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RESEARCH PAPER

Antibacterial Activity of Copper Nanoparticles Produced by Pulse Laser Ablation Against *E. Coli* and *Staphylococcus Aureus*

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ABSTRACT

Copper nanoparticles (CuNPs) were prepared in this work by laser ablation from a copper target immersed in deionized water. Q-switched Nd: YAG laser of fundamental wavelength (1064 nm) with different laser energies (600, 700, and 800 mJ) of 2 mm^2 laser spot area, using 600 plusses were used. Structural, morphological, and optical properties of the obtained CuNPs were examined. The characteristic absorption peak for Cu were observed in the UV region (649–653) nm. According to the X-ray diffraction (XRD) patterns, the CuNPs have a polycrystalline structure of a cubic phase. The crystallite size increased from 9.9 nm to 18.9 nm with increasing the laser energy from 600 to 800 mJ. The surface morphology was studied using SEM, which revealed spherical particles increased in dimension according to the laser energy. The diffusion well technique was used to examine the antimicrobial impact of the prepared Cu-NPs against *S. aureus* and *E. coli*. Cu-NPs inhibit the growth of gram-negative (*E. coli*) bacteria with an inhibitory zone larger than gram-positive (*S. aureus*). The nanoparticles created in this way have antibacterial properties which might be beneficial in the creation of high effective antibacterial agents.

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INTRODUCTION

Copper nanoparticles, particularly low-cost nanoparticles for electrical, biological, and pharmacological applications, have gotten a lot of interest in recent years and are likely to be as antibacterial agents [1]. Viruses, bacteria, and other microbes are affected by antimicrobial metal nanoparticles such as copper and silver [2]. Nanoparticles can easily interact with bacterial membranes [3]. Pulsed laser ablation in liquid (PLAL) method has been the most versatile physical method for producing metal nanoparticles [4]. Pulsed laser ablation in liquid (PLAL) has been utilized to make a variety of nanoparticles, including metals, oxides, alloys, semiconductors and functionalized nanomaterials [5]. The basic way that PLAL makes nanomaterials can be shown by the steps: laser-matter interaction (laser pulse absorbed by a material), formation of a plasma plume, expansion and cooling, production of shock waves, formation of bubbles, expansion and collapse, and release of nanomaterials in the liquid medium [6, 7]. PLAL has a number of advantageous features, It is a straight-forward, one-step technology that is effective for producing vast quantities of nanoparticles dispersed in

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liquid [8]. Metal particles' bactericidal effect has been related to their small size and high surface to volume ratio, which permit them to interact closely with microbial membranes [9]. Antibacterial activity of copper based nanoparticles has been reported against different pathogens by in numerous studies [10, 11]. The aim of this research is to use pulsed laser ablation on a bulk copper target to create and characterize the prepared copper nanoparticles in liquid. The major outcomes needed are the composition, size, shape, and the antibacterial activities of colloidal CuNPs against bacteria (*Escherichia coli*) and (*Staphylococcus aureus*).

MATERIALS AND METHODS

The copper nanoparticles were synthesized through the laser ablation technique in liquid from copper target as shown in Fig. 1. The setup consist of Q-switched Nd: YAG nanosecond laser source with a fundamental wavelength of 1064nm, of pulse duration of 10ns, and a repetition rate of 6Hz to create plasma from bulk copper target. Copper target of 99.99 % purity of 2×2 dimension and 2 millimeters thickness was used. The target was rinsed at 1 cm depth in deionized water. A quartz lens with 10 cm focal length focuses the laser beam perpendicularly onto the target's surface. Different laser pulse energies of 600, 700, and 800 mJ were used, at a focal length of 10 cm distance. A drop-casting technique was used to the colloidal copper nanoparticle solution on a glass substrate and drying it at 60 °C to examine the structural and morphological properties of the prepared nanoparticle. The X-ray diffraction (Shimadzu XRD 6000), scanning electron microscopy (JSM-7600F by JEOL Ltd), and UV-visible absorption (SP-8001 spectrophotometer) assay were used to examine the obtained CuNPs by laser ablation.

The antibacterial analysis using colloidal solution of copper nanoparticles, prepared at different laser energies, was evaluated by diffusion-well method on Mueller-Hinton agar against *Escherichia coli* and *Staphylococcus aureus*. To prepare the tested bacterium, young culture colonies (18–24 h) of the isolates bacteria were transferred to a 5 ml tube of normal saline to provide density of about 1.5×108 cells/ml equal to the No. 0.5 McFarland standard solution measured at 600 nm. The bacteria were supplied from the Environmental Laboratory/ College of Science/University of Baghdad.

After solidification of the MH media, 6 mm diameter wells were done by a cork borer. Bacterial culture spreader on each agar plate. For each well, 100 µl of the colloidal solutions of copper nanoparticles produced with laser energy of 600, 700, and 800 mJ are injected directly in the well for each type of bacteria. The plates were incubated for 24 hours at 37°C. After incubation, the zone of inhibition was determined.

RESULTS AND DISCUSSION

The XRD diffraction patterns show polycrystalline structure of cubic copper phase.

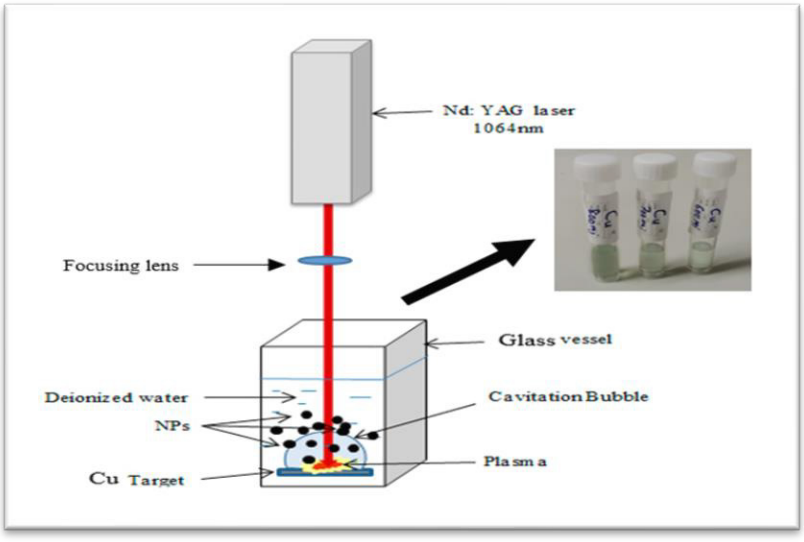

Fig. 1. Experimental setup of PLAL technique

The crystllinity enhanced with increasing the laser energy. The diffraction peaks located around 43.0 °, 50.4 °, and 74.5 ° corresponding to the diffraction peaks for CuNPs matches the planes have Miller indices of (111), (200), and (220) corresponding to the JCPDS standard card No. (96-901-1605). The preferred orientation along the (111) direction, which has the highest intensity as seen in Fig. 2. The crystallinety increased with increasing laser energy as a result of increasing the sputtered material.

The broad feature indicates on Nano crystalline structures. The line broadening were determine by Lorentzian fit and broadening errors were eliminated corresponding to $K_{\alpha 2}$ and instrument broadening using Xpowder software, as shown in Fig. 3. It seems that the full width at half maximum (FWHM) of the (111) diffraction line decreased with increasing the laser energy.

The crystallite sizes (*D)* of the Cu nanoparticles were measured using the Debye-Scherer equation [12]:

$$D = k\lambda/\beta \cos\theta \quad (1)$$

Where: *k* is the structure factor (0.9), λ is the X-ray wavelength (1.5406 Å), β is the full width at half maximum (FWHM) of the diffraction line at the diffracting angle 2ϑ.

Table 1 shows the structural properties of CuNPs, such as inter-planarity and crystallite size. It also indicates that the experimental and standard inter-planer spacing are quite equivalent. Cu crystallite size increased from 9.9 to 18.9 nm, along the preferred orientation, with increasing he laser energy from 600 t0 800 mJ.

Fig. 4 illustrates the variation of lattice constant, crystalline size, and lattice strain with laser energy for the prepared CuNPs. It seems that the lattice constant increased with increasing the laser energy as a result of increasing the crystalline size which cause reduce the lattice strain affected by the nano-size [13]. Fig. 3-B indicate the opposite behavior of the crystalline size, and lattice strain.

Scanning electron microscopy (SEM) were

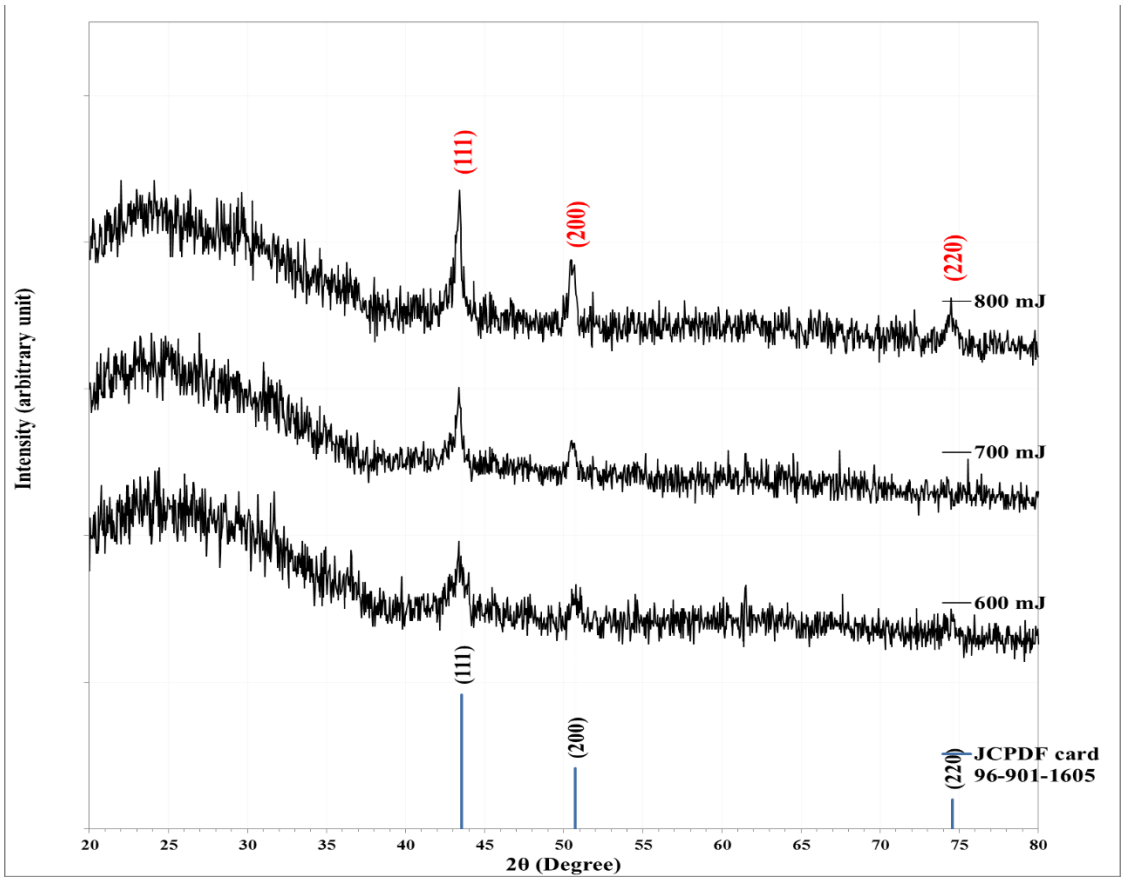

Fig. 2. The XRD spectrum of CuNPs for experimental pattern

used to examine the morphology of the prepared CuNPs utilizing the PLAL approach (Fig. 5). The SEM images revealed that the generated particles with nano size of nearly spherical shapes [14] with some agglomerations after drying process. Fig. 5 shows the morphologies of CuNPs produced by PLAL at laser energies of 600, 700, and 800 mJ.

The nanoparticle production was verified by the UV-visible absorption spectra of colloidal CuNPs. The absorbance spectra of the prepared CuNPs at different laser energies (600, 700, and 800 mJ), show broad band around (649–653) nm confirms the existence of localized surface Plasmon resonance (LSPR) absorptions related to the Nano sized as shown in Fig. 6 [15]. Increasing the laser energy cause slightly red-shift of the absorption peak indicate on increasing the particle size [16]. In addition, the Plasmon peak height increases with increasing laser power, indicates the presence of more nanoparticles (NPs) in the sample.

The antibacterial effect of copper nanoparticles prepared by the laser ablation method (PLAL) was investigated against human pathogens *Escherichia coli* and *Staphylococcus aureus*. The determination is based on the diffusion well method. Bacterial cell growth enhances the turbidity of a liquid nutrient medium because microbes have a higher refractive index than water, thus scattering incident light, while the inhibition zone of the agar appears as a more clear media. The inhibition was very distinct for all samples as shown in Fig. 7, as well as it was observed that the inhibition diameter

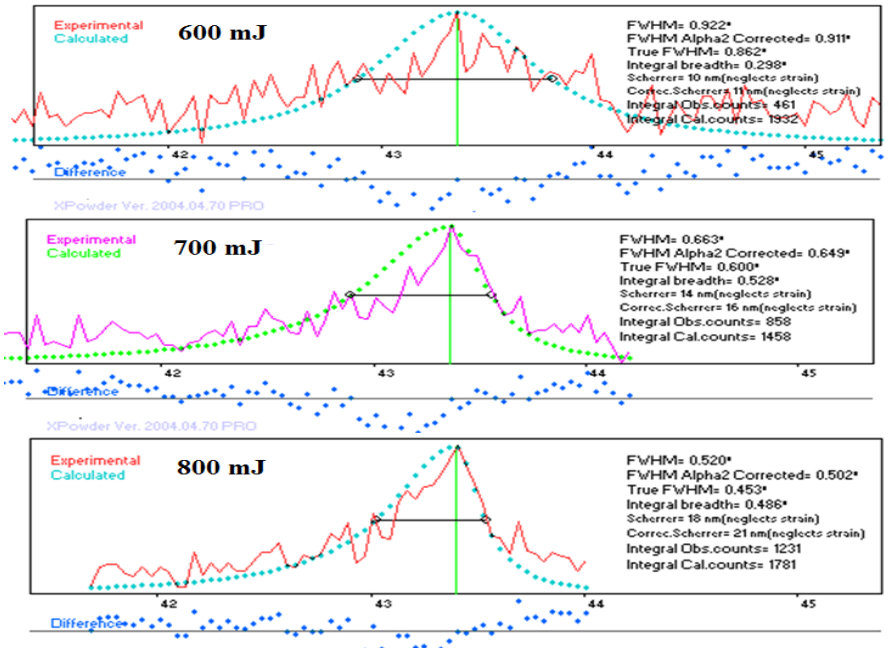

Fig. 3. The XRD spectrum of CuNPs for experimental pattern

Table 1. structural properties of CuNPs, such as inter-planarity and crystallite size.

E (mJ)	2θ (Deg.)	FWHM (Deg.)	d_{hkl} Exp.(Å)	C.S (nm)	d_{hkl} Std.(Å)	hkl
600	43.2918	0.8620	2.0883	9.9	2.0346	(111)
700	43.1516	0.6000	2.0947	14.2	2.0346	(111)
	50.6200	0.5400	1.8018	16.3	1.7620	(200)
800	43.0850	0.4530	2.0978	18.9	2.0346	(111)
	50.4480	0.5200	1.8075	16.9	1.7620	(200)
	74.5110	0.5430	1.2724	18.4	1.2717	(220)

increased as the laser energy increased for the two gram-negative and positive bacteria types as a result of increasing the sputtered particles yield with increasing the laser energy. Table.2 shows the inhibition zone diameters for Escherichia coli and Staphylococcus aureus due to the effect of CuNPs. The size and surface area of the CuNPs are crucial in the high efficiency of these particles. Copper has the potential to interfere with cellular activity in a variety of ways because it may prevent bacteria from developing copper resistance through a number of processes that may act in concert [17].

The release of copper ions in a liquid medium is an essential role to eliminate bacterial growth. The surface area that can interact with the surrounding medium determines the amount of emitted ions. In comparison to larger particles, nanoparticles are more effective against bacteria due to their large surface area. Because copper ions have a powerful reduction ability, they can kill bacteria by disordering their cell walls and membranes [18].

Gram-positive bacteria (*S. aureus*) and gram-negative bacteria (*E. coli*) have different characteristics, so they differ in their resistance to antibacterial agents [19]. It has been observed that the antibacterial activity against Gram-negative bacteria is generally stronger than Gram-positive bacteria. Although Gram-negative bacteria are often thought has more resistant to antibiotics and antibacterial agents than *E. coli*,

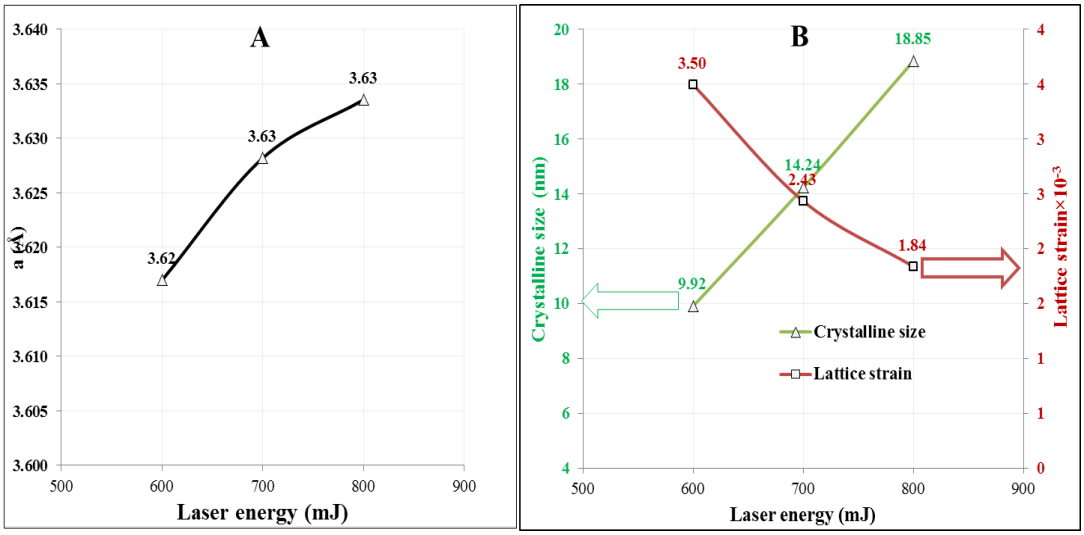

Fig. 4. Variation of XRD parameters with laser energy (A) lattice constant (B) crystalline size and lattice strain for CuNPs.

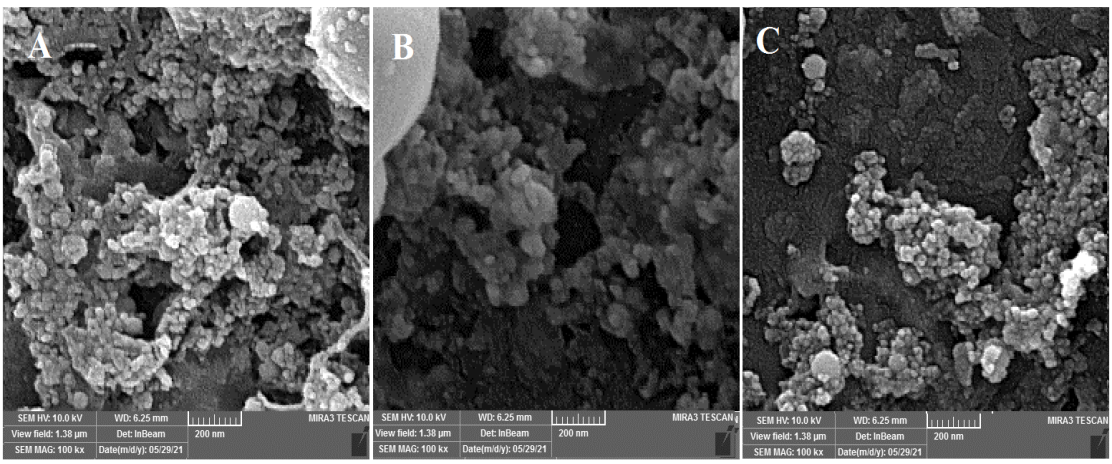

Fig. 5. SEM images of CuNPs synthesized by PLAL technique at different laser energies (A) 600, (B) 700, and (C) 800 mJ.

this bacteria may allow more Cu^{2+} ions to enter the plasma membrane [20]. The excess of carboxylic groups in the lipoproteins at the bacterial surface, which, upon dissociation, makes the cell surface

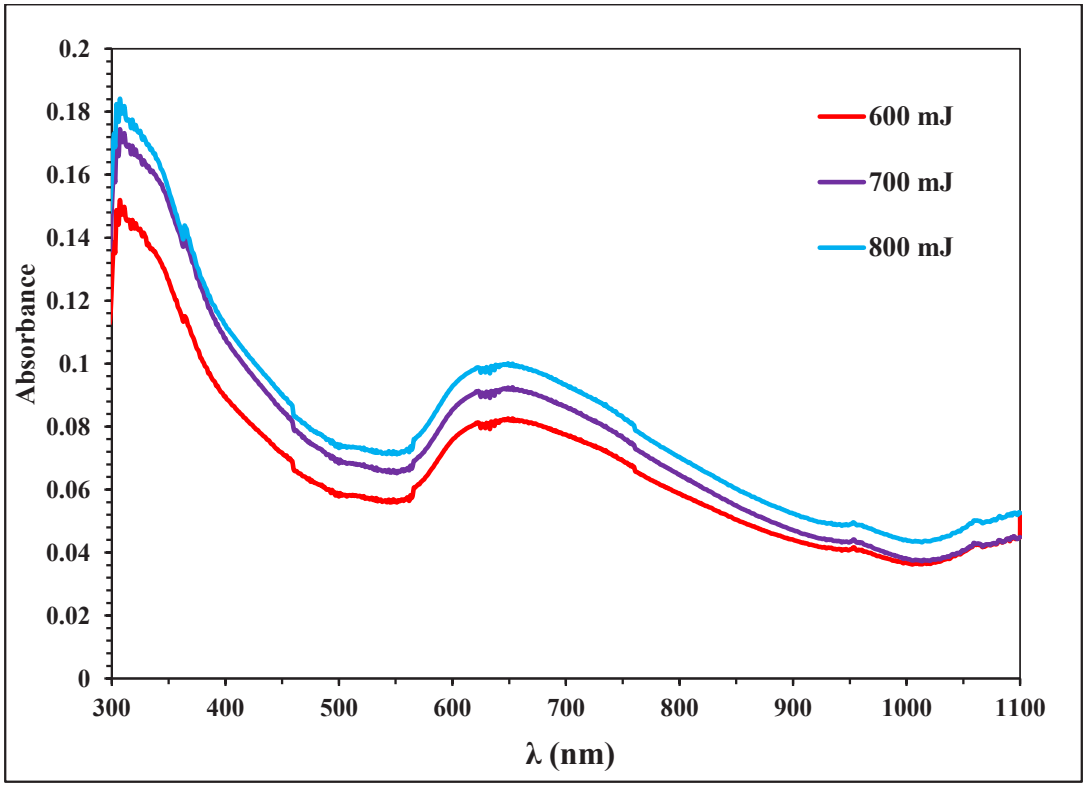

Fig. 6. UV-visible absorbance for CuNPs synthesized by PLAL technique using different laser energies

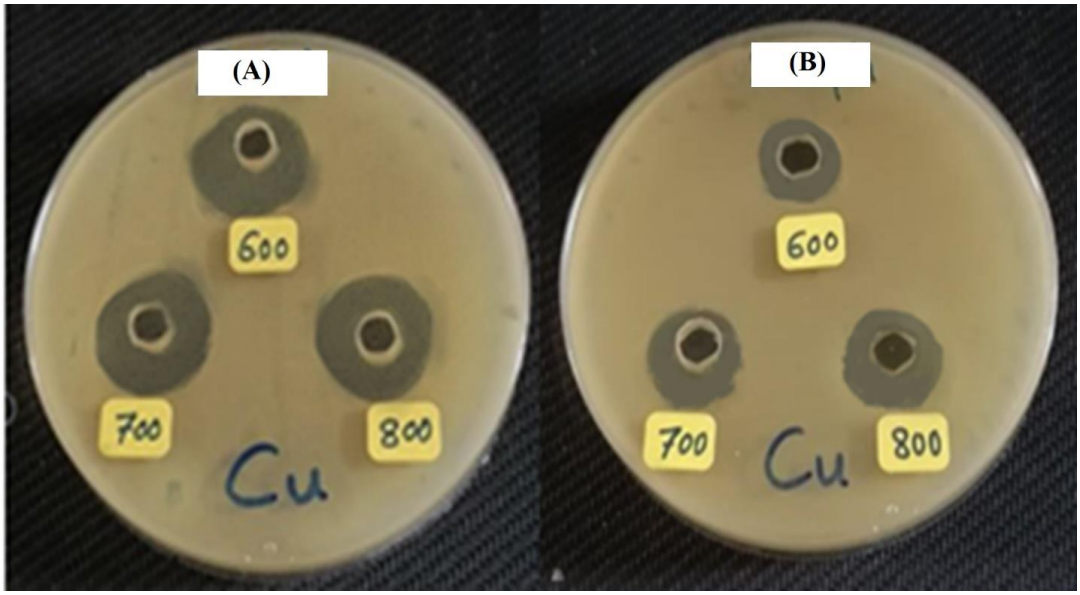

Fig. 7. The inhibition effect of CuNPs for (A) *E. coli* and (B) *S. aureus*.

Tale 2. Diameter of inhibition of CuNPs against *S. aureus* and *E. coli* for different laser energies

Bacteria	Laser energy (mJ)	Inhibition Zone (mm)
E. coli	600	18
	700	20
	800	21
S. aureus	600	13
	700	15
	800	16.5

negative, is what causes E. coli cells to have a negative overall charge [21]. Electrostatic forces are hypothesized to be responsible for causing adhesion and bioactivity between bacteria and copper ions produced by nanoparticles due to their opposing charges [22].

These ions attach to the cell wall and some of them enter inside the cell. Due to the strong tendency of the Cu^{2+} ions to interact electrostatically with the plasma membrane and then permeate the cellular membranes by opening or closing borins of the membrane. As a result, intracellular ions and low molecular weight metabolites seep out of the cells as a result of altering the cell membrane permeability [23]. In addition, the presence of copper nanoparticles in the growing media immobilizes and inactivates bacterial cells, which hinders their ability to replicate and leads to cell death. The inhibitory mechanism can also be explained by the fact that copper nanoparticles, which have a high tendency to react with substances containing phosphorus and sulfur, including deoxyribonucleic acid (DNA), cause degeneration that eventually results in protein denaturation and thus cell killing [24].

CONCLUSION

Copper nanoparticles (CuNPs) were prepared by simple physical technique using Q-switched Nd: YAG laser with different laser energies to examine the effect of laser energy on the prepared NPs properties and in turn its effect on its activity against two types of bacteria. All tested indicate the formation of nanoparticles with variety properties depend on the laser energy. The diffusion well technique against gram-negative and gram-positive bacteria indicates on the high activity of the prepared CuNPs, but with higer effect against *E. coli* than *S. aureus*. The nanoparticles created in this way have antibacterial properties which might be beneficial in the creation of high effective antibacterial agents. It is hoped that in the future copper nanoparticles will replace some antibiotics against pathogenic bacteria on medical equipment.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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