

RESEARCH PAPER

Preparing Copper Oxide Nanoparticles by Laser Ablation at Different Energies, Characterizing Them, and Studying Their Antibacterial and Antifungal Activity

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ARTICLE INFO

Article History:

Received 27 July 2026

Accepted 04 October 2026

Published 01 January 2027

Keywords:

Antibacterial Activity

Copper oxide

FESEM

Pulsed laser ablation (PLA)

XRD

ABSTRACT

In this study, copper oxide nanoparticles were prepared using laser ablation in liquid as a rapid and environmentally friendly method using a 532 nm Q-switched Nd:YAG laser for 500 pulses of varying energies (440, 520, and 600 mJ) were used to investigate the effects of these nanoparticles on different types of fungi and bacteria such as (*Candida albicans*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Klebsiella sp.*). The results of X-ray diffraction (XRD), atomic force microscopy (AFM), field emission scanning electron microscopy (FE-SEM), UV-Vis spectroscopy, and Fourier transform infrared spectroscopy (FTIR) confirmed the successful formation of the copper oxide nanoparticles and showed that increasing the laser energy directly modifies these properties, thus giving the prepared materials high efficacy as antibacterial and antifungal agents.

How to cite this article

Mahdi Z., Hassan N., A. Shatti W., Salh A., Sadiq Z., Abdulrahman H. Preparing Copper Oxide Nanoparticles by Laser Ablation at Different Energies, Characterizing Them, and Studying Their Antibacterial and Antifungal Activity. J Nanostruct, 2027; 17(1):12-23. DOI: 10.22052/JNS.2027.01.002

INTRODUCTION

Semiconductor metal oxide nanoparticles have emerged as essential building blocks in advanced nanotechnology due to their versatile physical and chemical properties. These nanostructures possess remarkable characteristics, including high ductility at elevated temperatures, nonlinear optical behavior, high sensitivity, cold welding capability, unique catalytic activity, and super paramagnetic performance. Moreover, their high diffusivities and outstanding adsorptive capacities make them very attractive for a wide variety of advanced applications [1- 3]. Cupric oxide (CuO) is one of the main p-type semiconductors with a narrow energy band gap among the transition

metal oxides [4–7]. It has a stable monoclinic crystal structure and exhibits excellent photo catalytic, photovoltaic and thermal transport properties with high antimicrobial efficiency [8–10]. Hence, CuO nanoparticles are well known for their extensive applications such as antibacterial agent, photo catalytic degradation of antibiotics [10, 11], high efficiency thermal conductors [12], magnetic recording media [13], gas sensors [14], solar cell devices [15], and energy storage systems [16, 17]. Despite the progress achieved in the synthesis and self-assembly of nanomaterials, the challenge to achieve a controlled and reproducible synthesis route still exists. Commonly used chemical and physical methods for the preparation of copper

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oxide nanoparticles suffer from mixed phase formation, complicated synthetic procedures and poor structural control, which limit their efficiency and purity. To overcome these limitations Pulsed Laser Ablation in Liquid (PLAL) offers a simpler, faster and highly efficient route for the synthesis of high purity nanoparticles [18, 19]. The ablation of a solid target immersed in a liquid medium yields a stable colloidal dispersion of nanoparticles. This technique has several advantages over conventional methods such as short reaction times, operation under mild conditions, complete elimination of chemical precursors and harmful by-products, single step synthesis and superior product purity. In light of these advantages [20,

21], the primary objective of this research is to synthesize copper oxide nanoparticles via the laser ablation in liquid technique and systematically investigate their structural, morphological, optical, and functional group properties. Furthermore, the study evaluates the biological and antibacterial efficacy of the synthesized nanoparticles against various pathogenic bacterial strains.

MATERIALS AND METHODS

Copper Target Preparation

8 grams of copper powder were accurately weighed and compressed using a 5-ton hydraulic press for one hour to form a compact copper target. The compressed copper granule was used



Fig. 1. Representing the target before and after drying.

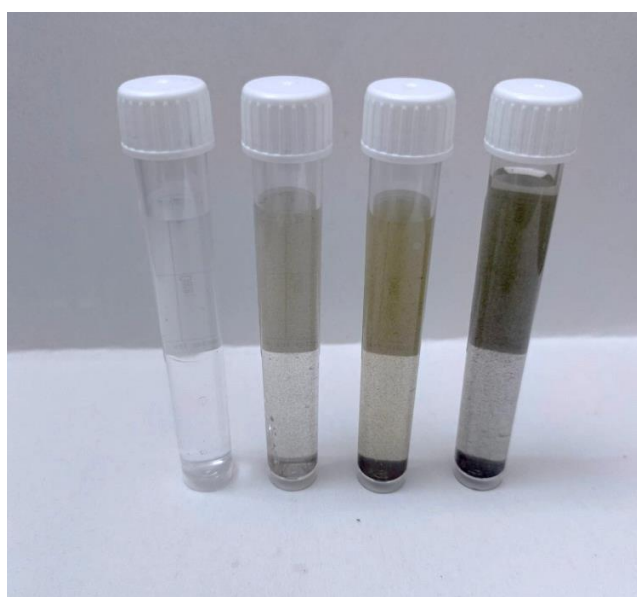


Fig. 2. represents the copper nanoparticle solution after laser ablation at laser energy (440,520,600) mJ.

as the target material for laser ablation. The target was then dried for two hours at a temperature of 200 degrees Celsius, as shown in Fig. 1.

Liquid Medium Preparation

Deionized water was used as the liquid medium for pulsed laser ablation. A suitable volume of 20 mL of deionized water was placed in a clean glass container, and the prepared copper target was completely immersed in the liquid.

Pulsed Laser Ablation in Liquid (PLAL)

The copper target was ablated using a pulsed Nd:YAG laser operating at a wavelength of 532 nm. The laser pulse energy was varied to 440, 520, and 600 mJ, for 500 pulse while the number of laser pulses was fixed at 500 per experiment. The laser beam was focused onto the surface of the submerged copper target to induce ablation and nanoparticle formation in the surrounding deionized water.

Drip Precipitation Procedure

Three colloidal solutions prepared using pulsed laser ablation in liquid (PLAL) with different laser energies were used to deposit thin films. The prepared solutions were precipitated at 50 °C to ensure stable precipitation conditions. A clean glass substrate was placed on a suitable horizontal surface, and five drops of each colloidal solution were carefully applied to the substrate using a micropipette. The drops were distributed across the substrate surface to achieve relatively uniform coverage, as shown in Fig. 3.

After precipitation, the samples were allowed to dry under controlled conditions until the solvent had completely evaporated, resulting in the formation of thin films on the substrate surface. The thickness of each deposited film was

measured and recorded (0.76, 0.82 and 0.93) μm which corresponds to the different laser energies (440, 520, 600) mJ used during the PLAL process, respectively.

Nanoparticle Formation

During laser irradiation, the high laser energy caused localized melting and vaporization of the copper target, followed by rapid cooling and condensation within the liquid medium. This process resulted in the formation of copper nanoparticles dispersed in the deionized water. Colloidal solutions obtained at different laser energies were collected separately for further analysis.

Post-processing of colloidal solutions

The prepared films were deposited onto a glass surface using thermal spraying, with a thickness not exceeding 1 micrometer.

Characterization of nanoparticles

The prepared nanoparticles were characterized using X-ray diffraction (XRD) to determine the crystal structure, atomic force microscopy (AFM) and field emission scanning electron microscopy (FESEM) to determine surface morphology and particle size, Fourier transform infrared spectroscopy (FTIR) to determine chemical bonds, and ultraviolet-visible spectroscopy (UV-Vis) to determine optical properties.

Antibacterial and Antifungal Activity

The biological activity of prepared copper nanoparticles against selected bacterial and fungal strains (*Candida albicans*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Klebsiella sp.*) was evaluated using the well diffusion method on agar agar. Suitable microbial cultures were prepared and



Fig. 3. Drop-casting method.

homogeneously inoculated onto appropriate agar media. Wells were then prepared in the inoculated agar plates, and different concentrations of the prepared nanoparticle suspensions were added using laser pulse energies of 440, 520, and 600 mJ. The plates were incubated under conditions suitable for the growth of the tested microorganisms. After incubation, the diameter of the inhibition zones (mm) surrounding each well was measured and recorded as an indicator of antimicrobial activity. A suitable positive control sample (standard antimicrobial agent) and a

negative control sample (deionized water) were included for comparison. The antibacterial and antifungal activity was subsequently compared as a function of the laser pulse energy and nanoparticle concentration.

RESULTS AND DISCUSSION

Structural and Morphological Properties

Fig. 4 shows the X-ray diffraction (XRD) patterns of the fabricated samples at different laser energies (440, 520, and 600 mJ). At the initial energy of 440 mJ, a single, low-intensity

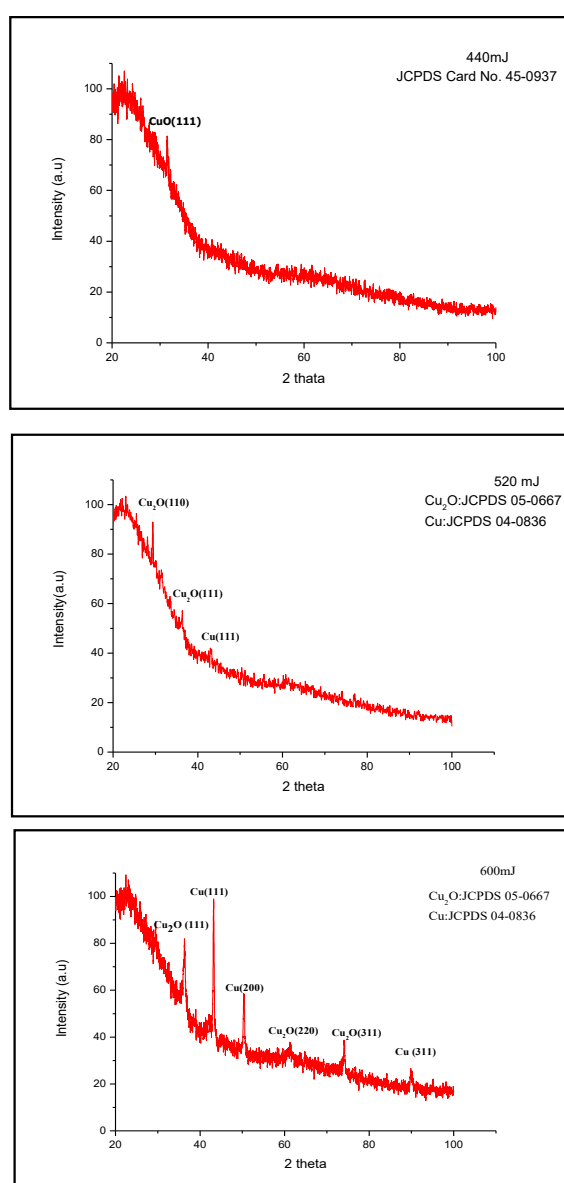


Fig. 4. XRD pattern for copper oxide at 440, 520 and 600 mJ

diffraction peak was observed at $2\theta \approx 32.5^\circ$, which corresponds to the (111) crystalline plane of monoclinic copper oxide (CuO), and is in perfect agreement with JCPDS card 45-0937 [22,23]. The presence of this single, weak reflection indicates that the energy of 440 mJ did not provide sufficient

thermodynamic energy for full crystallization, resulting in a weakly crystalline or nanocrystalline monoclinic copper oxide (CuO) phase embedded within a predominantly amorphous matrix [22,24]. Upon increasing the laser energy to 520 mJ and 600 mJ, a clear phase evolution toward

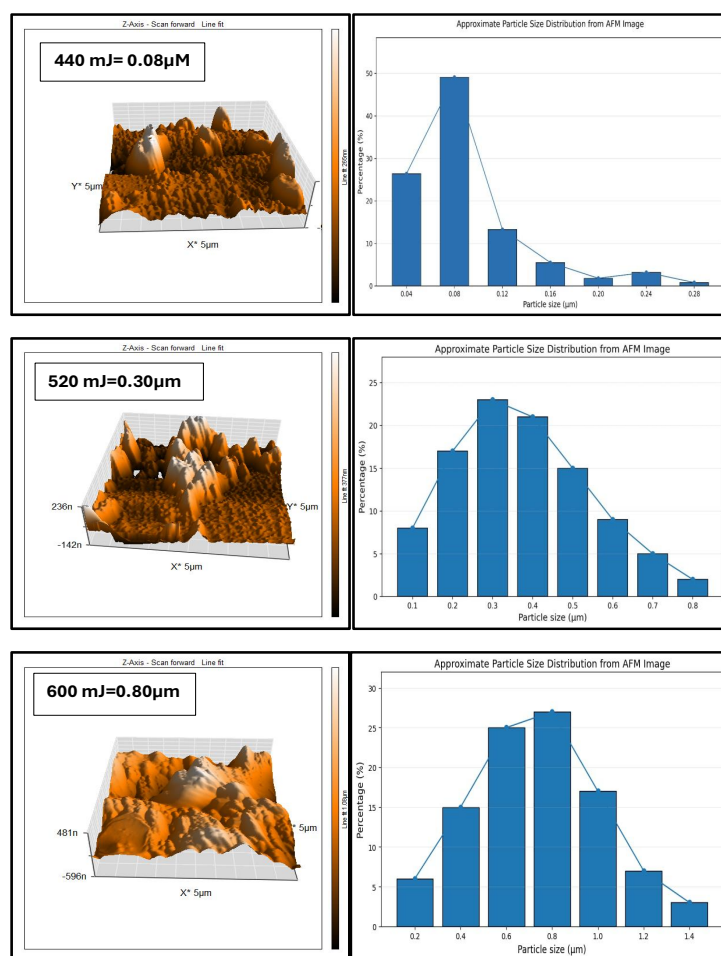


Fig. 5. 3D AFM images of for copper oxide and particle size distribution at 440, 520, and 600 mJ.

Table 1. XRD structural parameters and calculated crystallite sizes of the samples synthesized at different laser energies (440, 520, and 600) mJ.

Laser Energy (mJ)	Number of peaks	2 θ (deg)	FWHM (deg)	hkl	D (nm)
440	1	31.4	0.4399	CuO (111)	18.75
520	1	29.5	0.1217	Cu ₂ O (110)	67.5
	2	36.3	0.4399	Cu ₂ O (111)	20.7
	3	43.1	0.2229	Cu (111)	42.5
600	1	36.2	0.4399	Cu ₂ O (111)	20.7
	2	43.1	0.2229	Cu (111)	42.9
	3	50.3	0.2196	Cu (200)	44.9
	5	61.2	0.2160	Cu ₂ O(220)	42.7
	6	73.9	0.7164	Cu ₂ O(311)	8
	7	89.8	0.4938	Cu (311)	24

a two-phase mixture of monoclinic copper oxide (Cu_2O) and metallic copper (Cu) was observed [22,25]. The primary oxide phase transforms into cubic monocopper oxide (Cu_2O), which is confirmed by the diffraction reflections from the (110), (111), (220), and (311) levels. Notably, the high-intensity reflection at $2\theta = 29.5^\circ$ (110) shows a narrow half-height width (FWHM) of 0.1217° , indicating a much larger average crystal size of 67.5 nm [22]. Simultaneously, the characteristic diffraction peaks appearing at $2\theta = 43.1^\circ$, 50.3° , and 89.8° are attributed to the (111), (200), and (311) levels of face-centered cubic (FCC) metallic copper, respectively. The formation of the metallic copper phase is attributed to laser-stimulated photoreduction during the ablation process. The metallic copper bands Cu(111) and (200) exhibit enhanced structural regularity, with crystal sizes ranging from 42.5 nm to 44.9 nm. In contrast, the higher FWHM value of 0.7164° for the Cu₂O(311) reflection at $2\theta = 73.9^\circ$ leads to a sharp decrease of the crystal size to 8 nm, which is related to the localized micro-stress and structural defects in the oxide lattice [22,24]. The mean crystal size (D) at all levels of reflectance found at different energies was calculated using the Debye-Scherrer equation [26] and summarized in Table 1 [22].

$$D_{hkl} = k\lambda / (\beta_{hkl} \cos \theta) \quad (1)$$

The surface morphology, grain size distribution, and topographic surface roughness parameters of systematically synthesized copper oxide nanostructures were investigated using atomic force microscopy (AFM). Fig. 5 shows three-dimensional topographic images acquired by AFM, along with particle size distribution plots for samples prepared with different laser energies. A significant increase in particle size was observed, rising from $0.08 \mu\text{m}$ to $0.80 \mu\text{m}$, with increasing laser energy [27,28]. This dimensional expansion is primarily attributed to the increased plasma column density, which increases the particle collision rate and promotes thermal fusion and agglomeration during the cooling phase [28]. Simultaneously, both surface roughness parameters (Ra and RMS) showed a clear upward trend with increasing laser energy [29,30]. This behavior is attributed to the formation of coarse grain clusters and the redeposition of molten droplets ejected by the instability of high-energy shock waves [29].

Fig. 6 field-emission scanning electron microscopy (FE-SEM) images reveal a clear energy-dependent morphological shift in copper oxide nanoparticles synthesized by laser ablation [22, 31]. At an initial energy of 440 mJ, the nanostructures exhibit a spherical shape with a broad size distribution and marked agglomeration, resulting

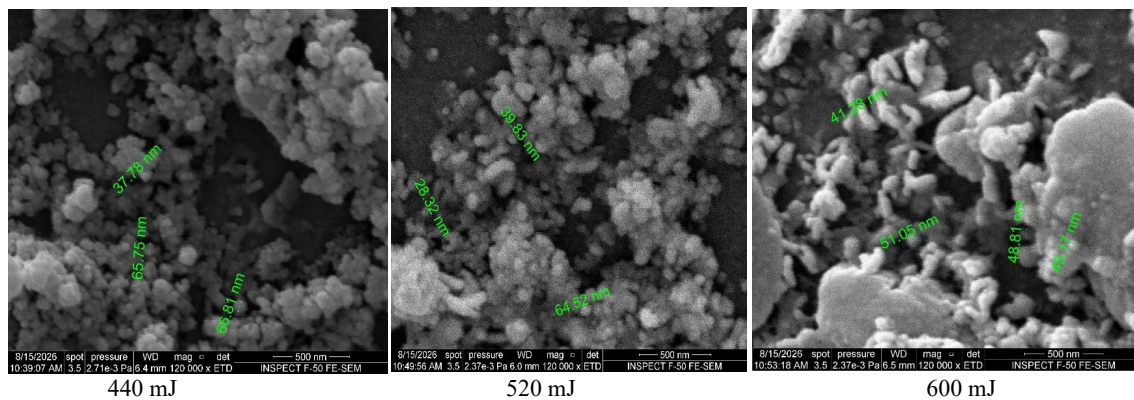


Fig. 6. FE-SEM images of copper oxide at 440, 520, and 600 mJ at a range of 500 nm.

Table 2. AFM Results of Copper oxide.

Laser Energy (mJ)	Particle size (μm)	Roughness average (nm)	Root Mean Square (nm)
440	0.08	44.046	55.059
520	0.30	58.696	77.141
600	0.80	120.18	154.66

in an average particle size of 56.78 nm [22]. Increasing the laser energy to 520 mJ enhances the electro thermal breakdown and secondary laser-induced fragmentation within the plasma column, sharply reducing the average particle size to a minimum of 44.20 nm with more pronounced granular boundaries [22, 31]. However, a further increase of the energy to 600 mJ leads to a slight re-expansion of the mean particle diameter to 47.57 nm, and appearance of nano-agglomerates

and chip-like properties [22, 31]. This behavior is due to the high plasma density and thermal fusion of the molten active species in the cavity bubble before cooling [31].

Optical Properties

The optical properties and electronic transitions of the synthesized copper oxide nanoparticles were studied at different laser ablation energies (440 mJ, 520 mJ and 600 mJ) as shown in Fig.

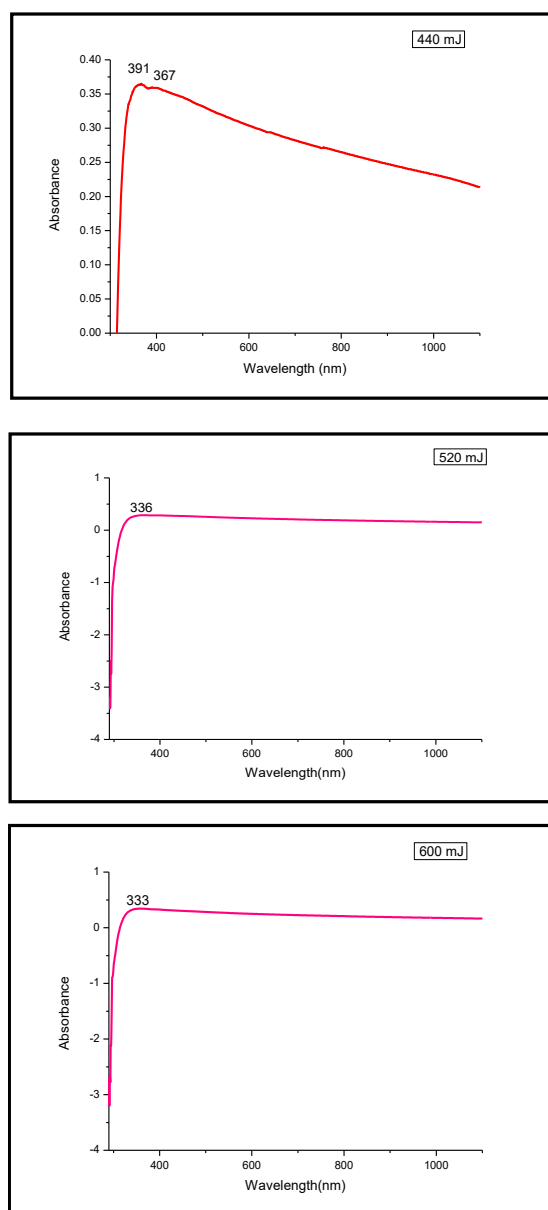


Fig. 7. UV-vis absorbance spectra of copper oxide at 440 mJoxide at 440, 520, and 600 mJ.

7. The absorption spectrum at 440 mJ shows a strong ultraviolet band with a major peak at 391 nm and a minor peak at 367 nm. These properties are attributed to the direct electronic transition between energy bands and the transition from the valence band to the conduction band in the quantum confinement effects range of 350-400 nm [32]. Moreover, the broad absorption tail covering the visible and near-infrared regions (400-1000 nm) is mainly caused by the surface defect states and particle aggregation in the aqueous

suspension. The absorption peaks exhibited a distinct blueshift upon increasing the laser power to 520 and 600 mJ from 391 nm to 336 nm and 333 nm, respectively [33]. High laser power density promotes fragmentation of particles into smaller copper oxide nanoparticles. This size reduction increases the quantum confinement effect thereby increasing the optical band gap and blue shifting the electronic transitions. These findings together support the idea that the optical properties of the quantum confinement semiconductor structure

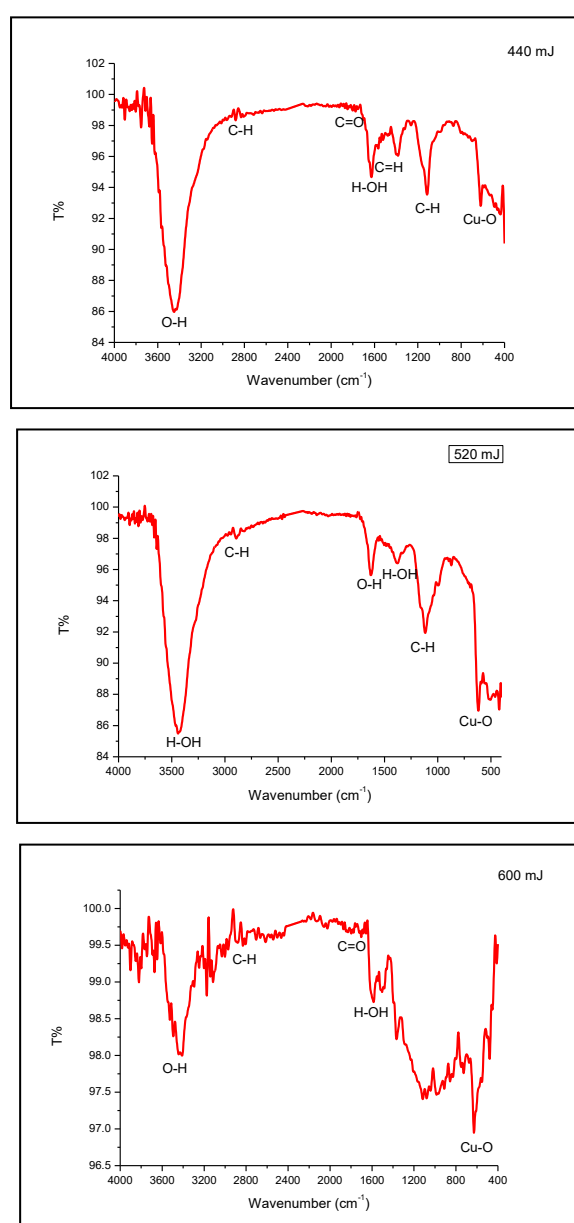


Fig. 8. FTIR spectra of copper oxide at 440, 520 and 600 mJ.

are governed by direct transitions between the energy bands [25].

FTIR Spectra Analysis and Effect of Laser Energy

The functional groups of copper oxides synthesized by the liquid-state laser ablation at three different energies (440, 520 and 600 mJ) in the range of 4000 to 400 cm^{-1} wavenumber were identified by Fourier transform infrared spectroscopy (FTIR). The influence of laser energy density on the chemical bonds is shown in Fig. 8. Hydroxyl Groups (O–H, H–OH) Stretching and Bending: A dominant broad absorption band appearing at about 3400–3440 (cm^{-1}) is attributed to the stretching vibrations of hydroxyl groups (O–H) and physically adsorbed water molecules on the surface of the nanoparticles [34]. Adsorbed water (H–OH) exhibits a characteristic peak at 1620–1640 (cm^{-1}) corresponding to the bending mode [35]. Interestingly, the higher laser energy of 600 mJ results in a dramatic drop in the transmittance intensity of these broad bands which is indicative of a decrease in surface absorbed moisture due to higher thermal ablation temperature. Carbon functional groups from impurities (C–H and C=O groups): The secondary bands at 2880–2970 cm^{-1} are attributed to the symmetric and asymmetric stretch of C–H bonds, while the peaks at 1110–1120 cm^{-1} are caused by the C–H bending or C–O single bond stretch [36]. In addition, a small peak near 1700 cm^{-1} (C=O) was observed at 440 and 600 mJ, which is assigned to the absorption of small amounts of atmospheric carbon during the synthesis process. The formation of the metal-oxygen (Cu–O) bond in the low frequency region below 700 cm^{-1} clearly indicates the copper oxide crystallization. The characteristic vibrational patterns of Cu–O bonds in copper oxide nanoparticles are observed as peaks in the range of 420–630 cm^{-1} [37]. The vibrational peak around 600–620 cm^{-1} confirms the formation of monoclinic (CuO) phase [38]. The effect of the laser power boost (440, 520 and 600 mJ) was observed as a drastic change of the spectral signature

region (Cu–O band). The Cu–O absorption curve at 600 mJ showed a distinct splitting of the peak and an increase of sharpness in comparison with lower energies. This structural change was due to the increased plasma temperature and the higher plasma energy produced at higher laser energies which helped to achieve better crystal arrangement, phase transition and grain growth of CuO nanostructures [39].

Antibacterial Activity

The antimicrobial efficacy of CuO NPs showed a clear dose and strain dependent activity against the range of tested microorganisms as summarized in the Figs. 9–12 as evidenced by the variations in the diameters of the inhibition zones Table 3. In general, the inhibition zones increased with the laser irradiation energy increased from 440 mJ to 600 mJ. This trend is in agreement with the mode of action for bacterial killing by copper oxide nanoparticles whereby higher energy levels generate smaller and more uniformly distributed nanoparticles with a larger surface area to volume ratio and hence quicker release of active copper (Cu^{+2}) ions into the growth medium [40]. The inhibition zone of *Streptococcus pneumoniae* was the highest, increasing significantly from 10 mm at 440 mJ to a maximum of 20 mm at 600 mJ. The gram negative *Klebsiella* sp. was also observed to increase in a linear fashion from 13 mm to 17 mm. This strong action against bacterial species is due to the first electrostatic attraction between positively charged copper oxide nanoparticles with negatively charged surfaces of bacterial cell walls, particularly the lipopolysaccharide (LPS) layer in Gram-negative bacteria and teichoic acids in Gram-positive bacteria [41]. Once adhered, the continuous leaching of copper ions (Cu^{+2}) allows interaction with sulfur-containing proteins in the bacterial cell membrane (e.g. cysteine-rich thiol groups), disrupting the membrane integrity, inducing enzyme inactivation and destabilizing the transport mechanisms [42]. In addition, the copper ions (Cu^{+2}) that are introduced bind to the

Table 3. explain the antibacterial activity of copper oxide.

Sample	440 mJ	520 mJ	600 mJ
<i>Candida albicans</i>	/	13	14
<i>Staphylococcus aureus</i>	14	13	14
<i>Streptococcus pneumoniae</i>	10	16	20
<i>Klebsiella</i> sp.	13	15	17

phosphate groups in bacterial DNA and inhibit the replication and transcription cycles [43]. Conversely, *Candida albicans* did not exhibit any significant inhibition zone at 440 mJ, and higher energy thresholds (520-600 mJ) were required

to induce significant inhibition 13-14 mm. This low resistance at low concentrations is due to the structural complexity of the cell walls of eukaryotic fungi, which are made up of rigid networks of chitin and glucan that act as physical barriers to

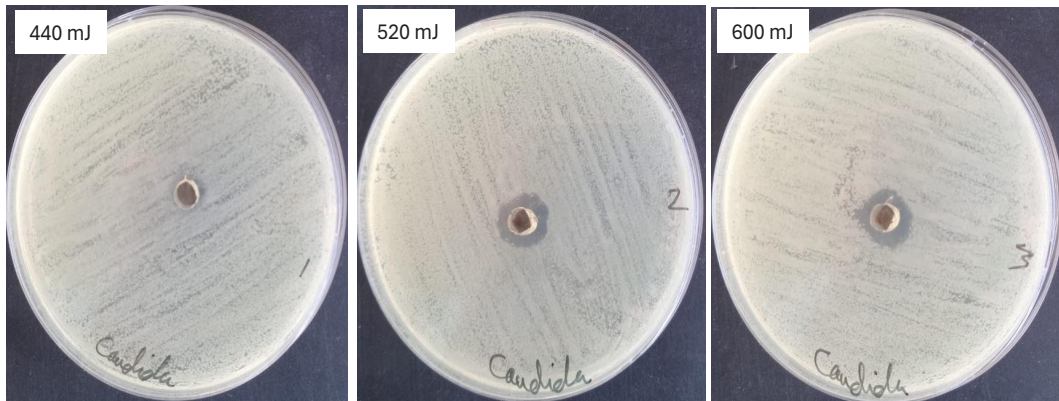


Fig. 9. Fungi activity of copper oxide against *Candida albicans* at 440, 520, and 600 mJ.

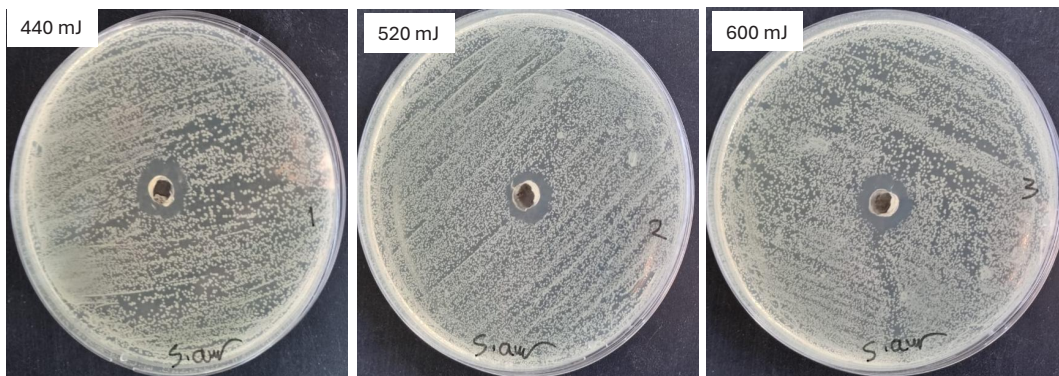


Fig. 10. Antibacterial activity of copper oxide against *Staphylococcus aureus* at 440, 520, and 600 mJ.

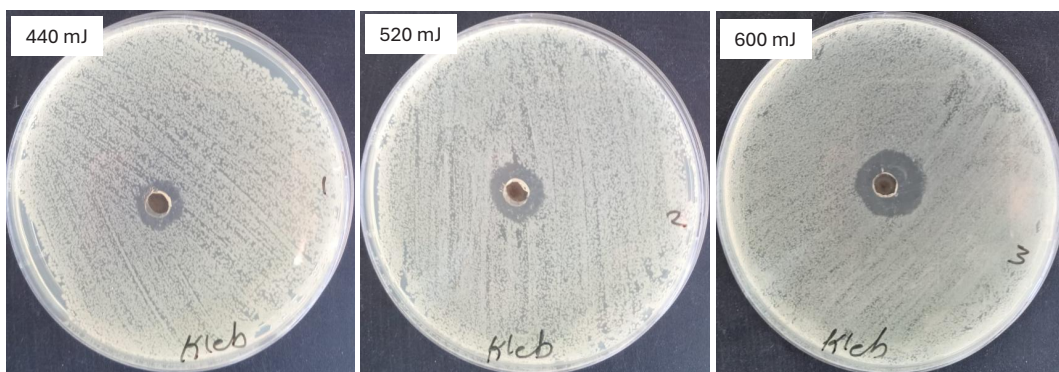


Fig. 11. Antibacterial activity of copper oxide against *Klebsiella sp.* at 440, 520, and 600 mJ.

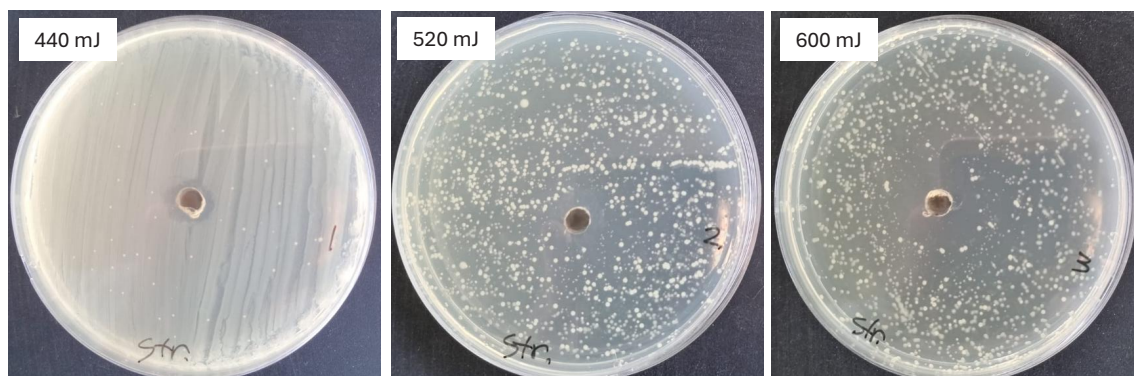


Fig. 12. Antibacterial activity of copper oxide against *Streptococcus pneumoniae* at 440, 520, and 600 mJ.

the penetration of nanoparticles [44]. However, at higher energy inputs (600 mJ), the increased flux of copper oxide nanoparticles (CuO NPs) generates severe oxidative stress and reactive oxygen species (ROS), which bypass the cell wall to damage membrane-associated organelles—specifically mitochondria—leading to mitochondrial membrane depolarization and apoptosis [44, 45]. At the same time, *Staphylococcus aureus* maintained a relatively stable inhibition zone (13–14 mm), reflecting a threshold saturation effect that is often observed in dense peptidoglycan matrices [41].

CONCLUSION

This study concludes that the preparation of copper oxide nanoparticles using liquid laser ablation is an environmentally friendly and efficient method, starting from compressed copper oxide discs and depositing the nanoparticle solutions onto glass substrates via spin coating. Structural, morphological, and optical analyses (XRD, FE-SEM, UV-Vis, and FTIR) showed that varying the laser power across three different levels significantly affects the particle size, morphological aggregation, and optical properties of the nanoparticles, thereby enhancing their applicability as antimicrobial agents (against bacteria and fungi).

CONFLICT OF INTEREST

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

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