/ml . significant antibiofilm activity with highest activity at concentration This results showed that the effects of CuoNPs synthesis by *L.plantarium* on bacterial inhibition.

Bacterial strain	**inhibition zone on MIC CuONPs 500µg/ml**	**inhibition zone on MIC CuONPs 250µg/ml**	**inhibition zone on MIC CuONPs 125µg/ml**
1- P. aeruginosa	40mm	28mm	21mm
2 E. faecalis,	26mm	25mm	20mm
3- K. pneumoniae	23mm	19mm	18mm

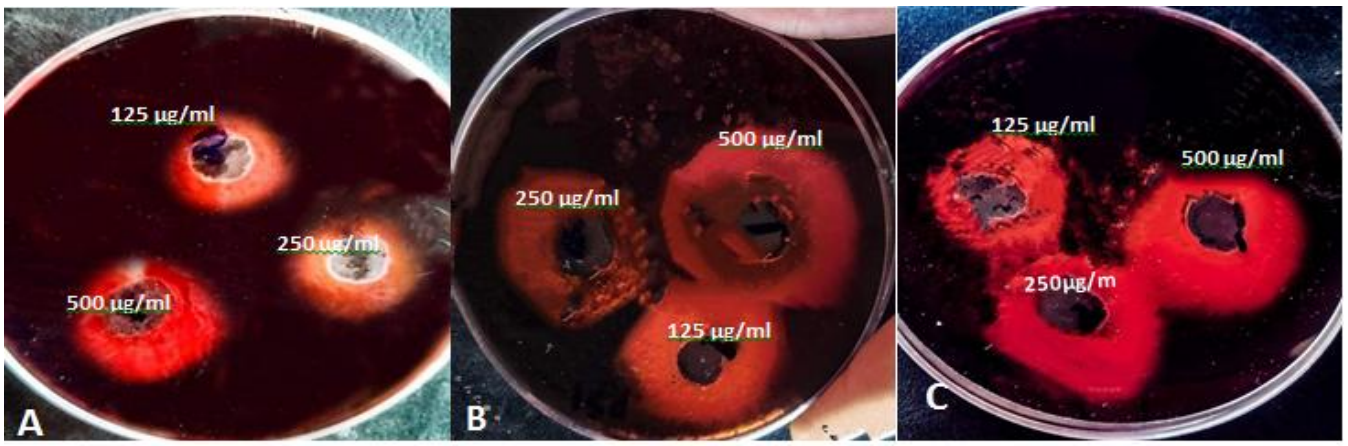

Figure (2) Antibiofilm activity of CuO nanoparticle by congo red agar method for A: *K.pneumoniae B: P. aeruginosa and C: E. faecalis* for 24 hours at 37C , B: congo red agar method

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Tube method

This method was also used for qualitative assessment for antibiofilm activity the result recorded as purple ring in the wall and bottom of the tube Figure (5) The biogenic CuO NPs synthesis by *L.plantarium* was prevented the formation of biofilm in many isolates such (*E. faecalis, K.pneumoniae and P. aeruginosa*) , Urease generation and various forms of motility are two of the most important bacterial virulence factors involved in biofilm development. Proteus sp., Klebsiella pneumoniae, Pseudomonas aeruginosa, and Serratia marcescens were the most urease-positive Enterobactericeae members. [30]

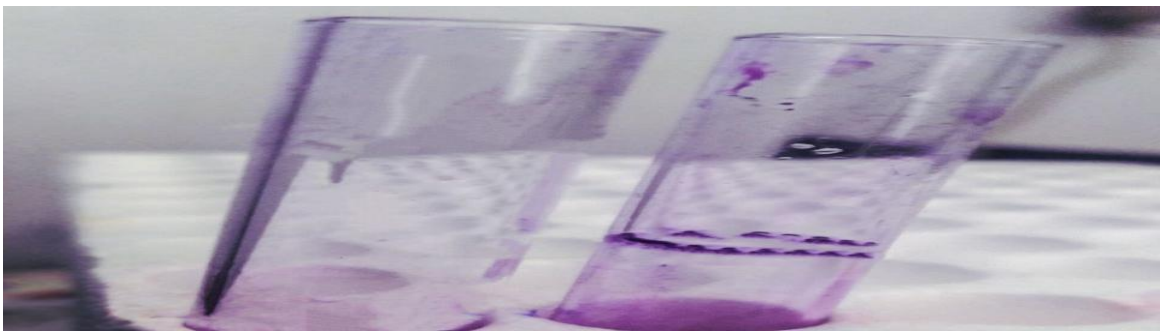

Figure (3) tube method used for detection the antibiofilm activity using *CuO NPs* the purple ring reveal positive result

Conclusion

Since conventional antibiotics are becoming increasingly toxic, nanoparticles are gaining interest for their possible antimicrobial effects and applications. This makes the possibility of using nanoparticles to cure infection quite appealing. Nanoparticle concentrations that suppress a number of bacteria species have been established in vitro studies. The efficiency of nanoparticles made of various materials and sizes varies. The dosage necessary to successfully suppress bacteria activity is determined by the extent of the nanoparticle's bactericidal/bacteriostatic impact.This means that CuONPS were more in inhibition the growth of pathogenic bacteria . The results represent a great potential benefit for a wide numbers of medical applications in the battle against antibiotic-resistant bacterial pathogens. the synthesized copper nanoparticles exert considerable anti-bacterial activity against *Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, and Enterococcus faecalis*, with MIC values 500 _g/mL. A significant interaction CuONPs in biofilm , the results demonstrate the greater antibiofilm activities of CuONPs on *E. faecalis, K.pneumoniae and P.aeruginosa*.We successfully screened the antioxidant activities of Copper Oxide Nanoparticles from *Lactobacillus plantarum* has the highest antioxidant at different concentration

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