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Antibacterial Activity of Copper Oxide Nanoparticles against *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923

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ABSTRACT

Background: Bacteria are the smallest and most numerous organisms that can cause many serious human diseases. Objective: This work was carried out to study the antibacterial activity of CuO nanoparticles (CuO NPs) which have been prepared by laser ablation of a copper target immersed in deionized water using Q-switched pulsed Nd:YAG laser. Results: UV-Vis spectrophotometer exhibited two peaks of absorption spectra of CuO NPs colloidal one peak at uv-region (205 nm) and another peak at visible region (636 nm) and transmission electron microscopy (TEM) showed the morphology of prepared CuO NPs that were spherical and the particle size ranged from 10-100 nm. CuO NPs exhibited significant reduction on the viability of bacteria by reduction their optical density. Conclusion: Pulsed laser ablation in liquid was a successful method to produce metal NPs with suitable particle size and the antibacterial activity of CuO NPs against *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 was effective especially at high concentrations.

KEYWORDS: Q-switched pulsed Nd:YAG laser, *CuO NPs, antibacterial activity*

INTRODUCTION

Antimicrobial agents like metal NPs have been used to inhibit a wide range of microbes such as bacteria and fungi [1]. *Staphylococcus aureus (S. aureus*) is a pathogenic microbe causes a wide range of diseases which includes skin infections, surgical site infections, bacteraemia and pneumonia [2]. *Escherichia coli* (*E. coli*) is on the preponderant facultative anaerobes in the human gastrointestinal tract. *E. coli* strains provide health benefit but may be harmful to the host. A pathogenic *E. coli* can cause diarrheal disease, serious sequelae, urinary tract infection, meningitis, and septicemia[3,4]. Bacterium cell is surrounded by the plasma membrane, a lipid bilayer which contains opposing monolayers, of phospholipids with the hydrophilic head groups facing the extracellular and intracellular solutions, and the hydrophobic tails facing each other [5]. Metal NPs have individual characteristics that are not available in bulk materials. Uniqueness characteristics of NPs attributed to their size, structures, high surface-to-volume ratios (increased the located atoms at the grain boundaries) thus they show significantly novel physical, chemical, and biological properties. Metal NPs have been used as antimicrobial agent against bacteria and other microbes because of their possibility to penetrate the membrane of bacterial cell

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due to their potential toxicity mechanism such as diffusion, endocytosis and channel implication [5-7]. Metal NPs provided wide range of application in varied fields including solar energy conversion, catalysis, optics, medicine, biotechnology and environmental electronics[8]. CuO is much cheaper than silver oxide and can be mixed with polymers more easily to obtain composites with unique chemical and physical properties [9]. Moreover, the extremely high surface areas, unusual crystal morphologies and distinctive catalytic activity endow CuO NPs with antimicrobial activity, and they dose-dependently inhibit *E. coli* strains[9-14]. Even today, the exact mechanism of antimicrobial action of the Cu NPs remains unknown. The general view seems to be a combination of several factors: releasing Cu ions, their penetration and disruption cell membrane and biochemical pathway by chelating cellular enzymes and DNA damage [15].

MATERIALS AND METHODS

Preparation of Colloidal NPs:

Copper target in the form of square-shaped was fixed at the bottom of a glass vessel containing 5 ml of distilled de-ionized water as shown in Fig.1. Copper target (98.5% purity) was focused by Q-switched Nd: YAG laser (pulse duration=10 ns, wavelength=1064 nm ,repetition rate= 1 Hz, focal length =10 cm and the number of pulses ranged from 200 to 500 pulse) operating at 280 mJ and 25 mJ/cm^2 respectively. Digital weigher was used to weigh the target sample before and after the ablation to determine the concentration of NPs.

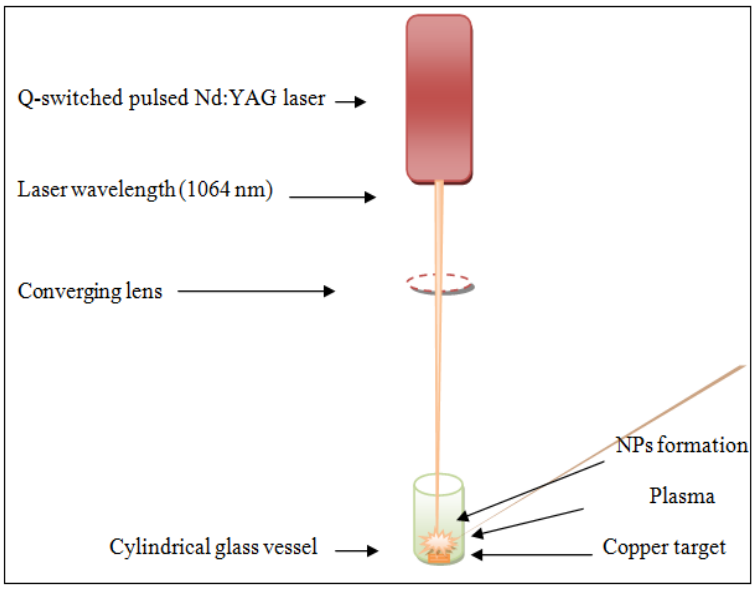

Fig. 1: experimental set up

Influence of CuO NPs on *E. coli* ATCC 25922 and *S. aureus* ATCC 25923

The antibacterial activity of CuO NPs were tested against *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 by broth dilution method [16]. *E. coli* ATCC 25922 was cultured in MacConkey agar while *S. aureus* ATCC 25923 was cultured and maintenance in mannitol salt agar. Both bacterial strains with 100 µl of 1× 10^6 cell/ml were inoculated in 5 ml of Muller Hinton broth (MHB) then (22, 45, 90) µg/ml and (100,200,300) µg/ml of prepared and standard CuO NPs were added, respectively . All tubes were incubated at 37° C for 24 hrs, bacterial growth was tested by measuring the optical density at 450 nm using UV-1100 spectrophotometers.

The inhibition rate of tested bacteria (%) was expressed as follows:

$$\text{Inhibition rate (\%)} = \left(\frac{\text{Control}-\text{Test}}{\text{Control}}\right) \times 100$$

Control = Bacterial absorbency at 450 nm wavelength.

Statistical Analysis

The data were statistically evaluated using ANOVAI for significance testing $p \leq 0.05$.

RESULTS AND DISCUSSION

Optical properties:

UV-Vis absorption spectrophotometer (Shimatzu SP8001) was used to determined the surface plasmon resonance (SPR) of the colloidal. When the laser beam incident on the immersed target, visible cloud was observed at the immersed target surface.

When the surface of the metal absorbed the laser radiation, high density plasma was formed followed by generation of shock waves propagated through the liquid and generation cavities bubble with high pressure, subsequently the cavities bubble exploded and NPs diffused in the liquid [17].

When laser pulses were enhanced the created particles increased and the solution changed to green color. UV-Vis spectrophotometer showed two peaks of absorption spectra in the ultraviolet region (205 nm) due to interband and in the visible region (636 nm) due to SPR shown in Fig 2. The result of absorption spectrum of CuO NPs prepared by laser agreed with reference[18].

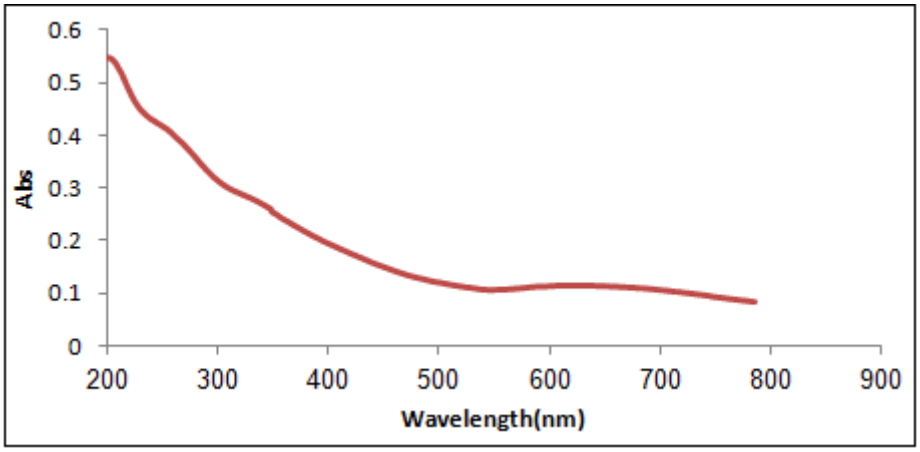

Fig. 2: The absorption spectra of CuO NPs at 200 pulse, repetition rate=1 Hz, λ=1064nm and 25 mJ/cm^2.

Transmission Electron Microscopy (TEM):

Particle size distribution and morphology of CuO NPs were characterized by Transmission Electron Microscopy (TEM) type CM10 pw6020, Philips-Germany**.** Figure 3 (a and b) shows particles shape (spherical shape) and distribution of particle size that ranged between 10 and 100 nm. The shape result of TEM image of CuO NPs agreed with references[19,20]

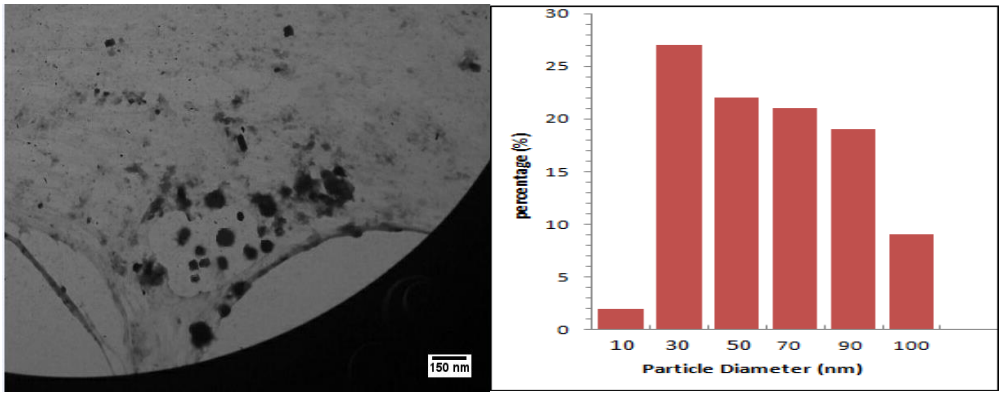

Fig. 3: The morphology and particles size distribution of CuO NPs with 500 pulse at 280jm, 1Hz and 1064 nm.

Antibacterial activity:

The bacterial optical density of bacterial species was measured using UV-Vis spectrophotometer as shown in Tables 1 and 2. The best suppressing bacterial strains growth was at high concentration of CuO NPs. It is recorded that CuO NPs have high antibacterial activity against both gram positive and gram negative strains of bacteria but their effect against gram negative bacteria was more than that in the gram positive bacteria as shown in Fig 4.

Table 1: Optical density of *E. coli* ATCC 25922 treated with different concentration of prepared and standard CuO NPs at 450 nm.

Con µg/mL	Optical density	Con µg/mL	Optical density
0.0	0.69±0.011	0.0	0.69±0.011
22	0. 41±0.01*	100	0.53±0.022*
45	0.39±0.01*	200	0.49±0.021*
90	0.29±0.01*	300	0.38±0.011*

- Each number represent M±SD for three replicates.
- * significant at (P≤0.05) .

Table 2: Optical density of *S. aureus* ATCC 25923 treated with different concentration of prepared and standard CuO NPs at 450 nm.

Con µg/mL	Optical density	Con µg/mL	Optical density
0.0	0.55±0.012	0.0	0.55±0.012
22	0. 52±0.02	100	0.50±0.02
45	0.44±0.01*	200	0.48±0.01*
90	0.39±0.01*	300	0.33±0.021*

- Each number represent M±SD for three replicates.
- * significant at (P≤0.05) .

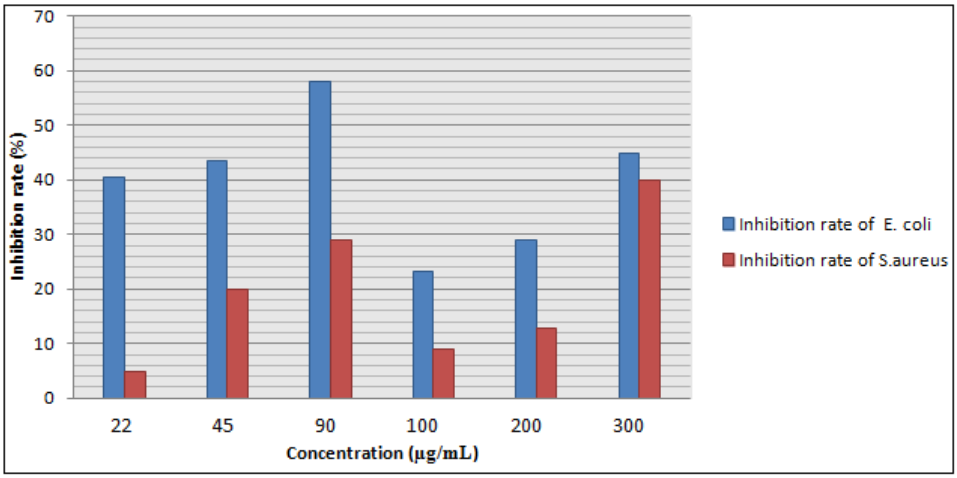

Fig. 4: Inhibition rate of tested bacteria with prepared and standard CuO NPs.

When NPs entering the cell, NPs can produce intracellular H_2O_2. This metabolite naturally produced via Cells. This is the reason why a specific mechanism occurs to counteract the presence of hydrogen peroxide for cell's detoxifying. Catalase is a tetrameric heme-containing enzyme, and is one of the key antioxidant enzymes show in almost every aerobic organisms, catalyzing the breakdown of hydrogen peroxide to water and molecular oxygen to protect cells against the toxic effects of hydrogen peroxide[5].

Conclusion:

This work demonstrated that the CuO NPs (10-100) nm can be easily generated by laser ablation technique of immersed metal in liquid media under ambient pressure and room temperature. UV-Vis absorption spectrophotometer exhibited two peaks of absorption spectra of CuO NPs colloidal one peak at 205 nm due to interband and the other peak located at 636 nm due to surface Plasmon resonance. Antibacterial activity was performed on various species of bacteria such as *E.coli* ATCC 25922 and *S.aurerus* ATCC 25923. The findings indicated that (22, 45, 90) µg/ml of prepared CuO NPs and (100, 200, 300) µg/ml of the standard have significant antibacterial activity against *E.coli* ATCC 25922 and *S.aurerus* ATCC 25923 specially at high concentrations and more significant effect on the viability of *E.coli* ATCC 25922 than *S.aurerus* ATCC 25923 therefore CuO NPs is a promising and suitable for biomedical applications.

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