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RESEARCH PAPER

Phytogenic Copper Oxide Nanoparticles Synthesized from Carica papaya Leaves: Characterization and Antibacterial–Antifungal Activity

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ABSTRACT

This work presents an eco-friendly method for synthesizing copper oxide nanoparticles (CuO NPs) using Carica papaya leaf extract as a natural reducing and stabilizing agent. The preparing NPs were characterized by Fourier Transform Infrared Spectroscopy (FTIR), Brunauer-Emmett-Teller (BET), Transmission Electron Microscopy (TEM), Scanning Electron Microscopy (SEM), and X-Ray Diffraction Spectroscopy (XRD), confirming a cubical shape with average size of 40 to 60 nm. Antibacterial activity was investigated using the ager well diffusion method against both gram negative (and P. aeruginosa K. pneumonia) and gram positive (S. epidermidis and S. aureus), as well as, the fungus Candida albicans. The CuO NPs appeared considerably antibacterial activity, with inhibition zone of up to 19 mm for C. albicans and 15 mm for S. aureus with concentration of 30 mg/ml. These results suggest that CuO NPs prepared by green method offer promising potential for antimicrobial application.

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INTRODUCTION

In last years, nanotechnology has rampaged materials science via cementing the application of nanoparticles with unrivaled optical, biological and structural features. Among nanomaterials, metal oxides have profited considerable interest because of their broad use in fields like catalyst, energy storage, biomedicine and electronics [1]. Specifically, the CuO are widely reported for their antioxidant, antimicrobial and catalytic activities, as well as it role in drug delivery and biosensors [2]. CuO nanoparticles have eligible features like a high surface area, narrow band gap and effective electron transfer properties. These properties make them auspicious elects for industrial and biomedical applications [3]. However, the traditional preparing methods including sol-gel, electrochemical and thermal decomposition methods typically need harsh reaction conditions, expensive equipment and toxic solvents, leading to limited and hazards scalability [4]. In contrast, green method gives a sustainable, safer and eco-friendly stand by employing biological existence such as microoganisms, plant extract to decrease and stabilize metal precursors in nanoparticles [5]. This method removes the need for hazardous

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materials and reagents, decrease waste and often improvements the nanoparticles biocompatibility [6]. In particular, plant synthesis supplies a plentiful and renewable source of phytochemical like alkaloids, flavonoids, phenolic and terpenoids compounds that work a capping agents and natural reducing [7]. Carica papaya, a equatorial of the caricaceae family, is wealthy in bioactive constituents containing polyphenols, papain, flavonoids and vitamin C [8]. These materials appear strong anti-inflammatory, oxidant and antimicrobial features, making C. papaya leaf extract a good candidate for green synthesis [9]. Furthermore, utilizing agricultural waste like papaya leaves participate to sustainable practices. Many works have investigated green preparation method for CuO NPs employing different plant extracts [10]. Ijaz et al., 2017 employed Abutilon indicum to prepare CuO NPs with dye degradation and antimicrobial activity [11]. Hariprasad et al., 2016 used Arevalanata leaves for antibacterial NPs formation [12]. Taha et al., 2020 synthesized CuO NPs employing Ficus carica leaves, investigating antifungal and antibacterial performance [13]. In spite of all thesis works confirm the green synthesis potential, but it appear many limitations. Previous studies didn't fully depict the nanoparticles employing a inclusive suite of techniques. For example, BET porosity and surface area are decisive for perception the reactivity of nanoparticles were neglected always [14]. in addition to, most works investigated only a tight spectrum of microbes, usually excluding fungal strains such as Candida albicans, which are clinically important [15]. On the other hand, the synthesis procedures were poorly optimized always, missing details about condition of reaction, pH, stirring rates and thermal treatment, which impact on the stability and morphology of nanoparticles [16]. Finally, the plant extract choice was arbitrary, without considering antimicrobial synergies and phytochemical richness [17]. In our study, we treated these gaps via evolving an easy and reproducible green preparation route utilizing Carica papaya leaf extract that have high content of phenolic, alkaloids and flavonoids content. On the other hand, we used a complete characterization techniques to determine the functional groups, surface area, crystallinity and morphology. Moreover, the study investigated both antifungel and antibacterial activity, containing Candida albicans, to supply a wider understanding of biological activity. So, this work not only illustrated an eco-friendly preparation of CuO NPs but also displays a more inclusive and biologically validated method, highlighting their potential as antimicrobial agent for pharmaceutical, food preservation and biomedical applications.

MATERIALS AND METHODS

Materials

All chemical materials were grade and used without future purification. Cupper (ll) acetate monohydrate [$Cu(CO_2CH_3)_2.H_2O$] and ammonium hydroxide (NH_4OH) was used as precursors and pH modifiers, respectively. Fresh leaves of Carica papaya have been collected from Salah al-Dain, Iraq. Bacteria strain (staphylococcus aureus, staphylococcus epidermidis, Klebsiella pneumonia and Pseudomonas aeruginosa) and the fungal strain candida albicans were obtained from local clinical isolates. Cultured media contained Mueller-Hinton ager, MacConkey agar, blood agar, and Sabouraud dextrose agar.

Synthesis of CuO

Firstly, fresh Carica papaya leaves were collected and completely washed by distilled water to remove surface impurities and dust. Then, the leaves were dried at room temperature and ground to powder by using a mortar. Subsequently, the fine powder was mixed with 100 ml of deionized water and heated at 50 °C under continuous stirring for 1h. Finally, the mixture was filtered using filter paper to get the leaf extract, which used as a natural capping and reducing agent. For synthesis of CuO NPs, 3 g of copper precursor was dissolved in 25 ml deionized water. After that, 10 ml of the freshly synthesized Carica papaya leaf extract was added dropwise to the Cu solution. To facilitate NPs production and adjust the pH, NH_4OH (5 ml, 1M) were added as dropwise to the mixture while keeping under constant stirring for 24 h at 25 °C to permit complete production of CuO NPs. The precipitate was isolated via decantation, fully washed with deionized water to remove residual ions, and dried at room temperature for 6h. Finally, the dried powder was calcined in tube furnace for 5 h at 550 °C to get the CuO NPs.

Characterization of CuO NPs

Many techniques were used to characterized the CuO NPs. The functional groups was investigated using Perkin elmer FTIR spectra that recorded

in the range of 400-4000 cm-1 with using KBr pellets. The CuO structure was investigated using X-ray diffraction (XRD) patterns (Philips PW 1730) with Cu Ka radiation in the range of 20° 80°. The morphology and the size of CuO was estimated by using TEM, while the pore size, surface area and average pore diameter were determined employing N_2 adsorption–desorption isotherms at 77 K with a BELSORP-MINI II BET analyzer.

Antimicrobial activity

Green synthesis CuO NPs antimicrobial activity was investigated utilizing the agar well diffusion process. The test was carried out against four bacterial genealogy, two Gram negative (Klebsiella pneumoniae, Pseudomonas aeruginosa) and two Gram-positive (Staphylococcus aureus, Staphylococcus epidermidis), as well as the fungel genealogy Candida albicans. To standardize the inoculum, the suspensions of bacterial were synthesized overnight cultures grown in BHI stock and kept for 24 h at 37 °C. The turbidity was regulated to correspond 0.5 McFarland standard (1.5×10^8 CFU/mL), employing a commercially prepared Biomerieux solution. The Mueller-Hinton agar was utilized as the base medium for testing of bacterial susceptibility, where the medium was fabricated by dissolving 40 g of dehydrated powder in 1 L of H_2O. Then, it autoclaving at 15 psi and 120 °C for 15 min. Subsequently, the sterilized medium was casted into sterile Petri dishes under dark condition and stored at 5 °C until utilize. The suspensions were equally wiped on the Mueller-Hinton agar surface plates utilizing sterile cotton wipes. After few minutes, when the plates were dried, 6 mm of wells were made utilizing a sterile cork borer. Each wells were loaded with 100 µL suspensions of CuO at 15 and 30 mg/ml. Finally, the plates were incubate for 24 h at 37 °C. Then, the inhibition zones diameters were determined in millimeters to estimate the antimicrobial efficiency. Same protocol was applied to investigated the antifungal activity utilizing Candida albicans, with Sabouraud dextrose agar as the culture medium.

RESULTS AND DISCUSSION

The crystalline structure of the prepared CuO NPs was investigated utilizing XRD, which detected clear and sharp diffraction peaks, confirming the crystalline nature. The XRD pattern appeared discrete diffraction peaks at 2q values of 28.76°, 32.83°, 35.86°, 39.05°, 39.28°, 49.06°, 53.92°, 58.64°, 62.07°, 66.93°, 68.73°, and 73.17°, which are in agreement with monoclinic crystal structure and correspond with (JCPDS: 00-041-0254) (Fig. 1) [18]. The eminent diffraction peaks at 2q = 35.85°, matched to the (002) crystal plane, is distinctive of monoclinic CuO. The noted diffraction peak positions match to the (110), (111), (002), (202), (020), (202), (020), (202), (113), and (311) lattice planes of monoclinic CuO, suggesting the successful production of a single-phase crystalline

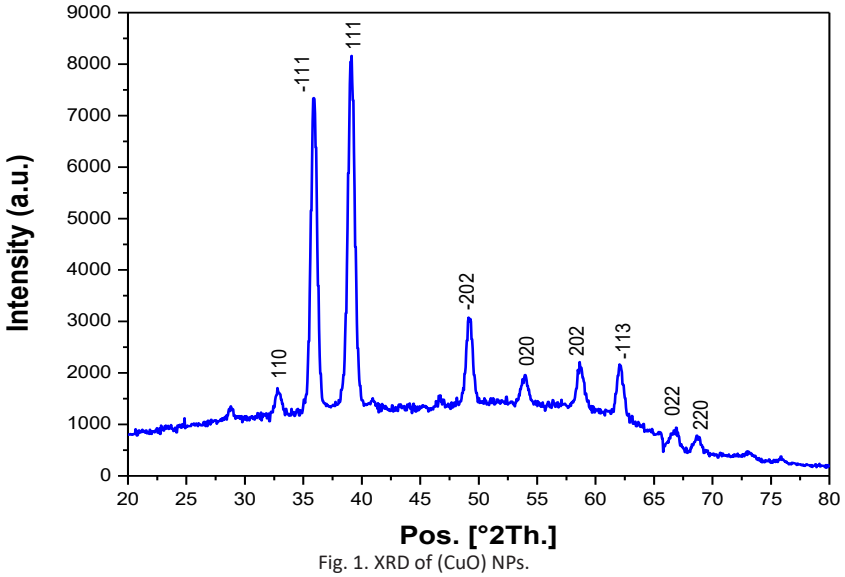

Fig. 1. XRD of (CuO) NPs.

CuO without reveal impurities [19]. The tight and well-defined diffraction peaks indicate that the prepared NPs have excellent crystallinity. The crystallite size of CuO NPs was investigated utilizing the Debye–Scherrer equation:

$$D = 0.9 \lambda/\beta \cos\theta$$

where θ is the Bragg's angle, β is the full width at half maximum (FWHM), λ is the radiation wave length, and D is the crystallite size. Depend on the diffraction peak (002), the crystallite size was calculated to be approximately 43.64 nm. The nanoscale dimensions and crystallinity of CuO NPs are crucial to their biological and reactivity performance. The crystalline structures improve the reactive oxygen species production, which is a recognized mechanism participating to their antimicrobial activity. Furthermore, monoclinic CuO has a tight band gap, which shores its redox conduct, making it a good candidate for biomedical application. As well as, the absence secondary phases suggests the validation Carica papaya leaf extract in synthesis pure CuO, which extended enough reducing and capping agents to stabilize the CuO nanoparticles and guide the production pure phase [19].

FTIR analysis was carried out to characterize the functional groups related with the prepared CuO NPs and to explain the Carica papaya leaf extract role. The spectrum (Fig. 2) shown a strong band located at 535.10 cm^{-1}, which is back to the Cu-O stretching vibrations, corroborating the successful production of CuO NPs. on the other hand, the results appeared broad band between 2800-3400 cm^{-1}, which related to O-H. This band are symptomatic of OH groups from alcohols, polyphenols or adsorbed H2O, ordinarily found in the plant extract. their existence indicates that OH-rich phytochemicals in Carica papaya worked a considerably role in reducing Cu ions and stabilizing the produced NPs. A discrete band at 1637.2 cm^{-1} was related to C=O stretching vibration band or COO^- bending vibration, emerging from aldehydes, ketones and carboxylic acids existent in the extract. These groups may participate to the capping and passivation the surface of CuO, prohibition aggregation and boosting the colloidal stability of NPs. These notes collectively suggest that Carica papaya extract worked a dual functions: working as a reducing agent to alter Cu ions to CuO, and as a capping agent, giving organic surface coating that improvement NPs dispersion and antimicrobial functionality. Moreover, the remain organic compounds may also participate synergistically to the antimicrobial activity of the CuO NPs [20,21].

TEM and FESEM analysis were used to estimate the size distribution, morphology and surface properties of prepared CuO NPs. For

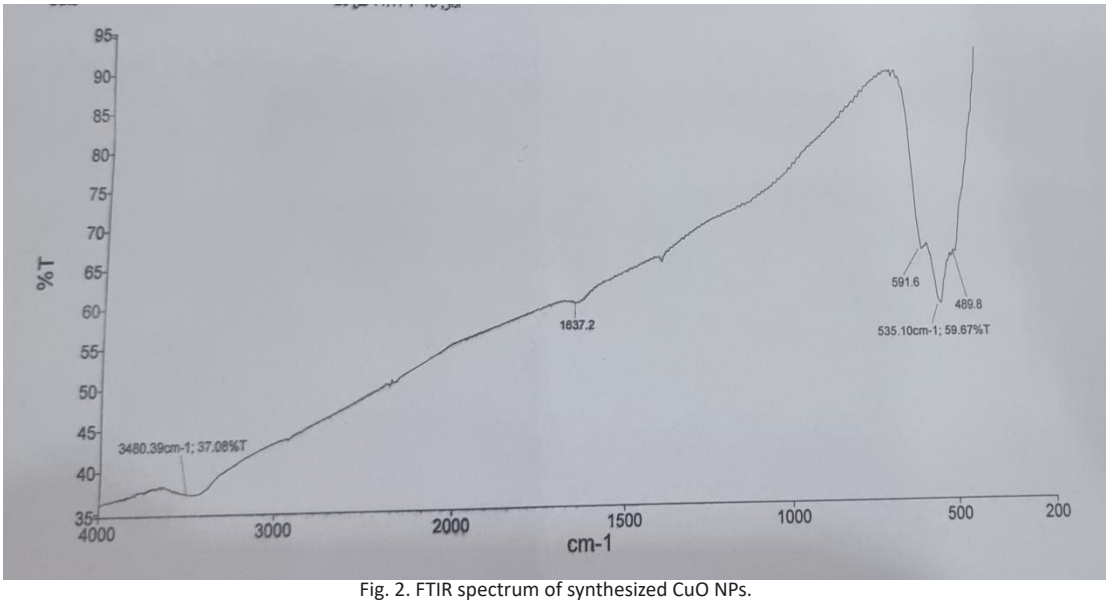

Fig. 2. FTIR spectrum of synthesized CuO NPs.

TEM analysis, the pictures appeared that the CuO NPs were polydispersed, with a mixture of quasi-spherical and cylindrical morphologies, which is common for NPs prepared employing green method. As shown in Fig. 3A and B, the synthesized particle size ranged between 40-55 nm, which in good agreement with XRD results. The existence of separating NPs marks effective capping via phytochemicals existing in extract that prevented agglomeration through nucleation and growth process, which is primary for applications needing redox activity. FESEM analysis gave more prudence into the surface morphology and agglomeration conduct of CuO NPs. The images (Fig. 3C) exposed large clusters of NPs, producing porous agglomerates with comparatively smooth surface. This agglomerating is typically in dried NPs powders and may produce capillary forces through the drying process. The comparatively smooth particle surfaces noted in FESEM images indicate that the NPs were capped with organic layers, possible derived from remain phytochemicals in prepared extract. These compounds not only work as stabilizing and reducing agents through prepare but also participate to particle dispersion surface functionality. The collective information from FESEM and TEM measurements emphasizes that the prepared CuO NPs are good crystallized with nanorange size and effectively stabilized via biomolecules. These properties are fundamental for maximizing surface to volume ratio and improving reaction with microbial membranes operators crucial to their noted antimicrobial efficiency [22-24].

BET surface area measurement was carried out to investigate the pore diameter and volume and specific area for the prepared CuO NPs. The N_2 adsorption-desorption isotherms were estimated at 77 K, and the results (Fig. 4) exposed a specific surface area, an average pore volume

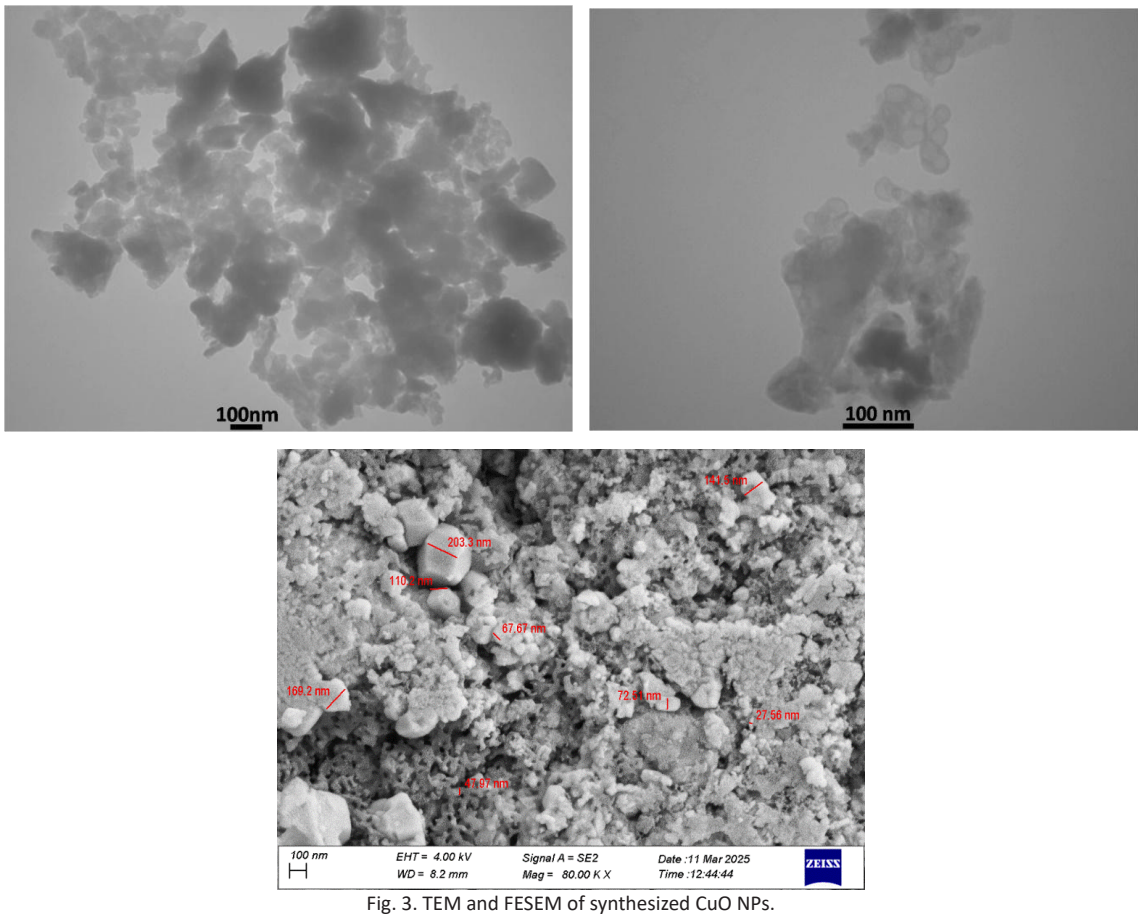

Fig. 3. TEM and FESEM of synthesized CuO NPs.

and diameter of 5.95 m^2/g, 0.08 cm^3/g and 53.73 nm, respectively, suggesting a mesoporous structure based on IUPAC classification. The mesoporous properties likely results from the soft template impact of the effective chemicals existence in Carica papaya extract that work not only as stabilizing and reducing agents but also effect on the nucleation and growth process of nanoparticles, inhibiting heavy agglomerating. These porous frameworks boost the accessibility of Cu ions sites and expedite the production of ROS when reaction with microbial cells, hence improving antimicrobial effectively. Furthermore, a balanced surface area with hold porosity is major in biomedical application, as it supplies an appropriate interface for microbial contact while averting excessive aggregation. BET results thus complements the structural and morphological analyses via supporting that the green prepared CuO NPs possess porosity and surface properties appropriate for utilize in antimicrobial platforms [25,26].

Antimicrobial activity

The antimicrobial action of the CuO NPs green synthesized was estimated against two Gram-positive bacteria (Staphylococcus aureus and Staphylococcus epidermidis), two Gram-negative bacteria (Pseudomonas aeruginosa and Klebsiella pneumoniae), and the fungal strain Candida albicans. The results (Fig. 5) appeared that CuO NPs display dose-dependent antimicrobial efficacy, with clear impacts at 30 mg/mL, while no inhibition was noted at 15 mg/mL versus the tested strains of bacterial. The results of inhibiting zone are summarized in Table. 1. The results propose that the CuO NPs are more active versus Gram-positive bacterial and strains of fungal because of the differences in cell wall permeability and composition. The eminent inhibition noted against S. sureus may be back to the missing of an outer membrane, which produces Gram-positive bacterial more liable to NPs-induced oxidative harm and ion uptake. on the other hand, Gram-negative bacterial have a covering outer lipopolysaccharide layer that work as a barrier to NPs admittance, decreasing their overall susceptibility. The good antifungal activity versus C. albicans may attribute from the porous cell wall structure, which expedite deeper permeation of NPs, permitting them to interfere with intracellular processes and compromise membrane safety. CuO antimicrobial activity NPs is widely famed to contain multiple integral mechanisms:

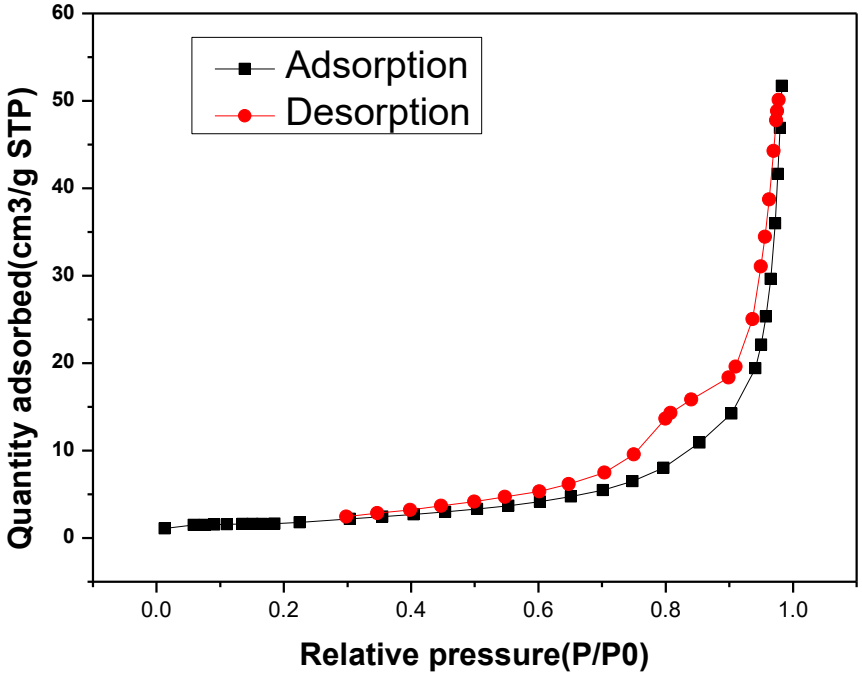

Fig. 4. BET of synthesized CuO NPs.

(A) Formation of ROS: CuO NPs can catalyzed the ROS production like O2-, OH* and H_2O_2, where it cause oxidative stress, lead to protein denaturation, lipid peroxidation and DNA degradation in microbial cells. (B) Liberation of Cu ions: The dissolution of CuO lead to the piecemeal ions that react with negatively charged microbial membranes. These Cu ions can infiltrate cells, connect to intracellular proteins, and dampen primary enzymatic activities, damaging metabolic pathways. (C) Membrane disruption: The mesoporous synthesized CuO NPs boost the surface contact with microbial cells and expedites direct interaction causing membrane thinning, pore production and cellysis. (D) Electostatic connection and surface adsorption: CuO NPs may adsorb on the surface of bacterial by electrostatic strain, particularly if stabilized via negatively charged phytochemicals. This damages membrane potential and permeability, leading to seep of intracellular compents. The collecting of these impacts results in synergistic toxicity, improving the activity of CuO NPs. As well as, the existence of surface bound phytochemicals form prepared extract may participate supplementary materials like alkaloids and flavonoids [27-32].

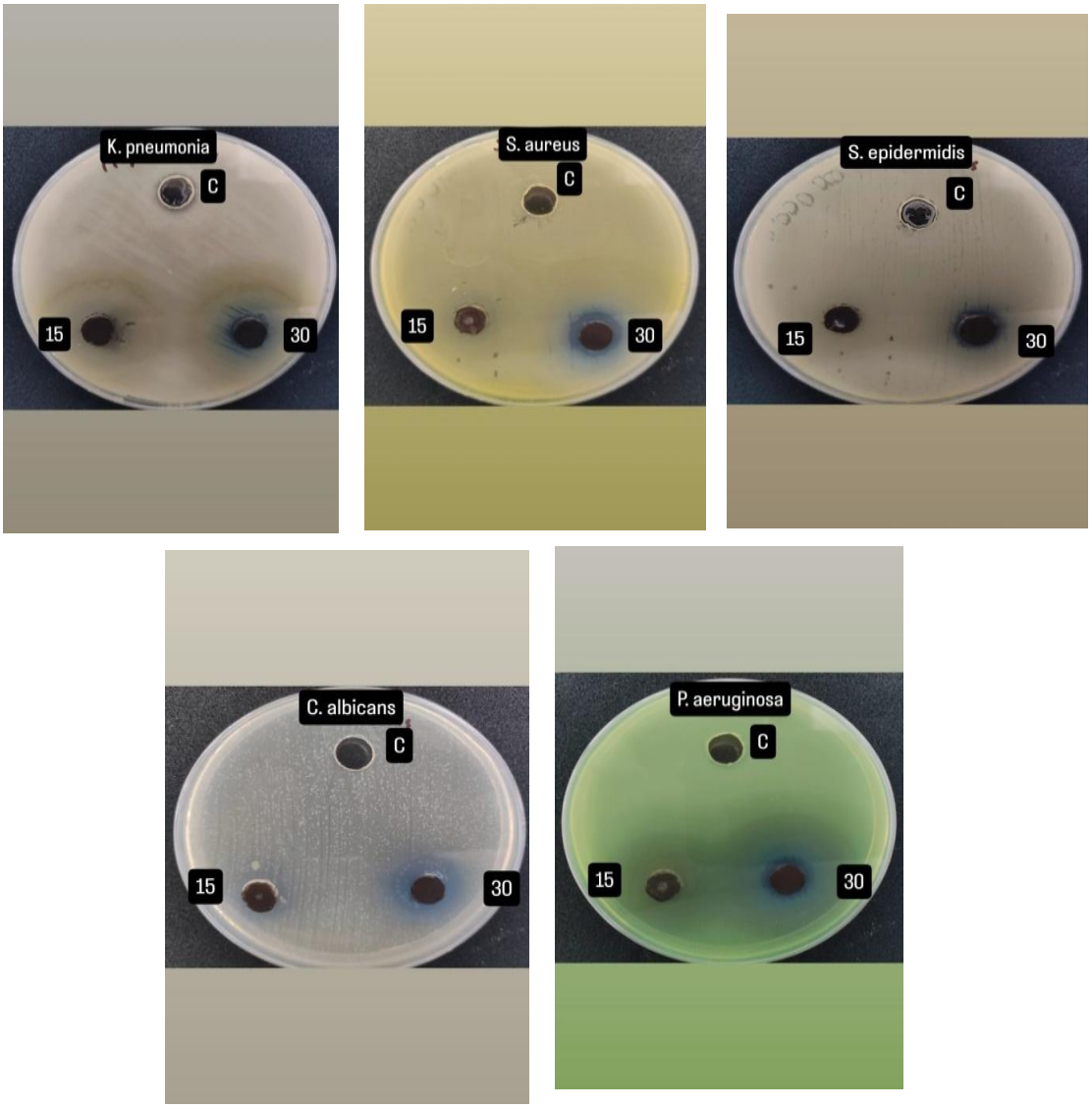

Fig. 5. Antimicrobial activity of CuO NPs.

Table 1. Shows diameter of the inhibition zone of bacteria and fungus against isolated pathogenic bacteria.

	S. aureus	S. epidermidis	K. pneumonia	P. aeruginosa	C. albicans
Z (30)	15 m m	12 m m	11 m m	13 m m	19 m m
Z (15)	0 m m	0 m m	0 m m	0 m m	13 m m

CONCLUSION

The CuO NPs was prepared in an environmentally friendly way using plant leaf extracts such as carica papaya. The shape, size and structure of the prepared CuO NPs was determined by XRD, FTIR, TEM, SEM and BET. The XRD pattern indicates that the CuO NPs has a centered cubic structure. With an average nanoparticle size of 47.97 nm, the produced CuO NPs exhibit a spherical formula in TEM and SEM pictures. The high surface to volume ratio of CuO NPs produced by carica papaya is further demonstrated by composition and size studies. According to BET results the surface area of CuO NPs is 5.9539 m^2/g. The prepared CuO NPs has antibacterial activity against S. aureus, S. epidermidis, K. pneumonia and P. aeruginosa. Depending on the results, CuO NPs is more effective against S. aureus than others. CuO NPs has also effective against fungi (C. albicans). As a result , the data show that the antifungal activity of the CuO NPs has a higher than its antibacterial activity. This study indicates which the prepared copper oxide NPs' may be applied in the health science, food storage industries, energy cell and electrochemical cell.

CONFLICT OF INTEREST

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

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