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ORIGINAL RESEARCH ARTICLE

Green synthesis and comparative evaluation of CuO and ZnO nanoparticles for antibacterial and anti-breast cancer applications

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ABSTRACT

Background: The growing resistance to conventional antibacterial and anticancer treatments necessitates innovative approaches such as nanotechnology, particularly green-synthesized nanoparticles (NPs). This work synthesizes eco-friendly copper oxide (CuO) and zinc oxide (ZnO) nanoparticles using lemon peel extract and tests their biological activities. The synthesis was confirmed through FTIR, EDX, and FESEM analyses, which revealed successful formation and biofunctionalization of the NPs. FTIR spectra identified phytochemical involvement, while EDX confirmed elemental composition. FESEM imaging showed agglomerated flake-like CuO particles and spherical ZnO particles. Antibacterial testing using the cup-plate agar method demonstrated significant inhibition zones: CuO NPs produced 14 mm and 20 mm against *Bacillus subtilis* and *E. coli* at 100 µg/mL. In comparison, ZnO NPs exhibited superior inhibition with 16 mm and 23 mm, respectively, even outperforming sulfadiazine. Concentration-dependent cytotoxicity was seen in MCF-7 breast cancer cells. At 320 ppm, CuO NPs reduced viability to 17.87%, while ZnO NPs further decreased it to 10.66%. The IC_{50} of CuO was calculated as 30.86 µg/mL, whereas ZnO demonstrated greater potency. These findings confirm that green-synthesized ZnO NPs possess more potent antibacterial and anticancer properties than CuO. If adopted clinically, such biogenic NPs could significantly mitigate multidrug resistance and enhance therapeutic outcomes with reduced environmental impact. Future work should focus on *in vivo* validation, standardization, and exploring synergistic applications with existing chemotherapeutics.

Keywords: antibacterial; CuO nanoparticles; cytotoxicity; IC50; green synthesis; nanomedicine; MCF-7; ZnO nanoparticles

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1. Introduction

The increasing prevalence of drug resistance in antibacterial and anti-breast cancer therapies presents one of the most formidable challenges in contemporary biomedical science. Resistance in bacterial pathogens and malignant cells emerges primarily due to genetic mutations, overuse and misuse of pharmaceuticals, and inadequate therapeutic strategies. Consequently, pathogens and tumor cells develop mechanisms to evade the cytotoxic effects of drugs, thereby diminishing treatment efficacy and increasing mortality rates[1]. For instance, multidrug-resistant (MDR) bacteria like Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa have evolved efflux pumps, enzymatic degradation, and biofilm formation, all contributing to resistance. If such resistance mechanisms remain unaddressed, they may undermine therapeutic outcomes and burden healthcare systems enormously[2,3].

Nanotechnology is one of several medication development methods being investigated to address these difficulties.

Nanotechnology improves antibacterial and anticancer drug pharmacology in a novel and adaptable way[4]. Nanoparticles (NPs) permeate cellular membranes better than typical medications owing to their tiny size, vast surface area, and variable surface chemistry[5]. They can also modulate medication release, increase solubility, and lower systemic toxicity. CuO and ZnO are particularly interesting for antibacterial and anticancer characteristics. These nanoparticles engage with microbial and cancer cell membranes, produce ROS-induced oxidative stress, and disrupt essential cellular functions, bypassing normal resistance mechanisms[6].

The manufacturing of CuO and ZnO nanoparticles is now an environmentally sustainable and economically viable replacement to traditional physical and chemical techniques. Traditional nanoparticle production uses dangerous chemicals, high energy consumption, and toxic byproducts, raising sustainability and biocompatibility problems. Instead, green synthesis uses plant extracts, bacteria, fungus, and algae as reducing and capping agents. Bioactive substances such phenolics, flavonoids, alkaloids, and proteins in these natural resources reduce metal ions and stabilize nanoparticles. Extracts from the plant Azadirachta indica (neem), the leaves of Camellia sinensis (green tea), and Ocimum sanctum (holy basil) have been utilised to synthesise CuO and ZnO nanoparticles exhibiting enhanced antibacterial and anticancer properties[7].

Recent research has shown that green-synthesized CuO and ZnO nanoparticles inhibit several bacterial strains, including MDR pathogens. This effectiveness is due to the nanoparticles' physicochemical qualities and the bioactive phytochemicals on their surfaces working together. ROS, mitochondrial malfunction, and apoptosis generally cause this anticancer impact. Additionally, conjugating these nanoparticles with recognized chemotherapeutic drugs or specific ligands might boost their therapeutic efficiency while avoiding tissue harm. Many obstacles remain despite these encouraging improvements. The repeatability and standardization of green synthesis technologies are key challenges. Since plant-derived biomolecules vary by species, growth conditions, and extraction techniques, nanoparticle size, shape, and biological activity may vary. In vivo data and clinical validations are few, however, in vitro studies have shown effectiveness. Long-term safety and therapeutic feasibility of nanoparticles are difficult to determine without toxicological and pharmacokinetic characteristics. Increasing multidisciplinary interactions between chemists, biologists, and doctors may overcome these restrictions[8].

Nanotechnology combined with molecular biology and genetics has expanded customized medicine. Such techniques might transform infectious illness and cancer therapy if they are effectively transferred from bench to bedside. The broad use of nanoparticle-based medicines also requires regulatory frameworks and public support. Governments and international organizations must set strict biosafety, effectiveness, and environmental impact requirements for nanomaterials. Public education initiatives must also eliminate nanomedicine myths and establish trust. These methods may speed nanotechnology-based therapies in mainstream healthcare if applied alongside scientific advances[9].

This study aims to synthesize CuO and ZnO NPs via a green method using lemon peel extract and to evaluate their antibacterial and anticancer efficacy. The research addresses environmental concerns associated with traditional nanoparticle synthesis by employing eco-friendly processes. Characterization techniques such as FTIR, EDX, XRD, and FESEM confirmed nanoparticle formation. The biological potential was assessed against Bacillus subtilis, E. coli, and MCF-7. The study intends to compare the performance of CuO and ZnO NPs, determine their IC_{50} values, and assess their potential as alternative therapeutic agents.

2. Method

2.1. Materials

This investigation employed analytical-grade chemicals and reagents without purification. Copper(II) sulphate pentahydrate ($CuSO_4 \cdot 5H_2O$, 99.9%) and 100% ethanol were purchased from Sigma-Aldrich (Germany). We obtained zinc nitrate hexahydrate ($Zn(NO_3)_2 \cdot 6H_2O$) from Himedia Laboratories Pvt. Ltd. in

India. All aqueous solutions were prepared using deionized water. Fresh lemon peels, collected from local markets in Baghdad, Iraq. All glassware used in the experimental procedures was thoroughly cleaned with distilled water and rinsed with ethanol prior to use to prevent contamination. The experiments were conducted under aseptic conditions where applicable, particularly during the antimicrobial testing procedures.

Several advanced analytical instruments were employed to synthesize and characterize the nanoparticles, as well as to evaluate their biological activities, such as FTIR, EDX, XRD, and FESEM, in addition to a centrifuge, incubator and Laminar Flow Hood, and Autoclave.

2.2. Methodology

Extraction of Lemon Peels: A distilled solution was used for clearing fresh lemon peels of dust and surface contaminants. Cut the cleaned peels into tiny pieces and shade-dry for 4-5 days to eliminate moisture. The peels were dried and pounded into a fine powder using a clean mortar and pestle. To make lemon peel extract, mix 10 g dry powder with 100 mL deionized water in a beaker. To extract bioactive parts, the mixture was heated at 70 °C for 30 minutes with constant stirring. After cooling to room temperature, Whatman No. 1 paper filtered the warm solution. Clear filtrate was stored at 4 °C for possible use as a reducing and stabilizing agent[10].

Green synthesis of CuO from Lemon peel: To make CuO nanoparticles, dissolve 2.49 g of salt in 110 mL of distilled water (D.W) to form a 0.1 M of $CuSO_4 \cdot 5H_2O$ solution. Next, put 50 mL of lemon peel extract into 50 mL of $CuSO_4$ solution and stir magnetically. For 2 hours, the reaction mixture was agitated constantly at 70 °C. A progressive colour shift from bright blue to dark brown indicated CuO NPs production. After the response, the solution was refrigerated and centrifuged at 10,000 rpm for 20 min. To eliminate contaminants, wood pellet was repeatedly washed with ethanol and distilled water. Nanoparticles were dried in a 60 °C hot air oven for 6 hours and collected as powder for characterization[11].

Green synthesis of ZnO from Lemon peel: To synthesize ZnO nanoparticles, dissolve 2.97 g of salt in 100 mL of D.W to create a 0.1 M zinc nitrate hexahydrate solution. Add 50 mL lemon peel extract dropwise to 50 mL zinc nitrate solution while magnetically stirring. After 2 hours at 70 °C, the reaction mixture changed from clear to milky white, indicating nanoparticle formation. After cooling, the liquid was centrifuged at 10,000 rpm for 15 min. The pellet was repeatedly cleaned with ethanol and distilled water to eliminate byproducts. The ZnO nanoparticles were dried in a 60 °C hot air oven for 6 hours and collected as powder for further analysis[12].

Nanoparticle Oxides Antibacterial Activity Study: Cup-plate agar diffusion tests the nanoparticle oxides' antibacterial properties. Growth occurs on Muller-Hinton agar. Pour sterile agar into Petri dishes to solidify. A sterile triangular loop spreads the microbial suspension uniformly over the surface. Aseptically positioned 12 mm stainless-steel cylinders produce wells. Testing commences after one hour of suspension diffusion. Bacillus subtilis and E. coli are tested. Tests compare nanoparticle oxides to sulfadiazine at 100 and 50 mg/ml. Sufadiazine is dissolved in sterile distilled water, whereas all other chemicals are in dimethyl sulfoxide. For 48 hours, plates are incubated at 37 °C. To assess antibacterial potential, inhibitory zones are determined in millimeters after incubation[13].

Anticancer Activity Evaluation: Green-synthesized CuO and ZnO nanoparticles were tested for anticancer efficacy against MCF-7 (ATCC HTB-22) breast cancer cells using the MTT assay. In a humidified incubator with 5% CO_2, cells were grown in high-glucose DMEM with 10% foetal bovine serum and 1% penicillin–streptomycin at 37 °C. In 96-well plates, MCF-7 cells (passages ≤20) were planted at a density of 6 × 10^3 cells/well and adhered for 24 hours. Nanoparticles were dispersed in sterile DMSO (≤0.5% v/v), sonicated to prevent aggregation, and diluted in complete medium to 0, 20, 40, 80, 160, and 320 µg/mL. Vehicle (DMSO) and positive control (5-fluorouracil) wells were treated in triplicate. Add 10 µL of MTT solution (5 mg/mL in PBS) to each well after 24 hours to reach 0.5 mg/mL. Plates were incubated for 4 hours.

Dissolve formazan crystals in 100 µL DMSO with careful shaking after removing the media. A microplate reader detected absorbance at 570 nm using 630 nm as a reference. Estimate cell viability (%) using (Abs_treated/Abs_vehicle control) × 100. Fit concentration-response curves to a four-parameter logistic model to determine inhibitory concentration (IC_{50}). Data was provided as mean ± SD (n = 3). One-way ANOVA and Tukey's post hoc test established statistical significance, with $p < 0.05$ deemed significant[14].

3. Results and discussion

The FTIR (cm^{-1}) spectrum of green-synthesized CuO nanoparticles reveals key functional groups from plant phytochemicals that aid in reduction and stabilization. A broad peak that appeared at 3283 cm^{-1} returns to the hydroxyl group. The 2917 and 2849 peaks return C–H of alkanes. The strong band at 1607 returns to C=C, while peaks near 1416 and 1242 may indicate C–N stretching of amines. The band at 1018 corresponds to C–O stretching[15]. Notably, peaks at 692, 618, and 527 confirm Cu–O vibrations, supporting successful CuO nanoparticle formation, as shown in **Figure 1**.

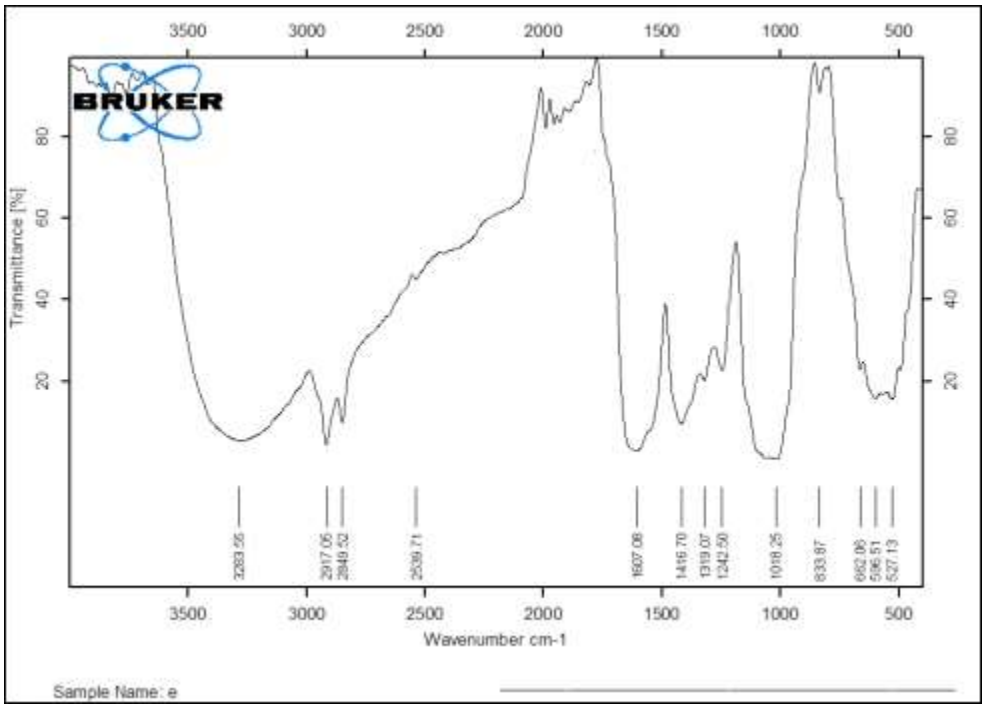

Figure 1. CuO NPs FTIR spectrum.

The FTIR (cm^{-1}) of ZnO from plant extract displays prominent bands that confirm the involvement of bioactive compounds in nanoparticle formation and stabilization. A peak at 3287 returns a hydroxyl group from alcohols or phenols. Bands at 2997 and 2593 cm^{-1} suggest C–H stretching vibrations [16]. Peaks at 1607, 1403, and 1320 are attributed to C=C and C–N bending, indicating amine and aromatic group presence. A peak at 1125 returns to carbon-oxygen atom stretching. Notably, absorption bands below 600, particularly around 499, confirm Zn–O stretching, validating successful ZnO nanoparticle synthesis, as shown in **Figure 2**.

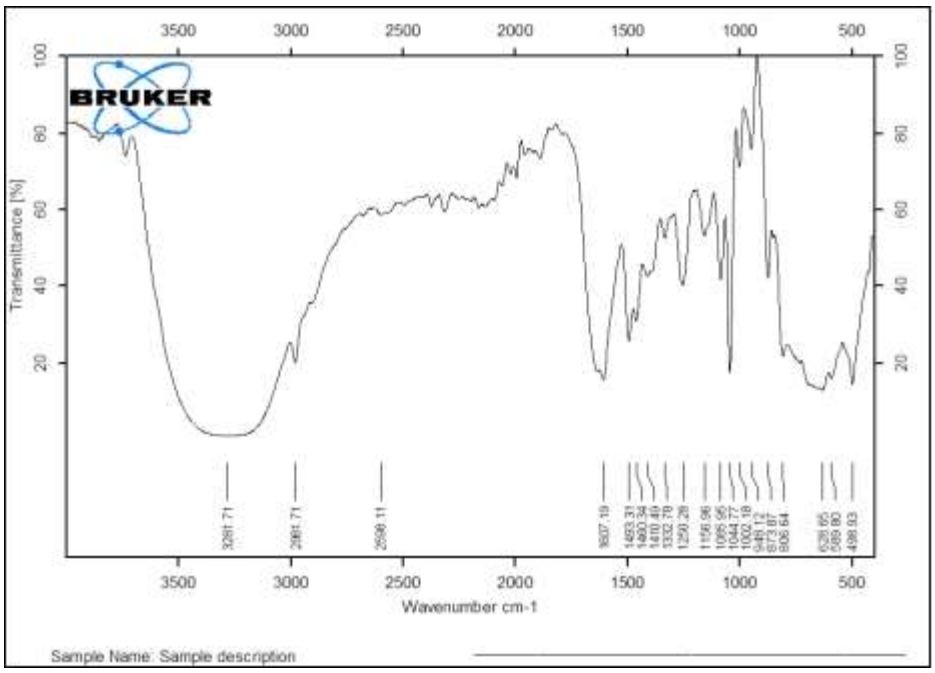

Figure 2. FTIR spectrum of ZnO NPs.

The EDX spectrum of CuO nanoparticles synthesized via green plant-based methods reveals the elemental composition confirming successful formation and capping. The prominent peaks correspond to oxygen (O) and carbon (C), indicating the presence of metal oxide and plant-derived organic molecules, respectively. Fluorine (F) is also detected, likely originating from bioactive compounds in the plant extract. A trace peak for nitrogen (N) suggests minor protein or amine group involvement[7]. The absence of impurities and the dominance of oxygen and copper-related signals support the purity of CuO NPs, as shown in **Figure 3**.

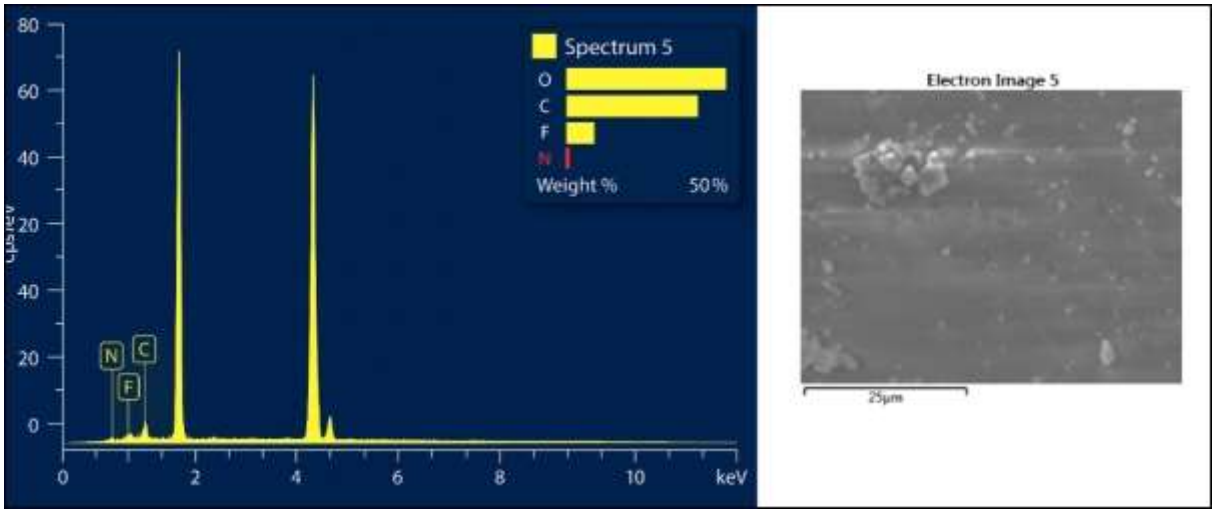

Figure 3. Edx of CuO NPs.

The EDX spectrum of ZnO nanoparticles synthesized via green plant methods shows distinct peaks for zinc, confirming successful nanoparticle formation. The presence of carbon and oxygen suggests involvement of the plant as a reducing and stabilizing agent. Minor peaks of nitrogen and fluorine indicate possible phytochemical residues. Notably, zinc's characteristic peaks around 1.0 keV and 8.6 - 9.6 keV further validate its incorporation. This elemental composition supports the efficiency and eco-friendliness of the plant-mediated synthesis route, as shown in **Figure 4**[17].

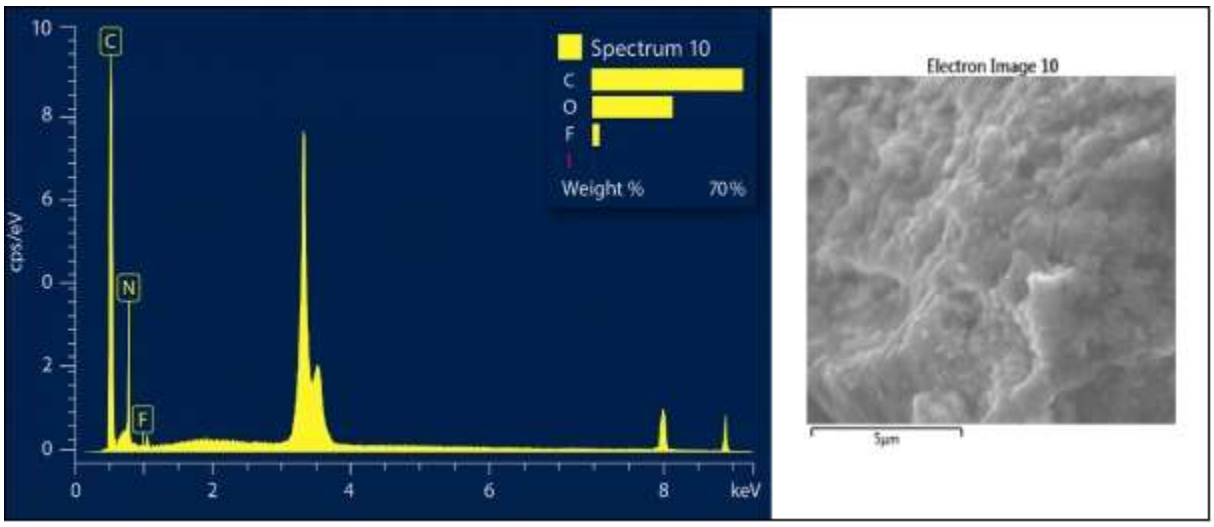

Figure 4. Edx of ZnO NPs.

The FESEM image of CuO nanoparticles synthesized via green methods using plant extract reveals agglomerated, sheet-like structures with irregular morphologies. These particles exhibit a rough surface and a flake-like architecture, which suggests anisotropic growth influenced by phytochemicals. Similar morphology has been previously reported in studies utilizing plant-mediated synthesis, indicating biomolecules reduce and stabilize nanoparticle formation[18]. The observed features imply a high surface area favorable for catalytic and antimicrobial applications[19], as shown in **Figure 5**.

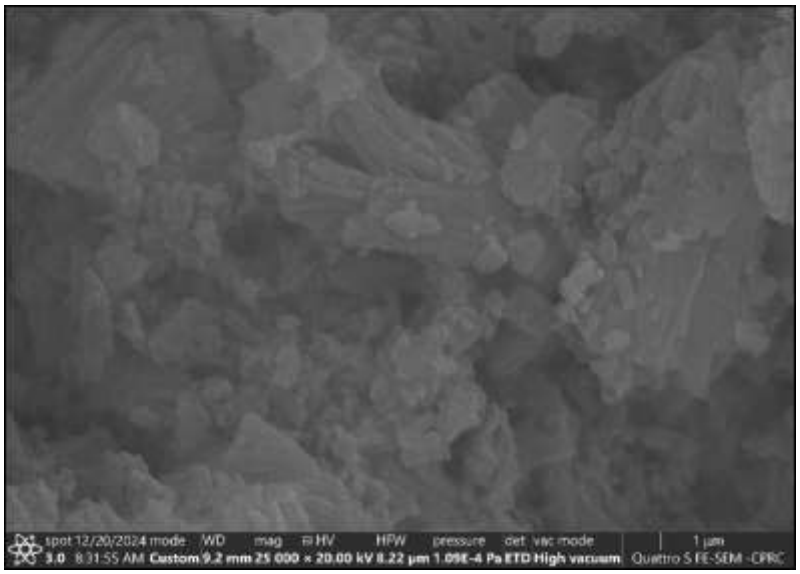

Figure 5. FESEM of CuO NPs.

The FESEM image of green-synthesized ZnO nanoparticles shows a dense distribution of nearly spherical particles with a high degree of agglomeration, suggesting strong interparticle interactions. The uniform morphology implies controlled nucleation and stabilization by plant phytochemicals. Previous studies have shown that plant extracts act as effective capping agents, influencing particle size and shape[20]. This morphology is advantageous for enhanced surface reactivity, making ZnO NPs suitable for antimicrobial and photocatalytic applications, as shown in **Figure 6**.

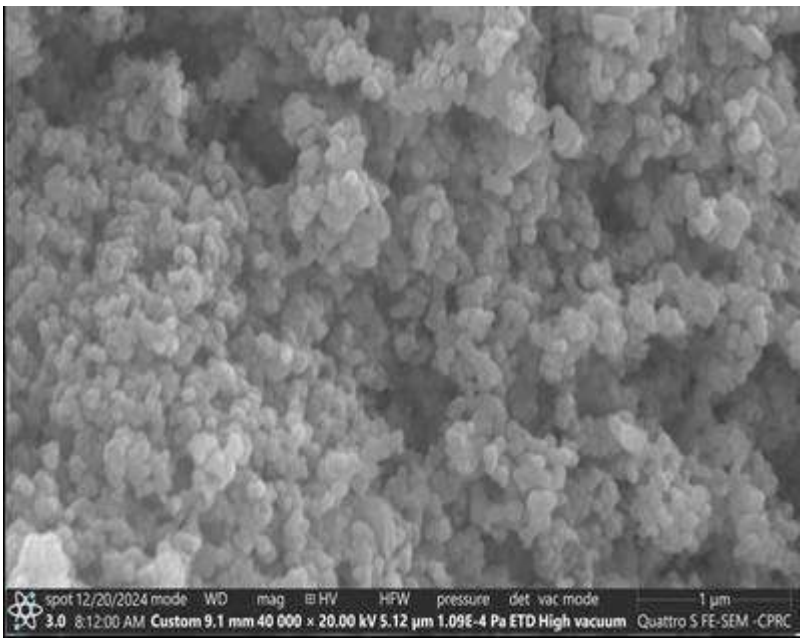

Figure 6. FESEM of ZnO NPs.

Figures 7 and 8 presented the antibacterial activities of CuO and ZnO nanoparticles synthesized via green methods compared with the standard drug sulfadiazine against Bacillus subtilis and E. coli. Against B. subtilis, sulfadiazine inhibited at 50 µg/mL and 100 µg/mL doses with zones of 11 and 19 mm, respectively. In comparison, CuO nanoparticles exhibited 14 mm and 20 mm zones, while ZnO showed superior activity with 16 mm and 23 mm. For E. coli, sulfadiazine produced 15 mm and 22 mm inhibition, while CuO (A1) gave 12 mm and 20 mm, and ZnO (A2) showed 14 mm and 23 mm at the same concentrations. These results indicated that ZnO nanoparticles displayed the most potent antibacterial effect among the tested samples at 100 µg/mL, outperforming even the standard drug. Overall, both CuO and ZnO nanoparticles exhibited concentration-dependent antimicrobial activities, with ZnO demonstrating the highest efficacy[21,22].

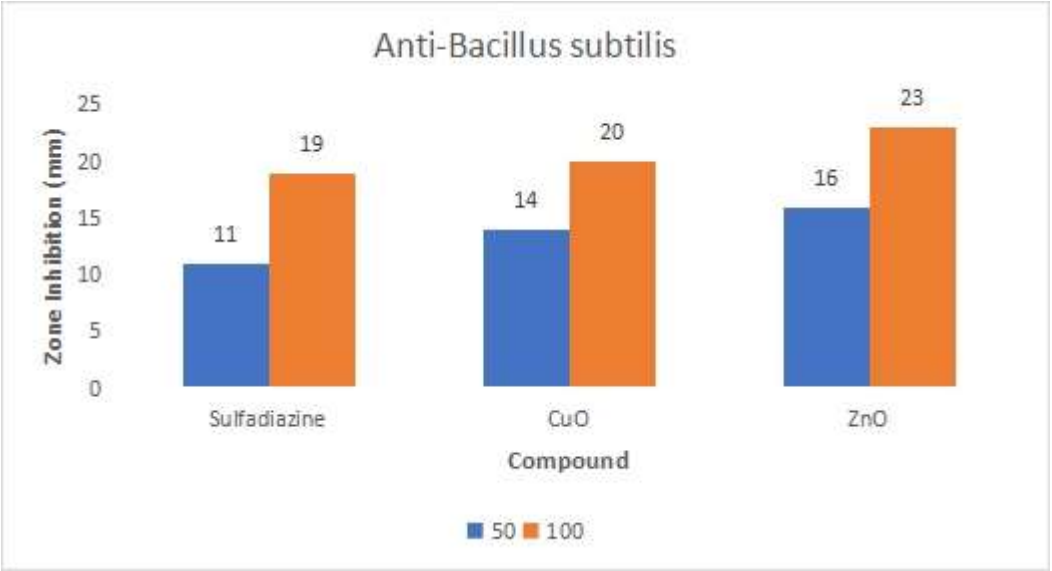

Figure 7. Biological activity of synthesized nanomaterial oxides against *B. subtilis*.

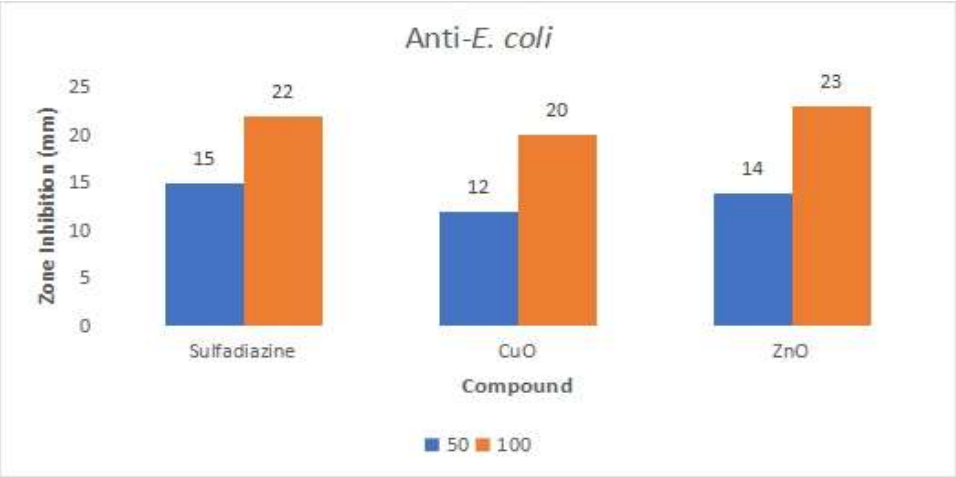

Figure 8. Biological activity of synthesized nanomaterial oxides against *E. Coli.*

Table 1 demonstrates the cytotoxic effects of CuO and ZnO NPs against MCF-7 across a range of concentrations. Both nanoparticles showed dose-dependent inhibition of cell viability, with ZnO NPs exhibiting greater cytotoxicity than CuO NPs at all tested concentrations. At 20 ppm, CuO NPs reduced cell viability to 67.01% (SD = 0.89), while ZnO NPs reduced it more significantly to 59.86% (SD = 1.67). This trend continued at higher concentrations: at 80 ppm, CuO caused 41.86% viability (SD = 2.50) versus 31.54% for ZnO (SD = 1.21). At the highest concentration tested (320 ppm), CuO NPs resulted in 17.87% viability (SD = 1.54), whereas ZnO NPs showed a more substantial effect with viability reduced to 10.66% (SD = 1.38). The calculated IC_{50} value for CuO NPs was approximately 30.86 µg/mL, indicating moderate cytotoxicity, while ZnO is expected to have a lower IC_{50}, confirming its superior anticancer activity, as shown in **Figures 9-12**[23,24].

Table 1. C50 rates of CuO and ZnO NPs on MCF-7 cell viability.

Concentration (PPM)	CuO NPs		ZnO NPs	
	Mean	SD	Mean	SD
0	100.9808	4.563203	100.624	2.393088
20	67.00633	0.893023	59.86013	1.668155
40	54.801	2.884989	48.07307	1.749642
80	41.86494	2.506247	31.54097	1.214433
160	27.82157	0.657361	18.66087	1.29343
320	17.866	1.53592	10.65667	1.378002

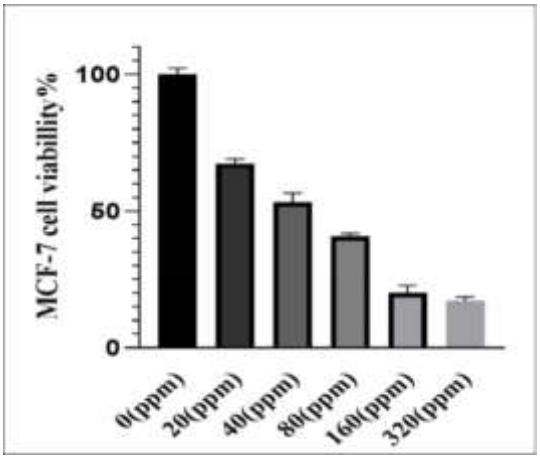

Figure 9. Cytotoxicity of CuO against MCF-7.

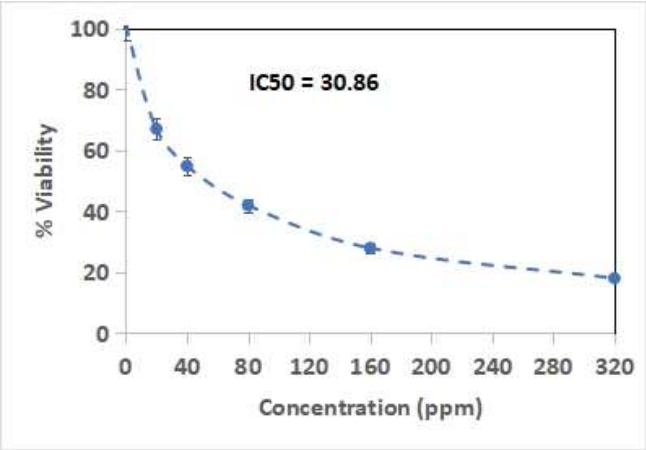

Figure 10. IC50 of CuO against MCF-7.

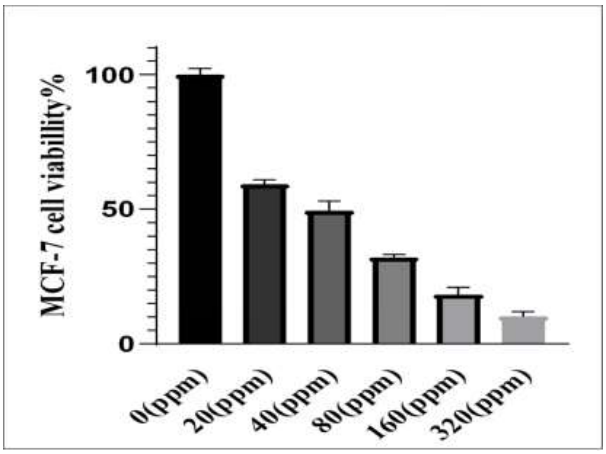

Figure 11. Cytotoxicity of ZnO against MCF-7.

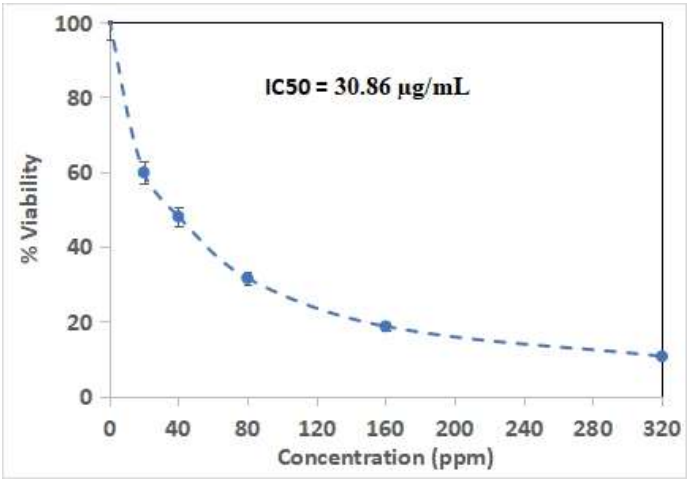

Figure 12. IC50 of ZnO against MCF-7.

4. Conclusion

This study demonstrates that green synthesis using lemon peel extract effectively produces CuO and ZnO nanoparticles with significant antibacterial and anticancer activities. The use of eco-friendly methods not only reduces environmental hazards but also introduces cost-effective strategies for biomedical applications. The characterized nanoparticles, confirmed via FTIR, FESEM, EDX, and XRD, exhibit distinct morphologies and bioactive surfaces that enhance their therapeutic potential. ZnO nanoparticles consistently outperform CuO in both antimicrobial and cytotoxic assays, likely due to their enhanced surface reactivity and stronger oxidative stress induction. Consequently, ZnO nanoparticles reduce MCF-7 cell viability more effectively and display lower IC_{50} values, indicating greater potency. If these findings are validated in vivo, they could lead to the development of novel nanomedicine alternatives to conventional drugs, especially in the context of antibiotic resistance and breast cancer treatment. Moreover, the plant-based synthesis route offers sustainable scalability for pharmaceutical use. We will complete the other applications by evaluating these nanoparticles in animal models and testing their biocompatibility and pharmacokinetics. Future studies should also investigate synergistic effects when these nanoparticles are combined with standard chemotherapeutic agents. Overall, this research lays a foundation for integrating green nanotechnology into next-generation therapeutic platforms.

Ethical issues

Not applicable.

Informed consent

Not applicable.

Funding

No funding is received for doing this study.

Data and Materials Availability

The research data are accessible to researchers, and the compiled data will be made available on reasonable request to the corresponding author.

Author contribution

Asmaa A. Jawad conceptualized the idea, and She collected the data and executed the work, and She prepared the draft version with revised the article draft.

Conflict of interest

No authors disclose conflicts of interest.

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