

RESEARCH PAPER

Green Synthesis of CuO NPs and Characterization with Antibacterial Activity

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ABSTRACT

The present study described green synthesis of inorganic oxide (CuO NPs) nanoparticles were prepared from ethanol: distill water ($V_{\text{ethanol}}:V_{\text{H}_2\text{O}}=7.5:7.5$) with diluted aqueous extract plant Wild Mint leaves ($V_{\text{extract}}:V_{\text{H}_2\text{O}}=3:10$). The absorbance, size, continent, morphology and nature of bonding for synthesized copper nanoparticles were evaluated by UV-Vis, X-ray Diffraction (XRD), Energy Dispersive Spectroscopy (EDX) Scanning Electron Microscopy (SEM), Raman spectroscopy, and Fourier Transform Infrared Spectroscopy FTIR. The results showed a color alteration from light green to dark brown, along with the formation of copper nanoparticles. The activities were tested against antibacterial of some pathogenic bacteria which was Staphylococcus aureus (S.a), Enterococcus faecalis (S.f), Escherichia coli (E.c), and Acinetobacter baumannii (A.b). The analysis process refers to good percent and activities for synthesized CuO NPs against Staphylococcus which may related to succeeded the method with extraction to forming copper oxide nanoparticle.

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INTRODUCTION

The growing global challenge of antibiotic resistance necessitates the development of alternative therapeutic strategies. Among these, metal oxide nanoparticles have emerged as a promising new class of biologically active agents [1-4]. This specialty is witnessing rapid development in diverse sectors that include: Healthcare, [5] biomedical science [6], cosmetics [7], food and feed industries [8], drug and gene delivery [9], environmental protection [10], public health technologies [11], chemical industries [12], microelectronics [13], chemical catalysis [14] as well as electrochemical applications in photochemistry [15]. This remarkable growth has

contributed to opening new application fields and establishing a strong scientific and technological foundation for this promising field [16]. The distinctive physicochemical properties of CuO nanoparticles (NPs) have increasingly attracted the attention of researchers exploring their potential in antibacterial, antifungal, and anticancer applications [17]. Compared to microbe-derived nanoparticles, plant-based synthesis offers greater stability and cost-effectiveness, making it a promising biological production route [18]. Moreover, the notable antibacterial efficacy of CuO NPs is attributed to their low toxicity, strong magnetic properties, and controlled particle size [19]. In this study, copper oxide nanoparticles

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(CuO NPs) were synthesized using wild mint extract under ambient conditions, following a procedure that differed from those reported in research. The synthesized CuO NPs exhibited high purity and Nano scale dimensions, reflecting the effectiveness of the adopted method. Notably, these nanoparticles demonstrated strong antimicrobial activity, particularly against *Escherichia coli*, a pathogen of growing concern due to increasing antibiotic resistance. The structural and morphological characteristics of the CuO NPs were thoroughly investigated using various analytical techniques. The synthesized CuO nanoparticles (NP) were characterized by UV/Vis Spectroscopy, FTIR spectroscopy, Raman spectroscopic, X-ray diffraction, and scanning electron microscopy. UV/Vis Spectroscopy were measured by (Shimadzu, model UV-2550, UV Vis spectrophotometer). These analyses provided insights into the functional groups, crystallinity, particle size, and surface morphology of the nanoparticles. Additionally, their antibacterial performance was evaluated using the agar diffusion method, confirming their potential as effective agents against pathogenic bacteria.

MATERIALS AND METHODS

Copper nitrate ($\text{Cu}(\text{NO}_3)_2 \cdot \text{H}_2\text{O}$) with purities 99.999% was supplied from Sigma-Aldrich, and Samples of Wild Mint leaves (*Mentha arvensis*), were collected from garden north of Baghdad. The leaves were washing and dried at 70 °C overnight before grinding using mortar. 30 grams of grinding material was dispersion in 500 m L of ethanol, then shaken for 2 hours at 15 °C by using Orbital

Shaker LOS-B10 with 100 rpm. The mixture was filtered by filter paper, then upper part of solution was separated as we reported in our previous work [20]. According to our work [20], the extraction solution was diluted to (3:10 ml) respectively. Copper nitrate (0.5 g) was dissolved in a mixture of 7.5 m L of ethanol and 7.5 m L of distilled water. This solution was placed on a magnetic stirrer with stirring 300 rpm for 30 minutes until the copper nitrate was completely dissolved. 5 m L from copper nitrate solution was drop wise with continuous stirring which shown change in color and that would be converted to black color after six hours. The precipitated materials in three beakers were separated by centrifuged at 3000 rpm for 10 minutes, before dried at 100 °C then calcination at 400°C with speed of heating 4 °C/ min by Muffle Furnace 1200 [21].

Antibacterial activity The antibacterial activity of copper oxide nanoparticles prepared biologically using plant extract was evaluated using the agar well diffusion method, in accordance with the standard protocols mentioned in the studies [22]. Four bacterial strains were selected for this test: two Gram-positive bacteria (*Staphylococcus aureus* and *Streptococcus facials*) and two Gram negative bacteria (*Escherichia coli* and *Acinetobacter baumannii*). Mueller Hinton Agar (MHA) plate, the wells were created with a Pasteur pipette, and different concentrations (50, 75, and 100 µg/ m L) of nanoparticles were loaded into the wells. Was used as the growth medium for bacteria. Mueller Hinton agar was prepared and sterilized in the autoclave then poured into sterile Petri dishes and left to solidify. The bacterial

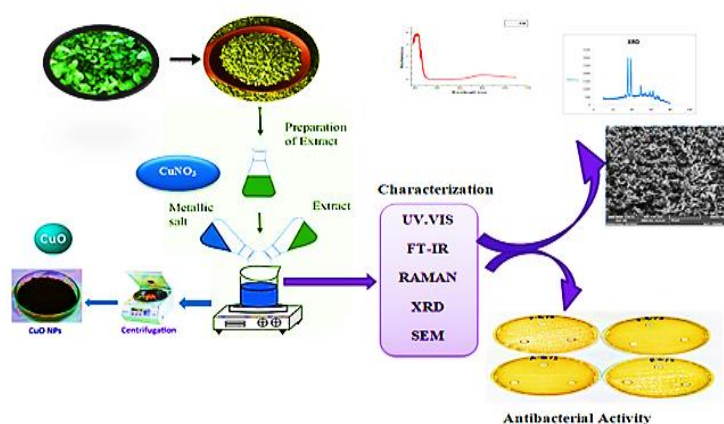


Fig. 1. The skim for the steps of synthesized and characterized CuO NPs.

inoculum was prepared with a concentration of 1.5×10^8 cells/ mL using sterile physiological saline, and the optical density was adjusted to match McFarland scale 0.5. The bacterial inoculum was distributed over the entire surface of the culture medium using sterile cotton swabs. Wells were made in the culture media using a sterile corn borer to load various solutions into them. The plates were incubated at 37°C in an incubator for 24 h. After incubation, the inhibition zones (IZ) were measured around the wells loaded with nanoparticles, to determine the biological activity. Both the bacteria-free medium and DMSO were used as negative control groups to ensure the absence of any interference or contamination in the results.

RESULTS AND DISCUSSION

according to analysis CuO NPs The progress of the reaction was visually indicated by a color change and further confirmed by measuring absorbance values around 327 nm for the synthesized sample. As shown in Fig. 2a, shows the absorbance for CuO NPs which shown absorbance in UV- reign. Fourier Transform Infrared Spectroscopy (Shimadzu- 8400S) were recorded in the range of $500\text{--}4000\text{ cm}^{-1}$ with using KBr, Sigma-Aldrich. The Fig. 2b reported the functional group for synthesized CuO NPs. The FTIR spectrum of the synthesized CuO nanoparticles

using (Wild Mint leaves) extract revealed several prominent absorption bands corresponding to various functional groups. Fig. 2c, reported Raman spectroscopy which measurements using a Reni Shaw in Via™ Raman spectrometer using a green laser with a wavelength of 532 nanometers. Fig. 2d include phase identification by matching the obtained X-ray diffraction (XRD) pattern with standard reference data from the JCPDS database. determine the phase and characterize the crystalline metallic AgO NPs, X-ray diffraction (XRD; D8ADVANCE, Bruker) was used. The size of the synthesized AgO NPs was evaluated using the Debye Scheier equation [21].

Fig. 3, reported the morphology and surface characteristics of the synthesized CuO nanoparticles, Scanning Electron Microscopy (SEM) analysis was performed using by FE SEM (JSM-6160)) microscope. For SEM preparation, approximately $200\text{ }\mu\text{L}$ of each nanoparticle suspension (undiluted) was transferred into tubes and centrifuged at for 10 minutes. The resulting pellet was carefully extracted, placed onto a conductive stub, and air dried at room temperature for 24 hours before imaging.

The UV–Vis absorption spectra were obtained within the wavelength range of $200\text{--}1200\text{ nm}$, as shown in Fig. 2a. This peak is attributed to electronic transitions characteristic of CuO NPs. A distinct absorption band observed at 285 nm confirms

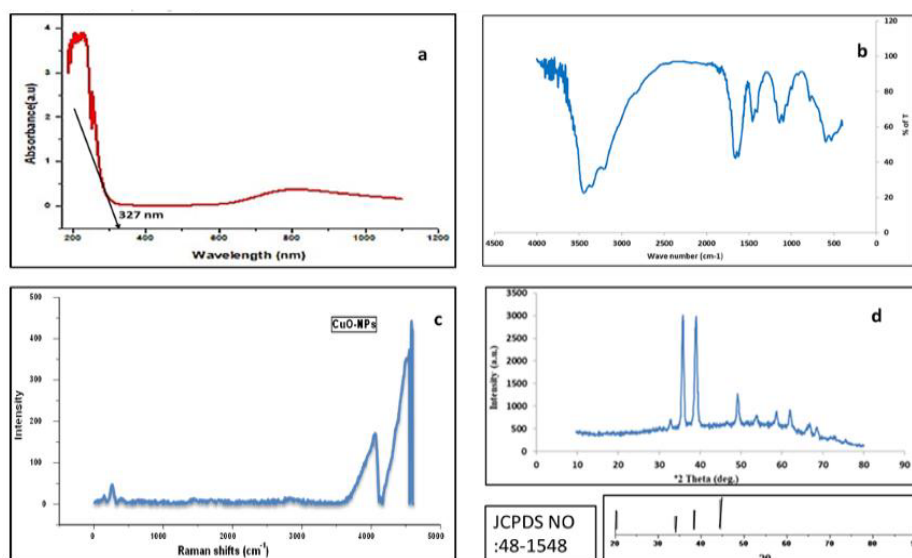


Fig. 2. The skim for analysis CuO NPs (a) UV-visible absorbance, (b) FTIR, (c) Raman spectroscopy, (d) XRD patterns.

the successful formation of CuO nanoparticles. [23]. May be the most important part in IR spectrum, Fig. 2b, can be related to the absorption band at 617 cm^{-1} for CuO stretching vibrations, confirming the successful formation of copper oxide nanoparticles. The Fig. 2b shows a broad peak at 3490 cm^{-1} is indicative of O–H stretching vibrations [24], which may originate from phenolic or alcoholic compounds present in the plant extract. The common absorption band at 2924 cm^{-1} can be related to C–H stretching of aliphatic hydrocarbons, typically found in organic plant constituents. the carbonyl-containing compounds such as esters or ketones were reported in distinct peak at 1733 cm^{-1} which is related to stretching vibration of C=O [25]. The peak at 1634 cm^{-1} may be associated with C=C stretching or N–H bending, supporting the involvement of amine or aromatic groups in nanoparticle formation. Further peaks at 1384 , 1161 , and 1027 cm^{-1} indicate CH_3 bending and C–O–C/C–O stretching modes, respectively, which mostly refers to alcohols, ethers, or esters in capping the nanoparticles. Raman spectroscopy as shown in Fig. 2c, included revealing vibrational

modes specific to CuO lattices, with peaks at 234 , 292 , and 348 cm^{-1} confirming copper oxide structures. Higher frequency bands indicated interactions between nanoparticles and plant-derived organic molecules, further confirming the capping role of phytochemicals. The Raman spectrum of the biosynthesized CuO nanoparticles using *Nepeta cataria* extract displayed several distinct vibrational modes. A prominent peak at 234 cm^{-1} is typically associated with CuO lattice vibrations, confirming the formation of copper oxide phases. Additional peaks at 292 and 348 cm^{-1} correspond to bending modes of CuO bonds, supporting the existence of monoclinic or ten rite (CuO) structures. The XRD analysis which reported in Fig. 2d, displayed multiple sharp and well-defined peaks, confirming the crystalline structure of the material. the diffraction peaks appear at $2\theta=35^\circ$ and $2\theta=39^\circ$, which can be related to the (111) and (200) planes of monoclinic CuO, in accordance with the JCPDS card No. 48-1548. [26]. These peaks confirm the formation of highly crystalline copper oxide nanoparticles. Additional diffraction peaks observed between $2\theta=49^\circ$ and

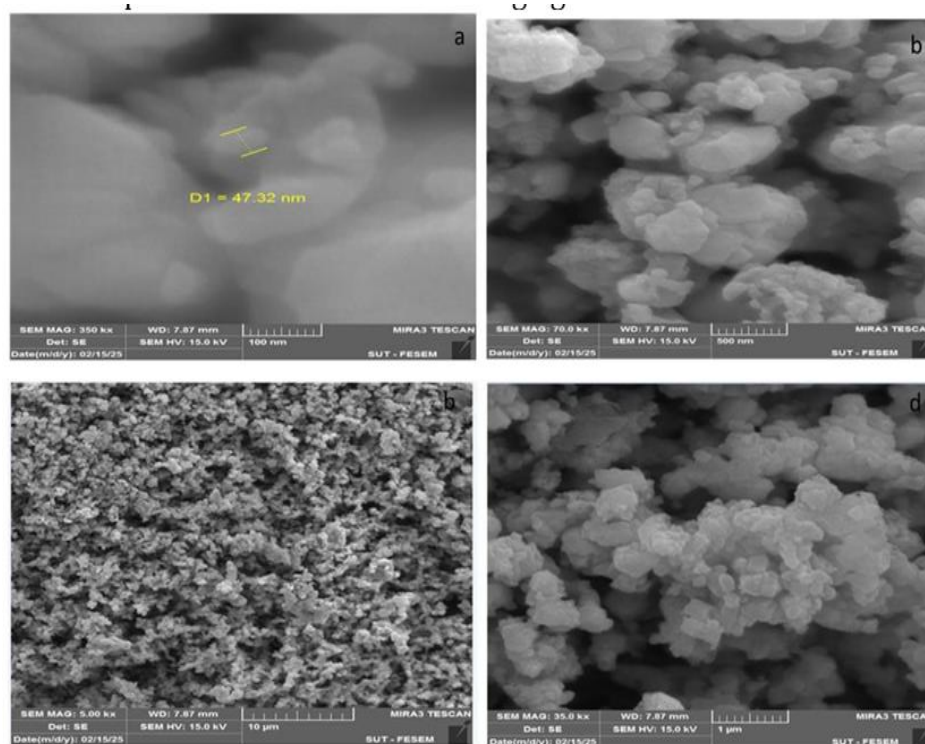


Fig. 3. The Figure of SEM images for synthesized CuO NPS at different magnification: (a) 350 kx (100 nm), (b) 70 kx (500 nm), (c) 5 kx (10 μm), and (d) 35 kx (1 μm).

72° suggest the presence of multiple crystalline planes, further supporting the successful formation of well defined Cu–O nanostructures. XRD analysis displayed distinct diffraction peaks at 35° and 39°, which correspond to the monoclinic CuO phase 202 planes. The high intensity and sharpness of these peaks confirm the crystalline nature and purity of the synthesized nanoparticles. The size of CuO nanoparticles according to this work was evaluated using the Scherrer equation, based on the full width at half maximum (FWHM) of the most prominent [27] diffraction peak observed in the XRD pattern. the equations is ($D=0.9\lambda/\beta \cos\theta$

) Where, D – Crystalline size (nm) , λ - Wavelength of the X-ray used (Å), β - Full width half maximum (radian), it is found to be 35nm. Table.1 reports that absence of spurious diffractions indicates the crystallographic purity. The 2θ peaks at 39.08° and 49.18° confirm its monoclinic structure [28].

The SEM micrographs of nanoparticles exhibit noticeable agglomeration, which is commonly, associated with green synthesis methods due to the presence of phytochemicals that facilitate inter particle interactions. Fig. 3 a-c, and d shows the estimated average particle size was approximately 47.32 nm, with non-uniform morphology. The

Table 1. estimation for synthesized CuO NPs from XRD analysis.

Pos. [°2Th.]	Height [cts]	FWHM Left [°2Th.]	d-spacing [Å]	Rel. Int. [%]	Tip Width
35.8654	2496.77	0.4920	2.50386	96.74	0.5904
39.0821	2580.94	0.1968	2.30487	100.00	0.2362
49.1813	922.78	0.3936	1.85262	35.75	0.4723
53.7744	469.77	0.7872	1.70472	18.20	0.9446
58.6403	599.08	0.3936	1.57434	23.21	0.4723
61.9309	636.57	0.5904	1.49836	24.66	0.7085
66.7893	348.08	1.1808	1.40068	13.49	1.4170
68.5746	298.45	0.5904	1.36850	11.56	0.7085
72.4980	115.71	1.5744	1.30381	4.48	1.8893

Table 2. The antibacterial activity of CuO Nano compound.

Co.50%	Co.75%	Co.100%	Levofloxacin	Name	Type Bacteria
17	29	32	19	<i>Staphylococcus aureus</i>	Gram-positive bacteria
19	25	31	20	<i>Streptococcus faecalis</i>	Gram-positive bacteria
11	19	19	16	<i>Escherichia coli</i>	Gram-negative bacteria
10	13	15	12	<i>Acinetobacter baumannii</i>	Gram-negative bacteria

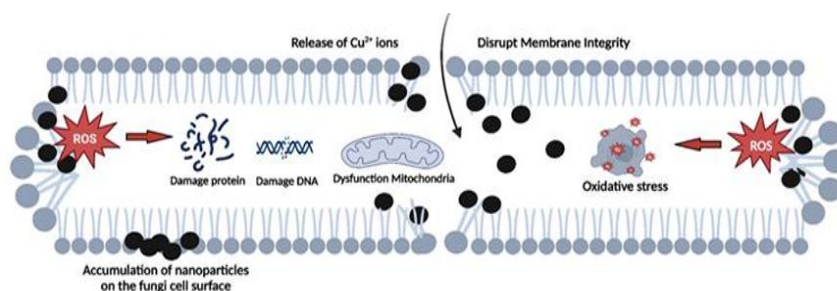


Fig. 4. Probable mechanism of pure CuO-NPs against bacterial cells.

results showed a difference in the inhibition zones among the different bacterial species, indicating the bioactivity of copper oxide nanoparticles against both Gram-positive and Gram-negative bacteria. Table 2 Shows the effectiveness test measured by the inhibition diameter around the prepared compounds against Gram-positive and Gram negative bacteria.

Four types of bacteria were tested: *Staphylococcus aureus* (S.a), *Enterococcus faecalis* (S.f), *Escherichia coli* (E.c), and *Acinetobacter baumannii* (A.b) [29]. A sample of 0.06 g of the inorganic oxide CuO-NPs was taken and then dissolved in 5 ml of dimethyl sulfoxide (DMSO), compared to the antibiotic used (levofloxacin), where the inhibition zone was smaller than (*Staphylococcus aureus* (Co.100%32*Streptococcus faecalis*)) for Gram-positive bacteria, and (19 mm) (75, 100%) against (*Escherichia coli*) and (20 mm) (100%) against (*A. baumannii*) for Gram-negative bacteria. The findings of this study offer compelling evidence supporting the traditional use of the Catnip (disambiguation). Plant in folk medicine for managing bacterial infections. The data demonstrated that the plant possesses broad spectrum antibacterial properties, showing effectiveness against multiple clinically important pathogens, including several commonly linked to hospital-acquired infections [30].

these findings provide strong evidence for the successful green synthesis of copper oxide nanoparticles using *Nepeta cataria* extract, with plant metabolites playing a vital role in nanoparticle formation, crystallization, and stabilization. Table 1, explains the FWHM and Crystallite size of CuO NPs. The observed agglomeration and irregular shapes are in agreement with the crystalline characteristics revealed by XRD analysis [31], supporting the formation of stable, crystalline Cu–O nanostructures under eco-friendly synthesis conditions.

CONCLUSION

Wild Mint leaves extraction was succeeded to make as reducing material and forming Cu-NPs with behave as control for building specific phase able to forming specific particle size with less agglomerations as reported in XRD and SEM images. The particles exhibited high surface activity due to the presence of numerous dangling bonds, which enhance their interaction with surrounding media and promote aggregation.

The antibacterial efficacy of the synthesized nanoparticles was assessed by measuring the inhibition zones, which were found to be 19 mm for the gram-negative *Escherichia coli* and 32 mm for the gram-positive *Staphylococcus aureus*. These results underscore the promising potential of CuO NPs as antimicrobial agents against harmful bacterial strains.

CONFLICT OF INTEREST

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

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