

Research Article

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Facile, polyherbal drug-mediated green synthesis of CuO nanoparticles and their potent biological applications

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Abstract: Copper oxide nanoparticles (CuO NPs) were synthesized using ayurvedic medicine septilin. The septilin-mediated CuO NPs were characterized using UV–Vis, fourier-transform infrared spectroscopy, X-ray diffraction (XRD), scanning electron microscope (SEM), and transmission electron microscope

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(TEM). The average particle size of CuO NPs was 8 nm as evident from TEM. Minimum inhibitory concentration of CuO NPs against *Escherichia coli*, *Pseudomonas aeruginosa*, methicillin-resistant *Staphylococcus aureus* (MRSA), and *Candida albicans* was found in the range of 1–2.5 mg·mL⁻¹. CuO NPs dose-dependently decreased the biofilm formation from 0.0315 to 2 mg·mL⁻¹, at the highest dose of 2 mg·mL⁻¹ of CuO NPs; 92.91%, 79.84%, and 71.57% decrease in biofilm was observed for *P. aeruginosa*, MRSA, and *C. albicans*, respectively. Down-regulation of biofilm upon treatment with nanoparticles (NPs) was also observed by SEM analysis. SEM analysis also showed the change in morphological structure, and deformities in bacterial and fungal cells upon treatment of NPs. Furthermore, the anticancer efficacy of NPs was assessed using colon cancer (HCT-116). The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay clearly showed the anticancer potential of NPs, as the concentration of CuO NPs increased, the number of viable cells decreased. The produced CuO NPs have promise for future investigations in many biological and therapeutic domains, including the treatment of microbial biofilm infections, as well as the inhibition of cancer cell growth.

Keywords: *C. albicans*, CuO NPs, multi-drug resistance, biofilm, colon cancer, polyherbal drug

1 Introduction

The multi-drug resistance (MDR) can be defined in a simpler form as the resistance developed by the microorganism against the lethal doses of antimicrobials. The MDR developed by microorganisms has become a health concern, specifically against pathogenic diseases [1]. The ineffectiveness of antimicrobials against MDR has risen many folds during the last few years, several reports have warned that MDR is a considerable risk to human health in European countries [2]. According to data from

the United States, the additional societal and healthcare costs associated with MDR diseases are estimated to 55 billion dollars per year [3]. The delay in developing new antimicrobial agents further worsens the situation [4]. The data collected from the different hospitals in the United States reports 40–60% of *Staphylococcus aureus* strains to be methicillin resistant and, in some cases, even vancomycin and carbapenem resistant [5].

Biofilms are the communities of microorganism that are complex and attach to the surface or substratum. These complex communities are enclosed in a self-polymer matrix which is composed of polysaccharide, proteins, and DNA [6]. The formation of biofilm by microorganisms is one of the adaptive features to survive in harsh environmental conditions [7]. The biofilm provides a better adaptive environment for survival compared to the planktonic cells, which includes a stronger ability to grow in an oligotrophic environment [8], and provides better nutrition [9] and improved organism productivity and their interactions [10].

Colon cancer, also called colorectal cancer (HCT-116), is one of the most common cancers in developing and developed nations [11,12]. Colorectal cancer is the third most cancer in the category of malignant tumors occurring in humans, and the mortality rate is increasing globally with a decrease in survival rate [13]. Treatment of colorectal cancer is still an unsolved issue, but for the sake of treatment, the only option left is chemotherapy [14].

Nanotechnology in today's world is not only confined to the electronics but also has shown promising results in biomedical, pharmaceutical, and environmental sciences [15]. Due to the involvement of nanotechnology in different aspects, it is believed that it can also play a key role in the improvement of human health [16]. Nanoparticles (NPs) can be produced via physical, chemical, or biological processes. A few drawbacks of physical and chemical processes are that they need high temperatures, while chemical methods need hazardous chemicals [17]. Biological method is the most preferred method since it is safer, cheaper, and nonhazardous and does not involve any toxic product [18,19]. Green route of NP synthesis has been used for different NPs, including silver [20], gold [21], platinum [22], and zinc [23]. The results from gold, silver, and platinum in terms of antimicrobial and antibiofilm are promising, but when it is compared with copper, it is cheaper and more effective than gold and silver [24]. Due to the easy availability of copper and being antimicrobial in nature, the copper oxide nanoparticles (CuO NPs) are gaining attention [25].

The use of nanomaterials in medicine is not new; it has been in practice since ancient times. Ayurveda is a traditional system of medicine that has been practiced in India

for seven centuries. In ancient times, people used metal ash, which is also called Bhasma, to treat different diseases [26]. Septilin is one of the ayurvedic drugs manufactured by Himalayan Drug Company (India), which contains extracts of several ayurvedic plants. Septilin drug has been extensively used in severe acute and chronic infections due to its antibacterial, anti-inflammatory, immunomodulatory, and immunopotentiating effects [27,28]. Keeping in view the importance of green synthesis of NPs, we have synthesized CuO NPs from the ayurvedic drug septilin and tested them for their potential pharmaceutical and medical applications, such as antimicrobial, antibiofilm, and anticancer.

2 Materials and methods

2.1 Preparation of septilin aqueous extract

Ayurvedic herbal medicine septilin (manufactured by Himalaya Company) was purchased from the local market. Few tablets (drugs) were taken and ground into the fine powder using mortar and pestle and passed through the muslin cloth. A rotary shaker was used for mixing 10 g of powdered septilin with 90 mL of sterile water for 30 min at 60 rpm. After that, the extract was filtered and preserved for subsequent use at 4°C.

2.2 Septilin-mediated biosynthesis of CuO NPs

Ten milliliters of septilin extract was added to 90 mL of copper nitrate solution. The solution was subsequently left on the stirrer for 24 h, after which an apparent change in color was seen.

2.3 Characterization techniques

The septilin-mediated NPs were scanned using UV-Vis Spectrophotometer (UV-Vis) at the wavelength of 230–330 nm. Fourier-transform infrared spectroscopy (FTIR) was performed in the range of 4,000–500 cm^{-1} at room temperature. FTIR has been used to identify the functional groups that are present in the synthesized CuO NPs. It utilizes the energy absorption bands associated with each chemical bond to determine the structural and bonding information of the complex. This allows for the identification of the bonding type and strength of the

bond. To know the morphology and size of NPs, scanning electron microscope (SEM) and transmission electron microscope (TEM) were performed by the method previously described [29]. X-ray diffraction (XRD) (Rigaku, Pittsburg, PA, USA) analysis was performed to know the nature of NPs, whether amorphous or crystalline at 2θ ranges from 20° to 70° at 40 keV.

2.4 Determination of minimum inhibitory concentration (MIC)

MIC of green-synthesized CuO NPs was determined using the microbroth dilution method previously described [30]. The bacterial strains, i.e., *Escherichia coli*, MDR-*Pseudomonas aeruginosa* (MDR-PA), methicillin-resistant *S. aureus* (MRSA), and *Candida albicans* cultures, were treated to two-fold serial dilutions of CuO NPs and then incubated at a 37°C for 24 h. The MIC was tested using the brain heart infusion broth. The MIC value refers to the initial concentration of NPs at which no observable growth is detected [30].

2.5 Antibiofilm using crystal violet assay

The antibiofilm potential of NPs was evaluated using the method previously described [30]. Briefly, $100\ \mu\text{L}$ of mid-exponential culture of bacteria and fungus were incubated in a 96-well culture plates at 37 and 28°C for 24 h in a shaking incubator with or without CuO NPs. The wells were washed and 0.1% w/v crystal violet was added in each well for 30 min. After further washing, wells were filled with ethanol, and optical density was recorded at 595 nm.

$$\begin{aligned} &\text{Percent inhibition of biofilm} \\ &= \frac{1 - \text{Absorbance of cells treated with nanoparticles}}{\text{Absorbance of untreated cells}} \\ &\quad \times 100 \end{aligned}$$

2.6 SEM analysis of interaction of CuO NPs with bacterial/fungal cells

The interaction of bacterial and fungal cells was observed using SEM as previously described [31]. Briefly, the overnight-grown bacterial and fungal cells were treated with CuO NPs and then incubated for 16 h at 37°C . After the period of incubation, the samples underwent centrifugation for a duration of 15 min. The resulting pellets were subsequently subjected to four rounds of washing using

PBS. Following a fixation step using 2.5% glutaraldehyde and 1% osmium tetroxide, it was dehydrated with 20, 30, 40, 50, 60, 70, 80, 90, and 100% ethanol. Subsequently, the samples were placed onto the aluminum stubs and then coated with gold. The impact of NPs on the structure of tested pathogens was examined using an SEM operating at 20 kV [30].

3 Cytotoxicity analysis

3.1 Anticancer activity

Cell proliferation test was used to examine the impact of different concentrations of NPs on colon cancer cell viability using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay as the method described in our previous study [30]. In brief, the cells were cultured at 37°C in dulbecco's modified eagle medium with 1% penicillin–streptomycin, 1% L-glutamine, and 10% fetal bovine serum. The culture was maintained in a humid room with a 5% CO_2 atmosphere. The cells were subsequently treated to a concentration range of $11\text{--}118\ \mu\text{g}\cdot\text{mL}^{-1}$ of NPs for a duration of 48 h, after which they were prepared for the cell viability experiment. No NPs were added to the untreated control. Following 4 h of MTT ($5.0\ \text{mg}\cdot\text{mL}^{-1}$) incubation, cell viability was calculated at 570 nm using the below formula:

$$\text{Cell viability (\%)} = \text{OD of sample} / \text{OD of control} \times 100$$

3.2 Microscopic analysis

Under an inverted microscope, the structural morphology of HCT-116 cells was examined. In brief, HCT-116 cells were exposed to different doses of CuO NPs and then incubated for a duration of 48 h. Following a 48-hour treatment period, the cells were rinsed and the impact of NPs on the morphology and cellular proliferation of HCT-116 was assessed by observing them under an inverted microscope.

4 Result

4.1 Synthesis and characterization of CuO NPs

The schematic illustration (Figure 1) shows the formation of CuO NPs from the drug septicin. UV–Vis spectra show the intense peak at 285 nm, which is due to the surface plasmon resonance (Figure 2). FTIR spectra showed

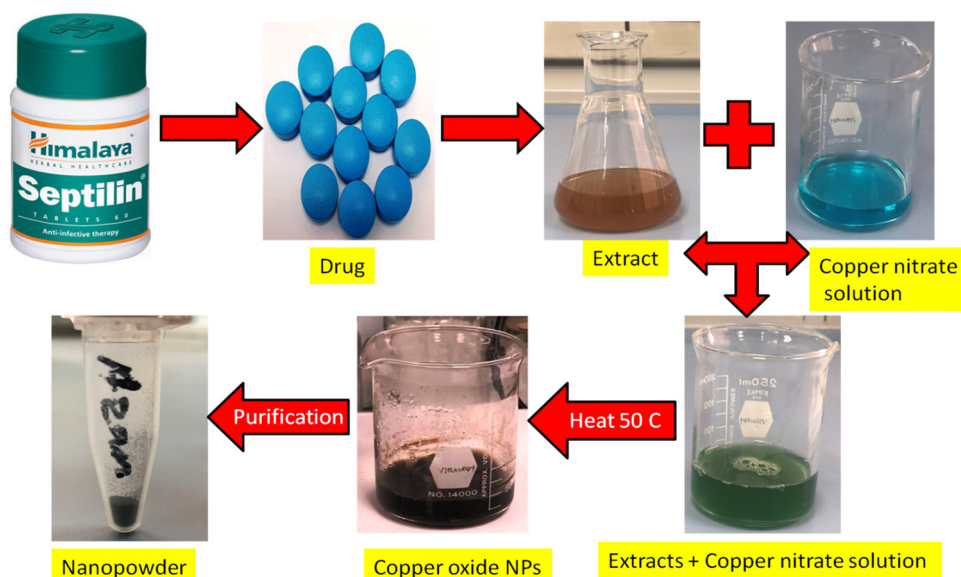


Figure1: Scheme of green synthesis of CuO NPs.

various peaks at 3,243, 2,925, 1,697, 1,576, 1,325, 1,019, 730, and 517, which corresponds to OH stretching, C–H asymmetric, C=C stretching, C=C stretching, OH bending, C–OH bending, C=C bending, and halo compounds, respectively (Figure 3). The XRD peaks of CuO NPs were located at $2\theta = 32.2^\circ, 35.2^\circ, 38.8^\circ, 48.6^\circ, 53.2^\circ, 57.8^\circ, 61.3^\circ,$ and 67.8° mainly attributed to the (110), (002), (111), (202), (020), (202), (113), and (220) planes, respectively, that represent the monoclinic structure of CuO NPs (Figure 4). The crystalline size calculated was 10.88 nm. SEM represents the surface morphology (Figure 5a), and it shows that the NPs formed are segregated not clumped, although the better picture is represented by TEM (Figure 5b), where the individual NPs can be more clearly seen and average size was found 8 nm (Figure 5c).

5 Antimicrobial activity

5.1 MIC

MIC for gram-negative *E. coli* and MDR-PA accounts for $2.5 \text{ mg}\cdot\text{mL}^{-1}$, whereas the MIC for gram-positive MRSA was $1 \text{ mg}\cdot\text{mL}^{-1}$ and it was $2.5 \text{ mg}\cdot\text{mL}^{-1}$ for *C. albicans*.

5.2 Antibiofilm activity

Inhibition of biofilm was observed at all the tested concentrations. The decrease in biofilm for *P. aeruginosa* ranged from 28.47% to 92.91%. At the lowest dose, i.e., $0.0315 \text{ mg}\cdot\text{mL}^{-1}$, a 28.47% decrease in biofilm was observed, whereas at the

highest dose, i.e., $2 \text{ mg}\cdot\text{mL}^{-1}$, the maximum reduction of 92.91% in biofilm was observed. Similarly, a 24.8–79.84% decrease in biofilm was observed for MRSA. The lowest concentration of CuO NPs ($0.0315 \text{ mg}\cdot\text{mL}^{-1}$) decreased the biofilm by 24.8% and, at $2 \text{ mg}\cdot\text{mL}^{-1}$ of CuO NPs, the maximum decrease of 79.84% was observed. For *C. albicans* decrease in biofilm ranged from 16.45% to 71.57%. At the lowest concentration of CuO NPs ($0.0315 \text{ mg}\cdot\text{mL}^{-1}$), a 16.45% decrease was observed, whereas a 71.5% decrease in biofilm was observed at $2 \text{ mg}\cdot\text{mL}^{-1}$ of CuO NPs (Figure 6).

5.3 Interaction of bacterial/fungal cells

SEM analysis revealed the microscopic changes in *P. aeruginosa*, MRSA, and *C. albicans* upon treatment with CuO

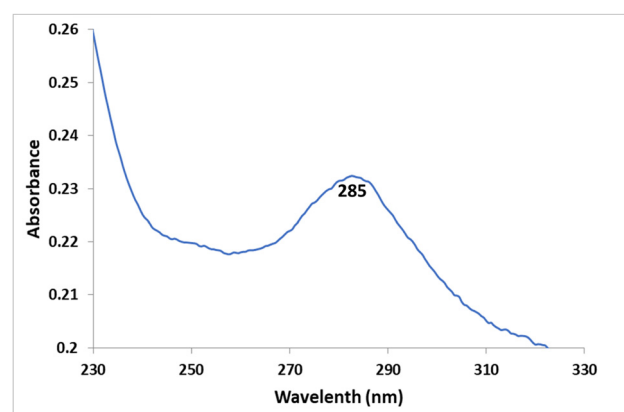


Figure 2: UV-Vis spectra of green-synthesized CuO NPs.

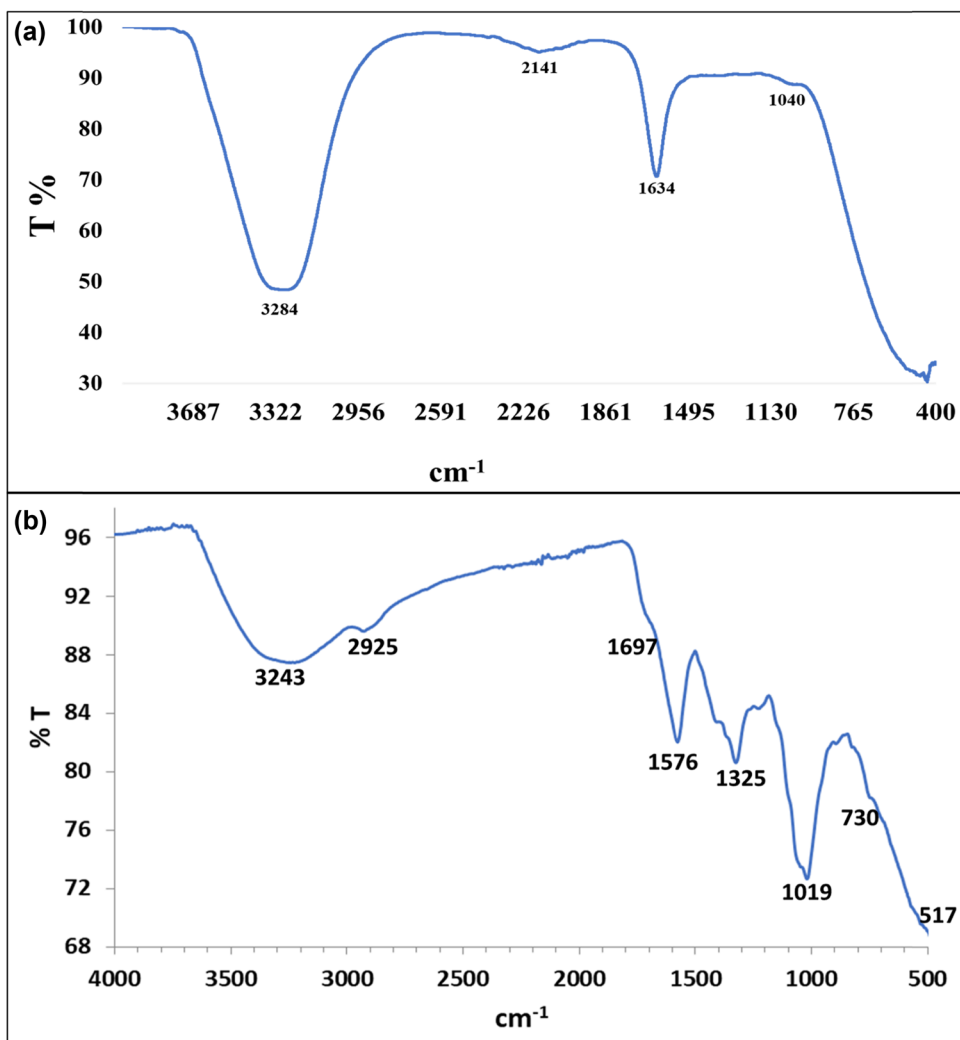


Figure 3: FTIR spectra of herbal drug extract (a) and green-synthesized CuO NPs (b).

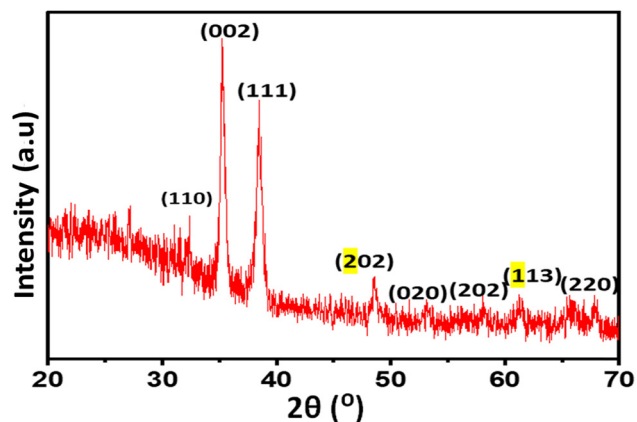


Figure 4: XRD pattern of as-prepared CuO NPs.

NPs. SEM images are indicative of structural changes in cells. Figure 7(a–c) shows the normal morphology of *P. aeruginosa*,

MRSA, and *C. albicans*. In contrast, treated cells Figure 7(a1–c1) shows the destruction in cell morphology, shrinkage in cell size, and distorted cell structures.

5.4 Anticancer potential of CuO NPs

A statistical decrease in cell viability (HCT-116 cell line) was observed upon treatment with CuO NPs at all tested concentrations as revealed by MTT (Figure 8). At 11.80 $\mu\text{g}\cdot\text{mL}^{-1}$ of NPs, 80.62% of cells were viable, whereas at 23.60 $\mu\text{g}\cdot\text{mL}^{-1}$, only 60% of cells were viable. Furthermore, an increase in CuO NPs to 59.0 $\mu\text{g}\cdot\text{mL}^{-1}$ down-regulated the viability, and only 37.03% of cells remain viable, and at the highest dose, i.e., 118 $\mu\text{g}\cdot\text{mL}^{-1}$, the maximum decrease in viability was observed and only 10.15% of cells remain viable (Figure 9).

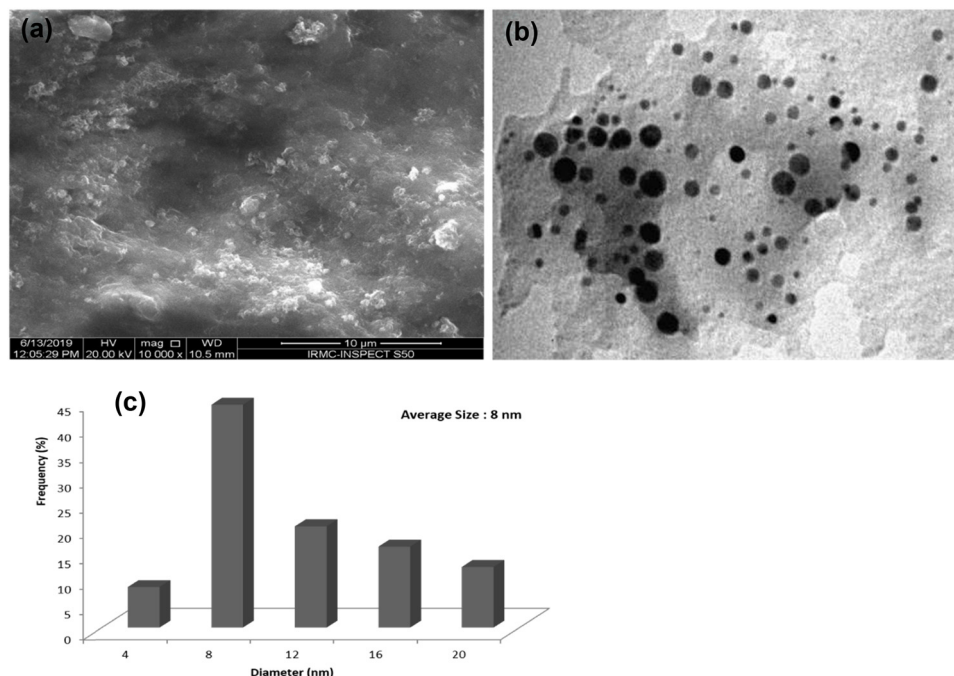


Figure 5: SEM (a), TEM, (b), and histogram (c) analysis.

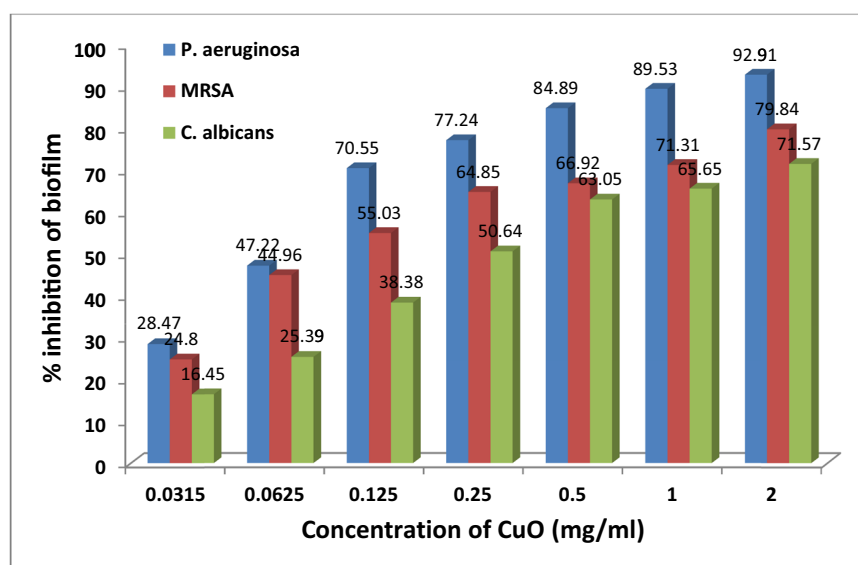


Figure 6: Effects of CuO NPs on biofilm-forming abilities of tested pathogens.

6 Discussion

The color change of septilin extract + copper nitrate solution was the initial sign of NP formation, which was further confirmed by the UV-Vis (Figure 1). The strong peaks detected at a wavelength of 285 nm are similar to the ultra-violet range of CuO NPs (Figure 2). An identical absorbance peak at a wavelength of 290 nm was observed for the CuO

NPs that were produced through green method [32]. The UV spectra results align with prior studies that have reported absorption peaks at around 250 and 272 nm for CuO NPs produced from *Caesalpinia bonducella* [33] and *Ephedra alata* [34] extracts, respectively. The highest absorbance peak of CuO NPs is influenced by various factors, including temperature, type precursor salts and plant extract, and the synthesis method used. However, values as low as 219–500 nm have

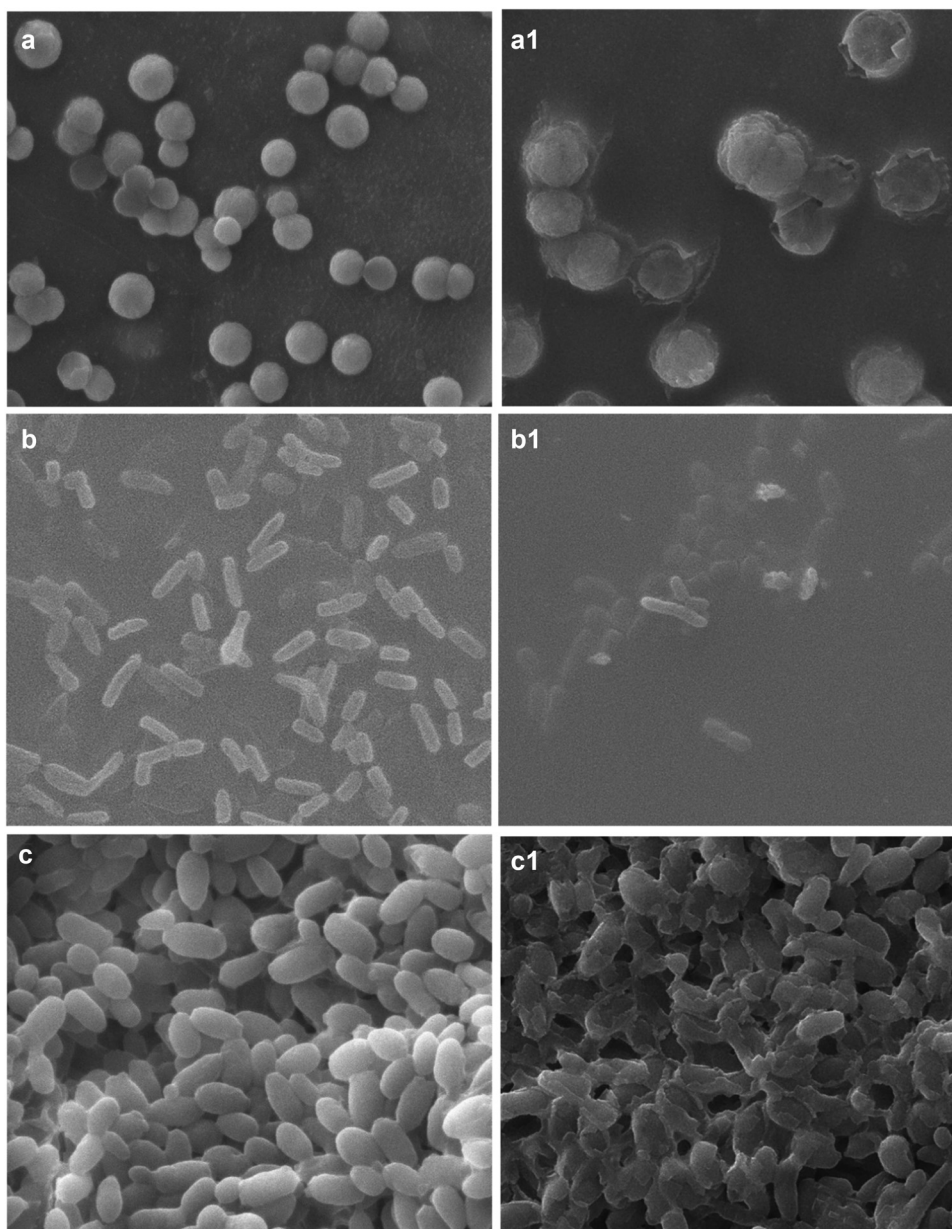


Figure 7: SEM analysis. (a) MRSA control and (a1) treated MRSA; (b) *P. aeruginosa* control and (b1) treated; and (c) *C. albicans* control and (c1) treated.

been documented for CuO NPs in the literature [35]. The medicinal extract from the septilin drug may function as a capping, reducer, and stabilizer agent, reducing copper nitrate into CuO NPs. FTIR analysis was conducted to locate the stretching and vibrating bonds in biosynthesized NPs and to locate the biomolecules present in the septilin extract that may act as reducing and capping agents (Figure 3). The peak at $3,284\text{ cm}^{-1}$ in extract (Figure 3a) and $3,243\text{ cm}^{-1}$ (Figure 3b) of green-synthesized CuO NPs correlates to the vibrational frequency of O–H stretching [35], whereas the peak in the herbal extract at $1,634\text{ cm}^{-1}$ indicates the C=O stretching of acids or ketones [33]. The spectral peaks seen at

$2,925\text{ cm}^{-1}$ correspond to the stretching of C–H bonds [34]. The prominent peaks observed at $1,325$, $1,576$, and $1,697\text{ cm}^{-1}$ correspond to the bending of phenolic O–H bonds and the stretching of C=O bonds in ketones [35,36]. The peaks at $1,019$ and 730 cm^{-1} corresponds C–O and C–H bonds, respectively [37]. The peaks at 517 cm^{-1} suggest the formation of a CuO nanostructure and the existence of Cu–O stretching [35,37]. The FTIR spectrum provides evidence that the plant extract contained carboxylic acid, carbohydrates, flavonoids, phenols, and alkaloids. These compounds facilitated the synthesis of NPs and functioned as capping and stabilizing agents [34,36].

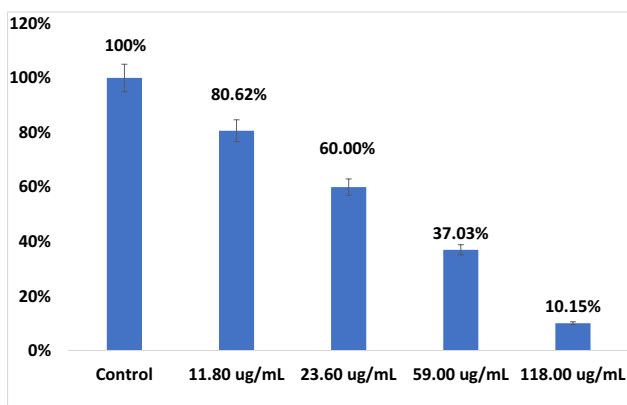


Figure 8: Cell viability analysis of HCT-116 cells by MTT assay.

The purity, crystalline nature, and size of NPs were assessed using XRD analysis. The XRD analysis revealed that the CuO NPs exhibited diffraction peaks at 2θ , namely 32.2° , 35.2° , 38.8° , 48.6° , 53.2° , 57.8° , 61.3° , and 67.8° . These peaks corresponded to the crystallographic planes (110), (002), (111), (202), (020), (202), (113) and (220), respectively (Figure 4). These findings confirm the successful green synthesis of pure and monoclinic structure of CuO NPs. The present findings align with prior studies on CuO NPs synthesized via environmentally friendly techniques [34,37,38]. The average crystallite size, as determined by the Debye–Scherer formula, was 10.88 nm. The current investigation is consistent with earlier research, which

found that *Seriphidium oliverianum* produced CuO NPs with a size of 12.44 nm [38].

The shape and size of the synthesized CuO NPs were assessed using SEM and TEM (Figure 5). The CuO NPs that were synthesized exhibit a distinct spherical shape, as depicted in the SEM and TEM images presented in Figure 5(a). It was observed that the biological synthesis of CuO NPs results in the production of small spherical particles with uniform dimensions. The presence of biological ingredients in the extract demonstrates the relatively small aggregation. The CuO NPs produced from the herbal drug extract exhibited a spherical morphology, aligning with earlier research findings [37,39–41]. Furthermore, the size and shape of the manufactured CuO NPs were also observed by TEM (Figure 5b). The TEM micrographs demonstrated that the NPs had an almost spherical shape and were in the range of 4–20 nm (average size ~ 8 nm). This is similar to earlier investigations [32,40,42].

Standard broth dilution techniques were used to assess the antibacterial and antifungal activity (MIC) that were found in the range of $1\text{--}2.5\text{ mg}\cdot\text{mL}^{-1}$ against tested pathogens. The study demonstrated that CuO NPs had better antibacterial efficacy against MRSA in comparison to MDR-PA and *C. albicans*. The results of MIC values align with the findings reported by Javadhesari *et al.* [43] in their earlier research. In a previous study an MIC value of 2.5 to $3.5\text{ mg}\cdot\text{mL}^{-1}$ has been reported for *E. coli* and *S. aureus*, respectively [43]. Amiri *et al.* also observed a comparable

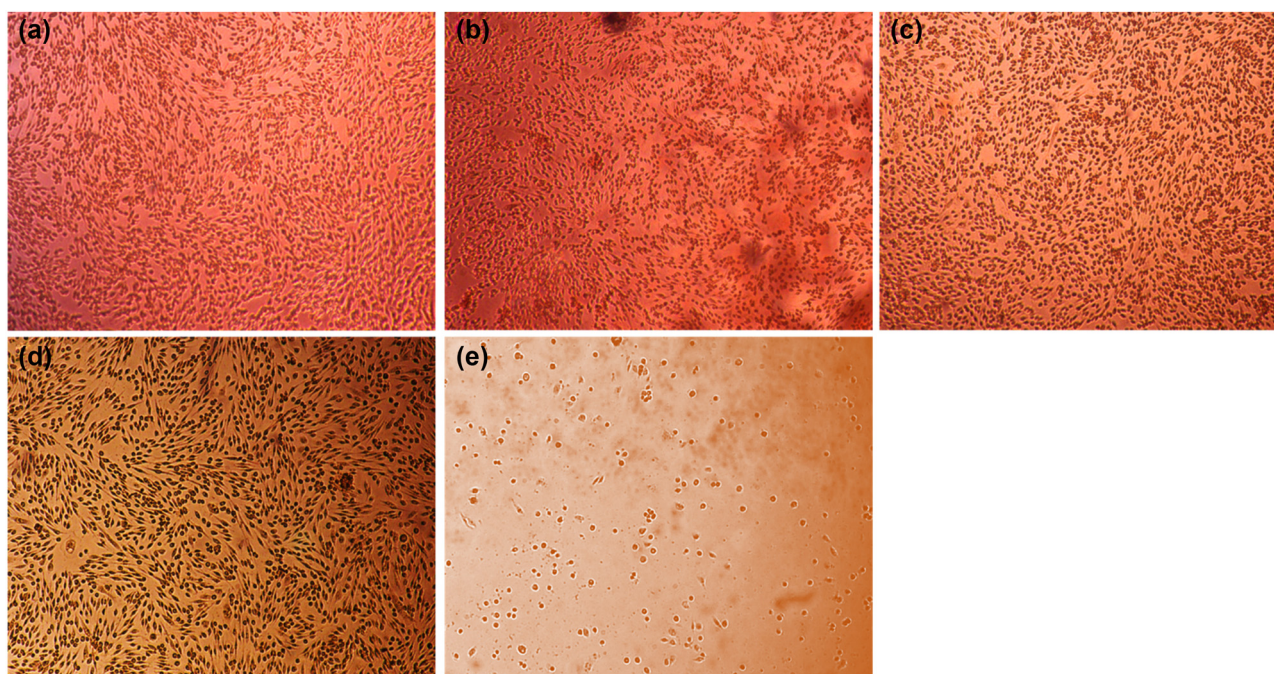


Figure 9: Microscopic analysis of the HCT-116 cells. (a) Control and (b–e) treated with 11.8, 23.6, 59, and $118\text{ }\mu\text{g}\cdot\text{mL}^{-1}$ of NPs, respectively.

trend in the MIC of CuO NPs against *Candida* species. The MIC₅₀ of CuO NPs against *Candida* species was reported as 1,000 $\mu\text{g}\cdot\text{mL}^{-1}$ [3,44]. The CuO NPs produced by *Solanum tuberosum* extract have been shown to have the antimicrobial activity of 0.2–1 $\text{mg}\cdot\text{mL}^{-1}$ [41]. Ren et al. previously documented that CuO NPs had antibacterial efficacy within the concentration range of 2,500–5,000 $\mu\text{g}\cdot\text{mL}^{-1}$ against several bacterial strains [45].

In addition, the impact of CuO NPs on the physical characteristics of MRSA, MDR-PA, and *C. albicans* was also examined by SEM (Figure 7). The control without treatment MRSA cells exhibited a smooth and intact cell surface, displaying typical, regular, and spherical morphological characteristics (Figure 7a). Nevertheless, the application of CuO NPs to MRSA cells demonstrated substantial impairment of the microorganisms and a significant decrease in their cell population. The cell wall and membrane exhibited distortions, irregularities, roughness, and lack of integrity, suggesting the loss of membrane integrity that ultimately results in cell death (Figure 7a1). Similarly, the MDR-PA cells that were not treated exhibited a healthy, normal, and rod-like architecture with a smooth cellular membrane (Figure 7b). However, when MDR-PA cells were exposed to CuO NPs, the cells exhibited severe damage. The cell membrane and wall were found to be disrupted, deformed, inconsistent, and coarse indicating a lack of cellular membrane integrity (Figure 7b1). The *C. albicans* cells that were not treated exhibited a sleek cell architecture characterized by an undamaged oval-shaped appearance (Figure 7c). The *C. albicans* cells that were subjected to CuO NPs exhibited an irregular and arbitrary cell surface, resulting in significant damage to the cells. In addition, the cells that were severely damaged were no longer in their original state, which could ultimately result in cell death (Figure 7c1). It was suggested that the Cu^{2+} ions have the potential to be easily released from CuO NPs, enabling them to interact more efficiently with membrane lipids and potentially trigger oxidation [35,36]. These interaction causes the membrane to collapse, resulting in the release of intracellular substances and enabling the interaction of CuO NPs with bacterial DNA, enzymes, and proteins [34]. Furthermore, it has been stated that the production of reactive oxygen species is responsible for CuO's antibacterial action [35,36]. These processes can diminish the potential for survival of the cells and result in cellular death.

Furthermore, inhibition of biofilm formation by CuO NPs was investigated by microtiter crystal violet assay (Figure 6). Microorganisms adhere irreversibly to surfaces to form biofilms, which are the sources of subsequent infections. CuO NPs dose-dependently decreased the biofilm formation in tested pathogens. As the concentration of CuO NPs

increased, the decrease in biofilm was observed. The highest concentration of 2 $\text{mg}\cdot\text{mL}^{-1}$ decreased the biofilm by 92.91%, 79.84%, and 71.57% for *P. aeruginosa*, MRSA, and *C. albicans*, respectively (Figure 6). The findings of our study align well with prior research, which has demonstrated the suppression of biofilm formation through the utilization of CuO NPs. Bai et al. [46] observed a significant reduction of 90% in *S. aureus* biofilm when exposed to 1,000 $\mu\text{g}\cdot\text{mL}^{-1}$ of CuO NPs. CuO NPs were found to suppress *C. albicans* biofilm development in a dose-dependent manner and inhibit it by 75% at 500 $\mu\text{g}\cdot\text{mL}^{-1}$ [47]. LewisOscar et al. [48] also reported a 90% decrease in biofilm by copper NPs. We are also of the opinion that the reduction in cell numbers in treated samples may be due to the absence of exopolysaccharides (EPS) secretions that did not allow the microorganism to adhere to the surface. It has been reported that EPS is crucial for the persistent adhesion of microbes to a surface [49]. Additionally, the EPS layers serve as a barrier to protect the microorganisms from adverse environmental conditions [50]. Furthermore, research has indicated that biofilm inhibition by CuO NPs is might be due to the suppression of enzymes, EPS, and virulence factors [46].

To predict the probable side effects of a drug, toxicity analysis is a critical component of toxicity studies. The cytotoxicity of CuO NPs has been further examined using MTT (Figure 8) and microscopic (Figure 9) techniques. MTT assay is associated with measuring material cytotoxicity dose dependently [51]. The assay relies on the concept that viable cells possess the ability to convert the MTT dye into a purple crystalline formazan product by the action of the NAD(P)H-dependent oxidoreductase enzyme, resulting in a color change from yellow to insoluble purple. The findings demonstrated a reduction in cell viability that was directly proportional to the dosage when treated with CuO NPs. It was found that there was an 89.85% decline in cell viability at the highest concentration of NPs i.e., 118 $\mu\text{g}\cdot\text{mL}^{-1}$ (Figure 8). Furthermore, in comparison to the control, the microscopic images revealed a decrease in the number of viable cells (Figure 9). After being exposed to NPs, HCT-116 cells may experience morphological changes that are obvious signs of the NPs' cytotoxicity. The microscopic images shown in Figure 9 illustrate the apparent loss of the original shape and size of cells under the experimental treatments. The cells treated with 11.8, 23.6, 59, and 118 $\mu\text{g}\cdot\text{mL}^{-1}$ of CuO NPs notably show a considerable loss in plasma membrane and integrity of structure when compared to the control group without treatment (Figure 9). This was most likely the result of effective contact between the CuO NPs and HCT-116 cells, which increased stress and may cause cell death. It is clear that the internalization of CuO NPs via cell membrane penetration served as the

primary mechanism for cell death in HCT-116 cells due to a substantial damage of cytoplasm [52]. Our findings align with the prior research conducted by Tabrez *et al.* [53], which demonstrated the suppression of the HCT-116 cell line through the use of CuO NPs and found that when exposed to a concentration of $35 \mu\text{g}\cdot\text{mL}^{-1}$, only 21% of the cells were able to survive. In addition, Gnanavel *et al.* [54] showed that when $100 \mu\text{g}\cdot\text{mL}^{-1}$ of CuO NPs are applied to the HCT-116, 22% of the cells remain viable. It has already been shown that CuO NPs in the range of $100\text{--}5,000 \mu\text{g}\cdot\text{mL}^{-1}$ did not show any cytotoxic effect on human cells [55]. In addition, CuO NPs have been utilized since the nineteenth century as a secure and efficient antibacterial agent [56]. From a cytotoxic perspective, the US Environmental Protection Agency has found that CuO NPs are safe for human usage because they are low in toxicity and environmentally safe [57,58].

7 Conclusion

NPs can also be synthesized from herbal drugs since previous reports are only regarding synthesizing CuO NPs from plants or microorganisms. The production of CuO NPs from the herbal drug septilin is described for the first time in this report. Green-synthesized CuO NPs from the drug septilin can be effectively used as antimicrobial against gram-positive, gram-negative, as well as fungus. CuO NPs also possess antibiofilm efficacy as they downregulated the biofilm formation at all concentrations tested. Furthermore, the CuO NPs also showed anticancer activity as tested on human colorectal cancer (HCT-116). The number of viable cells (HCT-116) decreased upon treatment with NPs. The present study emphasizes a new and innovative approach for generating NPs with significant potential in the field of biotechnology. This method offers a viable avenue for large-scale production of NPs using environmentally sustainable and cost-effective procedures. Hence, the produced CuO NPs have promise for future investigations in many biological and therapeutic domains, including the treatment of microbial biofilm infections, as well as the inhibition of cancer cell growth.

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