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Synthesis of Copper Oxide Nanoparticles Using Fruit Peel Extract of *Actinidia deliciosa* (Kiwi) for Antimicrobial Activity

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ABSTRACT

Copper oxide nanoparticles (CuO NPs) were synthesized using *Actinidia deliciosa* (Kiwi) fruit peel extract via a microwave-assisted green synthesis method. The X-ray diffraction (XRD) analysis confirmed the monoclinic CuO phase, with crystallite sizes calculated at approximately 9 nm. Fourier-transform infrared spectroscopy (FTIR) results revealed characteristic bands for O-H, C-H, C=C, C-OH, and Cu-O functional groups, confirming the presence of bioactive molecules from the kiwi peel

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extract stabilizing the nanoparticles. Scanning electron microscopy (SEM) revealed spherical and agglomerated particles, while energy dispersive X-ray (EDX) spectroscopy confirmed the elemental composition of copper (36.95%) and oxygen (52.09%). Antibacterial activity was evaluated using the agar well diffusion method against four pathogens: *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, and *Pseudomonas aeruginosa*. Significant zones of inhibition were observed, with inhibition zones ranging from 9.5 to 17.8 mm, demonstrating strong antimicrobial activity. The CuO NPs exhibited concentration-dependent antibacterial effects, with higher concentrations (600 µg/mL) showing maximum inhibition. These results support the potential of kiwi peel-derived CuO NPs as an eco-friendly antimicrobial agent.

Keywords: Copper oxide nanoparticles, Green synthesis, Antibacterial activity, *Actinidia deliciosa*, Microwave-assisted synthesis

1. Introduction

Antimicrobial resistance (AMR) had intensified into a major global health challenge, driven by the overuse and misuse of antibiotics and the slow pace of discovery of new antimicrobial agents. International policy and research agendas had therefore emphasized the need for alternative strategies that could either complement existing antibiotics or provide new broad-spectrum antimicrobial platforms, particularly against clinically relevant Gram-positive and Gram-negative pathogens [1], [2]. Among the emerging options, nanotechnology-based antimicrobials had attracted sustained attention because nanoscale materials had offered high surface reactivity, tunable physicochemical properties, and multi-target mechanisms that were harder for microbes to evade through single-gene resistance pathways [3-6].

Metal and metal-oxide nanoparticles had been widely investigated as bactericidal agents, and copper oxide (CuO) nanoparticles had stood out due to their relative affordability, ease of synthesis, and strong antimicrobial performance. Earlier studies had demonstrated that metal-oxide nanoparticles could inactivate bacteria through combined effects such as membrane damage, oxidative stress, and disruption of intracellular biomolecules [3-5]. Specifically, CuO nanoparticles had been reported to exert antibacterial effects through (i) contact-mediated injury to the bacterial envelope, (ii) generation of reactive oxygen species (ROS) that induced

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oxidative stress, and (iii) release of copper ions that interfered with proteins and nucleic acids, collectively impairing essential cellular processes [6-8]. Because these actions had occurred simultaneously, CuO nanoparticles had been considered promising candidates for applications ranging from surface disinfection to biomedical materials and dentistry-related antimicrobial formulations [7], [8].

Despite their promise, conventional CuO nanoparticle synthesis routes had often relied on harsh reagents, high energy inputs, and conditions that generated undesirable byproducts. In response, "green synthesis" approaches had been increasingly adopted to reduce environmental burden and improve biocompatibility by using biological materials as reducing and stabilizing agents [9-14]. Plant-mediated synthesis had been particularly attractive because plant extracts had contained diverse phytochemicals (e.g., phenolics, flavonoids, sugars, and organic acids) that could coordinate metal ions, promote nucleation, and cap growing nanoparticles, thereby influencing particle size, surface chemistry, and stability [13], [14]. Review evidence had indicated that greener CuO synthesis strategies had not only reduced chemical hazards but had also produced functional nanoparticles suitable for antimicrobial and environmental applications [9-11]. In addition, microwave-assisted processing had been used to accelerate reaction kinetics, shorten synthesis time, and enhance product uniformity, making it a practical intensification technique for green routes [15], [16].

Agro-waste and fruit peels had been increasingly viewed as valuable bioresources for nanoparticle synthesis because they had been abundant, low-cost, and rich in bioactive compounds. Recent studies had used peel-derived extracts to produce CuO nanostructures, including microwave-assisted methods and hybrid approaches that improved efficiency and functionality [17-19]. In this context, the peel of *Actinidia deliciosa* (kiwi fruit) had been a particularly relevant waste stream, because kiwifruit peel had contained substantial phenolic content and antioxidant constituents that could support metal-ion reduction and nanoparticle stabilization [20-23]. Analytical profiling studies had shown that kiwifruit peel had a stronger phenolic profile than inner tissues in many cultivars, supporting its potential as a green synthesis medium as well as a source of bioactive surface ligands that could

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contribute to antimicrobial action [20-23].

Based on these considerations, the present work had aimed to develop an eco-friendly synthesis of CuO nanoparticles using peel extract of *Actinidia deliciosa* (kiwi fruit) and to explore their antimicrobial potential. A microwave-assisted, plant-mediated route had been selected to combine the benefits of rapid energy delivery with phytochemical-driven reduction/capping [16], [19]. The antibacterial performance had been positioned within the broader trend of CuO nanoparticle deployment in biomedical-relevant contexts, including antimicrobial scaffolds, dentistry-related materials, and pathogen control studies [24-28]. Overall, synthesizing CuO nanoparticles from kiwi peel extract had aligned with waste valorization and green chemistry goals while addressing the urgent demand for effective antimicrobial alternatives in the AMR era [1], [2], [8].

2. Experimental

2.1. Materials

All chemical reagents used were of analytical grade, including cupric acetate monohydrate ($Cu(CH_3COO)_2 \cdot H_2O$) (UNI-CHEM, 99%) and sodium hydroxide (NaOH) (Loba, 98%). The peel of *Actinidia deliciosa* (Kiwi) fruit was collected from Baffa, Mansehra, Pakistan. Deionized water (DW) was used throughout the experimental work.

2.2. Extract Preparation

The peel of *Actinidia deliciosa* (Kiwi) fruit was washed thoroughly several times with tap water followed by DW to remove adhered dust and surface impurities, and it was then air-dried. After drying, the peel material was ground into a fine powder using a grinder. For extraction, 20 g of the powder was mixed with 200 mL DW in a 1000 mL conical flask and stirred for 20 min. The mixture was heated to the boiling point and boiled for 10 min under continuous stirring. After cooling to room temperature, stirring was continued for an additional 20 min. The extract was then filtered using Whatman filter paper, and the resulting filtrate was stored at approximately 4 °C for subsequent use.

2.3. CuO Synthesis

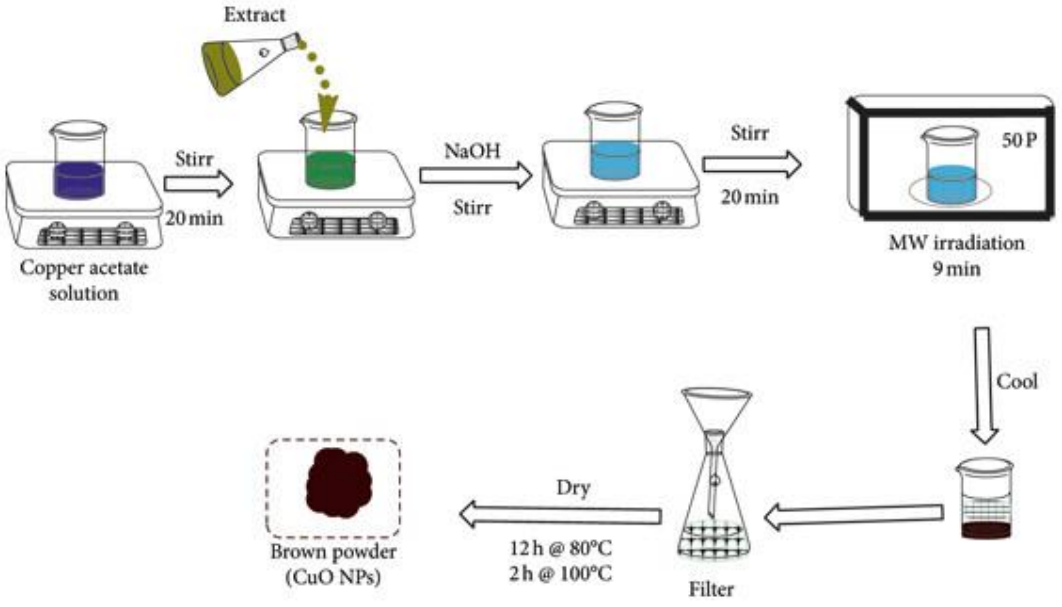
CuO nanoparticles (CuO NPs) were synthesized using a microwave (MW)-assisted

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modified method with $Cu(CH_3COO)_2 \cdot H_2O$ and the peel of *Actinidia deliciosa* (Kiwi) fruit extract. In a typical procedure, 6.2 g of $Cu(CH_3COO)_2 \cdot H_2O$ was dissolved in 100 mL DW in a 1000 mL beaker and stirred for 20 min. Subsequently, 10 mL of the peel extract was added to the copper precursor solution under constant stirring. The pH of the reaction mixture was adjusted by adding NaOH solution dropwise while stirring. After 20 min of stirring, the mixture was transferred to a domestic microwave oven (maximum power 1000 W), and the reaction was carried out at 50% power for 9 min. The resulting brown precipitate was collected and washed sequentially with DW and ethanol. The product was dried at 80 °C for 12 h, followed by further drying at 100 °C for 2 h. Finally, the dried material was ground into a fine powder using a mortar and pestle. The synthesis workflow is presented in Scheme 1.



Scheme 1: Flow chart for CuO NPs synthesis by the MW method [14]

2.4. Characterization

The crystal structure and phase composition of the as-prepared samples were analyzed by X-ray diffraction (XRD) using a Shimadzu XRD-7000 diffractometer with Cu Kα radiation (λ = 1.5406 Å). Fourier transform infrared (FTIR) spectra were recorded using a PerkinElmer Spectrum 65 FT-IR spectrometer with a

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resolution of 4 cm^{-1} over the range 4000–400 cm^{-1}, employing KBr pellets. Surface morphology and elemental composition were examined using SEM-EDX (COXIEM-30).

2.5. Evaluation of Antibacterial Activity Using the Agar Well Diffusion Method

The antibacterial activity of CuO NPs was evaluated using the agar well diffusion method against four pathogenic bacterial strains: *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 19433), *Escherichia coli* (ATCC 25922), and *Pseudomonas aeruginosa* (ATCC 25668). The bacterial strains were obtained from the Applied Microbiology Laboratory, Department of Microbiology, Hazara University, Mansehra. An 18 h bacterial culture was prepared in nutrient broth. Muller Hinton agar (MHA) was prepared, poured into Petri dishes, and allowed to solidify. The bacterial cultures were evenly spread on the MHA surface using sterile cotton swabs. Wells of 6 mm diameter were made in the agar using a sterile cork borer. A stock suspension of CuO NPs was prepared by dispersing 500 mg of CuO NPs in DMSO to obtain a concentration of 500 mg/mL. Working concentrations of 150.00, 300.00, 450.00, and 600.00 µg/mL were prepared from the stock solution. The required volume (30 µL) for each concentration was calculated using the $C_1V_1 = C_2V_2$ relationship. Then, 30 µL of each CuO NP concentration was introduced into the corresponding wells. Amoxicillin (AMX) and DMSO were used as positive and negative controls, respectively. All plates were incubated at 37 °C for 24 h. After incubation, the diameters of inhibition zones around the wells were measured. Each experiment was performed in triplicate to ensure reproducibility.

3. Results and Discussion

3.1. XRD Analysis of CuO Nanoparticles

To investigate the crystallinity and phase of the synthesized material, the XRD technique was used. The XRD pattern of the CuO NPs obtained using peel of *Actinidia deliciosa* (Kiwi) fruit extract is displayed in Figure 1. The noticeable peaks positioned at 2θ values of 32.44°, 35.46°, 38.68°,48.7°, 53.50°, 58.0°, 61.49°, 66.14°, 67.86°, 72.38°, and 74.89° correspond to (110), (002), (111), (20–2), (020), (202), (11–3), (31–1), (113), (311), and (004) crystal planes, respectively. These values agree

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with the monoclinic CuO phase (JCPDS Card number 48–1548, C2/c space group). The absence of peaks matching $Cu(OH)_2$ and Cu_2O in the pattern with the appearance of all peaks corresponding to the monoclinic CuO phase signifies the formation of pure crystalline CuO by MW-assisted synthesis method. The crystallite size of the sample was calculated using the Scherrer equation:

$$D = \frac{k\lambda}{\beta \cos \theta}, \quad (3)$$

where D = crystallite size, λ = the wavelength of the X-ray source applied, β = the width of the peak at half of its height (FWHM), θ = the Bragg angle, and k = the shape constant ≈ 0.9. The average crystallite size of the CuO NPs calculated for the highest peaks at 35.46° and 38.68° was around 9 nm. The resolved sharp peaks with the crystallite size of about 9 nm suggest the formation of the nanocrystalline CuO [12].

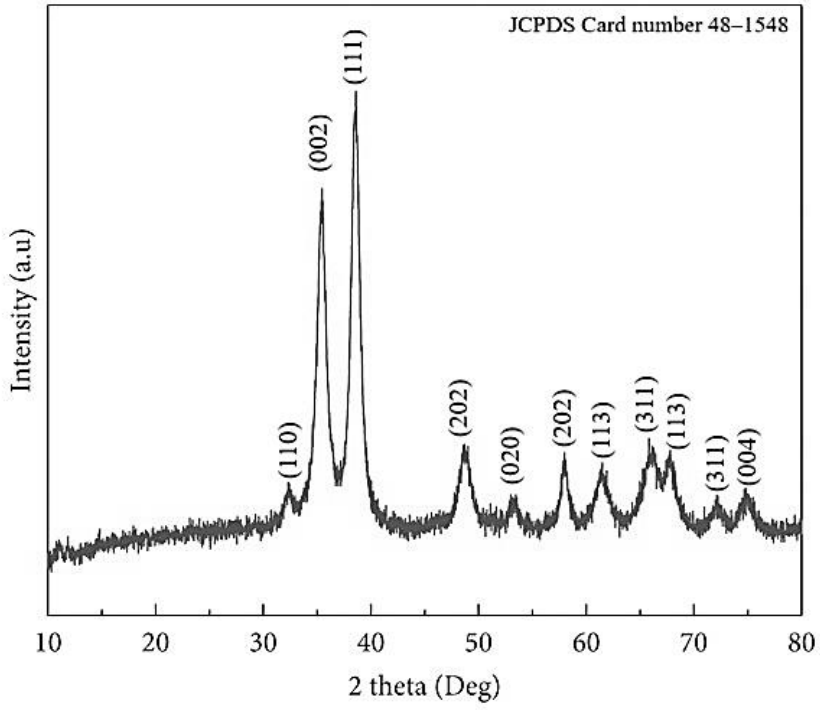


Figure 1: FTIR spectra of the CuO NPs synthesized from the peel of Actinidia deliciosa (Kiwi) fruit

3.1. FTIR of CuO Nanoparticles

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The FTIR spectra of the CuO NPs synthesized from the peel of *Actinidia deliciosa* (Kiwi) fruit are shown in Figure 2. Several characteristic bands were shown in the spectra of CuO NPs synthesized using the extract (Figure 2). The bands at 3400 and 2920 cm^{-1} correspond to the O–H and C-H functional groups stretching vibrational frequencies, respectively. Similarly, the bands at 1603 and 1300 to 1000 cm^{-1} (Figure 2) belong to the stretching vibrations of the C=C aromatic ring and C–OH. The sharp peaks that appeared at 1555 and 1393 cm^{-1} (Figure 2) could be attributed to the carboxylate C-O stretching vibrations (asymmetric and symmetric). The bands arising due to the vibrational frequencies of the metal-oxygen (M–O) bond generally appear in the region below 1000 cm^{-1}. Hence, the peaks positioned at 497 and 591 cm^{-1} belong to characteristic vibrations of Cu–O bond, revealing CuO NPs formation. No infrared active modes from Cu_2O were detected, indicating that, under the conditions stated in the synthesis method, only the CuO phase was selectively produced. Furthermore, the FTIR results indicate the formation of the CuO phase supporting the XRD result [13].

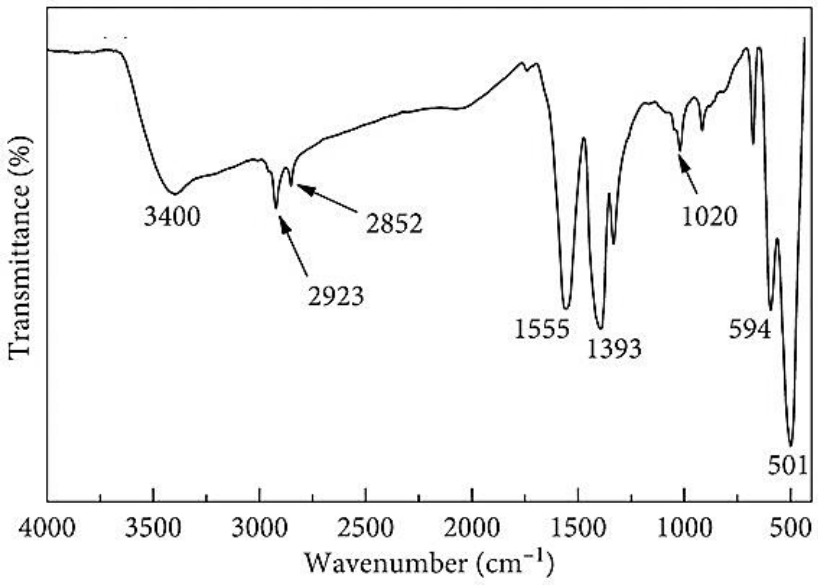


Figure 2: FTIR spectra of the CuO NPs synthesized from the peel of Actinidia deliciosa (Kiwi) fruit

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3.3. Morphology Analysis

The morphological and structural properties of the CuO nanoparticles were observed using SEM. The resultant images are shown in Figure 3. The synthesized nanoparticles had sizes which are in the nanometer range and their structures were not homogenous. These images indicate that only few nanoparticles with spherical shape was synthesized. Some nanoparticles were well separated from each other while most were present in agglomerated form. Thus, these SEM results confirmed the nanostructure behavior of the synthesized particles. The results are in agreement with previous reports [3,15] although with some slight differences due to chemical composition.

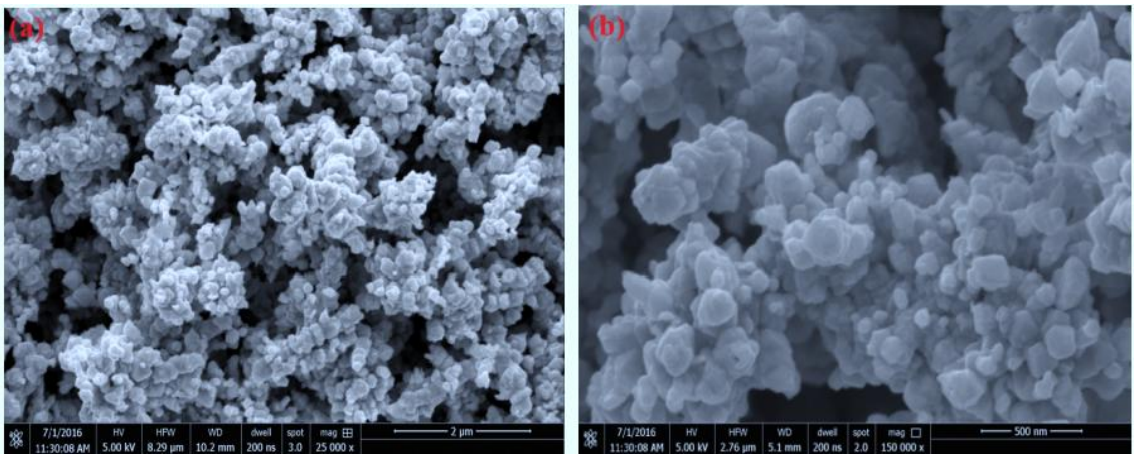


Figure 3: (A) SEM image of CuO nanoparticles taken at 2 µm, (B) SEM image of CuO nanoparticles taken at 500 µm

3.4. Energy dispersive x-ray spectra

EDX studies were carried out in order to confirm presence of CuO nanoparticles and also to examine the chemical formation and composition of the prepared sample. The EDX spectra are shown in Figure 4. It confirms the presence of all constituent elements, i.e., copper (Cu), oxygen (O), sulphur (S), phosphorus (P) and silicon (Si) in the CuO nanoparticles synthesized. The constituent elements are represented in Table 1. The EDX spectra also confirmed the presence CuO in the prepared sample. It is clear from Figure 3 that the EDX pattern confirmed successful formation of CuO nanoparticles with the leaf extract of Abutilon indicum. The EDX peak positions were consistent with copper oxide, and the sharp peaks of EDX indicated

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that the synthesized CuO nanoparticles had a crystalline structure. The other peaks observed in the EDX spectrum were due to impurities from Si, P and S which were present in the substrate. The strong intensity and narrow width of CuO diffraction peaks indicate that the resultant products were highly crystalline in nature. Hence we can conclude that the green fuel played profound role in controlling particle size. These findings are in close agreement with previous reports [16] but with slight difference due to variations in chemical composition.

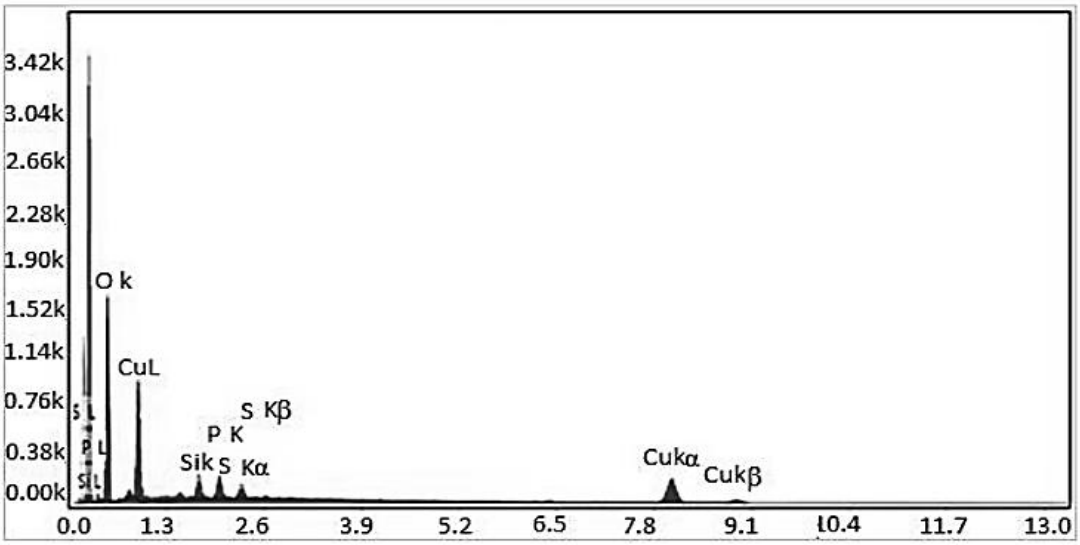


Figure 4: EDX spectra of copper oxide nanoparticles

Table 1: ***Constituent elements and their percentage values***

Element	Atomic (%)	Weight (%)	Net Int.	Error (%)
O-K	52.09	77.5	264	7.64
Si-K	3.8	3.22	36.89	13.45
P-K	4.2	3.47	41.31	13.09
S-K	2.64	1.96	27.02	15.78
Cu-K	36.95	13.84	90.14	5.11

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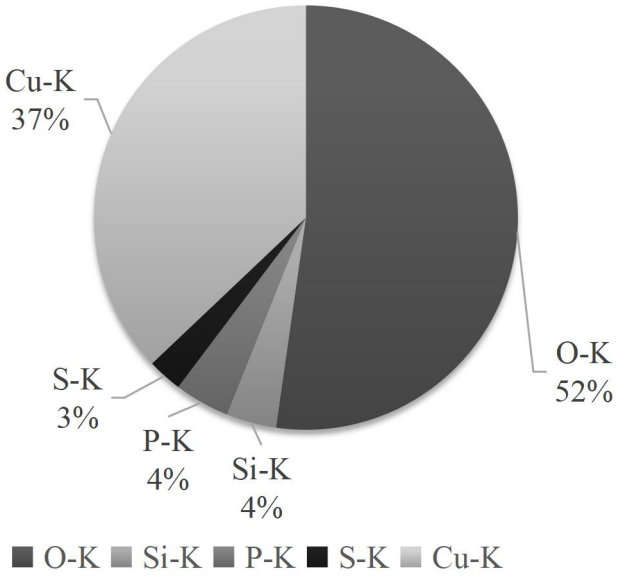


Figure 5: Elemental Composition of CuO nanoparticles

3.5. Antibacterial Activity

The biosynthesized CuO-NPs exhibited pronounced antibacterial efficacy against a range of bacterial pathogens. Significant zones of inhibition were observed for pathogens such as *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, and *Pseudomonas aeruginosa*, as shown in Figure 6a [28]. In contrast, dimethyl sulfoxide (DMSO) was used as a negative control and did not demonstrate any inhibitory activity against bacterial cells, thereby underscoring the antibacterial ability of CuO-NPs [29].

A prominent mechanism through which CuO-NPs exert their antibacterial action is believed to be their interaction with bacterial cells, primarily occurring via the thiol groups present in bacterial proteins. This interaction synergistically amplifies the inherent antibacterial properties of CuO-NPs. Moreover, a concentration-dependent trend was observed, where the zone of inhibition expanded with increasing doses of CuO-NPs, suggesting a dose-dependent relationship between their antibacterial efficacies (Figure 6). In the broader spectrum of nanoparticle research, metal oxide nanoparticles such as CuO-NPs have been spotlighted for their potential in antimicrobial applications. Their mode of action,

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which involves the disruption of bacterial cell membranes or interference with essential cellular processes, provides an avenue for potential therapeutic applications, especially in an era in which antibiotic resistance is a rising concern [33,34].

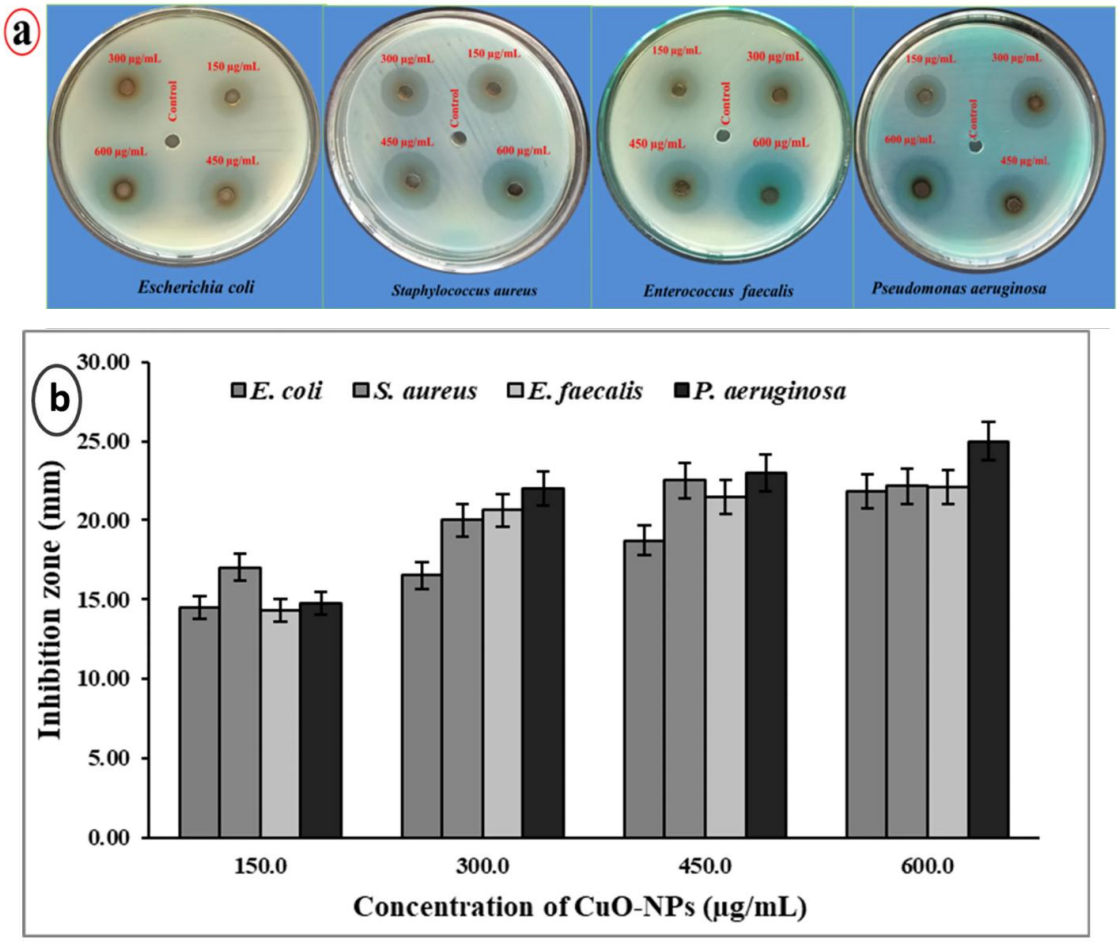


Figure 6. (a) Antibacterial activity of CuO-NPs exerted via agar well diffusion and (b) zone of inhibition with bacterial pathogens.

Conclusion

In this study, copper oxide nanoparticles (CuO NPs) were successfully synthesized using the peel extract of *Actinidia deliciosa* (Kiwi) fruit through a microwave-assisted green synthesis method. The X-ray diffraction (XRD) analysis confirmed the formation of crystalline CuO with a monoclinic phase, and the crystallite size was estimated to be approximately 9 nm. Fourier-transform infrared spectroscopy (FTIR) revealed characteristic functional groups, including O-H, C-H, C=C, and Cu-O vibrations, which indicated the involvement of bioactive molecules from the kiwi

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peel extract in stabilizing the nanoparticles. Scanning electron microscopy (SEM) images showed spherical nanoparticles, but agglomeration was observed, indicating the typical behavior of nanoparticles during drying. Energy dispersive X-ray (EDX) analysis verified the elemental composition, with copper accounting for 36.95% and oxygen for 52.09%, confirming the successful synthesis of CuO NPs.The antimicrobial potential of the synthesized CuO NPs was evaluated against four pathogenic bacterial strains: *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, and *Pseudomonas aeruginosa*. The CuO NPs exhibited significant antibacterial activity, with inhibition zones ranging from 9.5 mm to 17.8 mm, depending on the concentration. The highest concentration (600 µg/mL) showed maximum inhibition, confirming a concentration-dependent antibacterial effect. Notably, CuO NPs demonstrated stronger inhibition against Gram-positive bacteria (*Staphylococcus aureus* and *Enterococcus faecalis*) compared to Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*), likely due to differences in bacterial cell wall structure. This study highlights the effective and eco-friendly synthesis of CuO NPs using kiwi peel extract, showcasing their potential as a green antimicrobial agent. The results demonstrate that CuO NPs synthesized through this method have promising applications in medical and environmental fields, offering an alternative to traditional chemical-based antimicrobial agents.

Declaration of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper. The research was conducted independently, and all authors have contributed equally to the design, analysis, and writing of the manuscript. No financial or personal relationships have influenced the research outcomes.

Authors' Contributions

Irum Aziz & Maryam Mazam: Drafted the manuscript and provided critical revisions. Dr. Irfan Ullah Khan reviewed the manuscript revisions. Rida Zameer assisted with the preparation of the *Actinidia deliciosa* (Kiwi) fruit peel extract, conducted the XRD and FTIR analyses, and analyzed the experimental data. Masab Saeed contributed to the experimental design, statistical analysis, and interpretation of results. Samira Raouf provided guidance on the overall methodology and reviewed

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and finalized the manuscript. All authors have read and approved the final manuscript for submission.

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