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Green synthesized copper oxide nanoparticles from *Ocimum tenuiflorum* fabricated in PVC for antibacterial, antioxidant, and anticancer biomedical applications

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Nanoparticle-coated membranes are valued for their optical, catalytic, and conductive properties, with applications in food, medicine, and electronics. This study explores the biomedical potential of green-synthesized copper oxide nanoparticles (Ot-CuO NPs) from *Ocimum tenuiflorum*, loaded on PVC membranes, for antibacterial, antioxidant, and anticancer activity. Copper oxide nanoparticles were synthesized via a green method, characterized using FTIR, XRD, and TEM, and assessed for bioactivity. Results revealed excellent antibacterial and antioxidant properties, enhanced by ROS production, along with anticancer potential. The eco-friendly synthesis reduces costs and environmental impact, showcasing Ot-CuO NPs-loaded PVC membranes as promising materials for biomedical applications.

Keywords: Antibacterial, Antioxidant, Biosynthesis, Copper oxide, Nanoparticle, *Ocimum tenuiflorum*, Polyvinyl chloride

Nanoparticles exhibit unique properties that make them invaluable across the medicine, nutrition, and energy sectors. Their size and shape significantly influence their optical, catalytic, and conductive characteristics, leading to diverse applications[1]. Because of their controlled physicochemical properties, such as their melting point, catalytic action, light absorption and scattering, electrical and thermal conductivity, and absorption of light, nanoparticles and nanostructured materials have significantly contributed to scientific breakthroughs over their bulk counterparts. Among these many uses with excellent properties, employing metal nanoparticles has produced noteworthy outcomes in several fields, including nanochemistry in the domains of biomedicine, energy, and the environment[2,3]. Copper oxide (CuO) nanoparticles, in particular, have demonstrated great potential in various biomedical applications due to their cost-effectiveness, stability, and versatile bioactivities when compared to other metal nanoparticles such as silver (Ag), gold (Au), and zinc oxide (ZnO) nanoparticles[4,5]. While Ag NPs are well known for their exceptional antibacterial activity, their expensive cost and probable cytotoxicity limit their use in biomedical applications. Au NPs, while biocompatible, have less antibacterial activity than CuO NPs. ZnO NPs have excellent antibacterial and antioxidant capabilities, although photocatalytic breakdown under UV exposure may limit long-term stability. In contrast, CuO NPs strike a balance between cost-effectiveness, broad-spectrum bioactivity, and stability, making them an attractive option for biomedical applications[6,7].

Green synthesis of nanoparticles is an eco-friendly and sustainable method that has gained popularity as an alternative to conventional chemical and physical nanoparticle synthesis techniques[8,9]. This method significantly reduces energy consumption by up to 30% and can lower production costs by 40% compared to conventional techniques[10]. Utilizing biological entities such as plant extracts, microorganisms, or other natural agents as reducing and stabilizing agents, green synthesis eliminates the use of toxic chemicals, making it safer and more environmentally compatible[11]. Copper oxide nanoparticles synthesized using green methods have been shown to possess enhanced biological activities, including antimicrobial and antioxidant properties, which are critical in combating bacterial infections and oxidative stress-induced damage[12].

The antibacterial activity of copper oxide nanoparticles is largely attributed to their ability to generate reactive oxygen species (ROS), disrupt bacterial cell walls, and interfere with microbial enzymes and DNA[13]. These properties make CuO nanoparticles a potent tool in addressing antibiotic

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resistance, a growing concern in modern medicine. Additionally, their antioxidant activity plays a crucial role in neutralizing free radicals, thus preventing cellular damage caused by oxidative stress[14]. This dual functionality makes green synthesized CuO nanoparticles promising candidates for various biomedical applications, including wound healing, drug delivery, and tissue engineering.

In this study, we focus on evaluating the antibacterial and antioxidant activities of copper oxide nanoparticles synthesized through a green method using *O. tenuiflorum*, exploring their potential use in the development of novel therapeutic agents for managing infections and oxidative damage. The investigation of these properties is critical for advancing Ot-CuO NPs loaded PVC membrane as a safe and effective alternative in biomedical applications.

Material and methods

Preparation of *O. tenuiflorum* Leaf Extract

Fresh leaves of *O. tenuiflorum* were collected, thoroughly washed with tap water, followed by deionized water to remove dust and impurities. The clean leaves were shade-dried for 2-3 days to remove excess moisture, then ground into a fine powder. Approximately 20 g of powdered leaves were added to 200 mL of deionized water and boiled for 15 min to extract the phytochemicals. The mixture was cooled to room temperature and filtered through Whatman No. 1 filter paper to obtain a clear aqueous extract. This extract was used for the green synthesis of copper oxide nanoparticles.

Synthesis of Copper Oxide Nanoparticles (CuO NPs)

A 0.1 M solution of copper (II) sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$) was prepared by dissolving 2.5 g of $CuSO_4$ in 100 mL of deionized water. To this solution, 50 mL of *O. tenuiflorum* leaf extract was added dropwise under continuous stirring using a magnetic stirrer. The mixture was stirred at 70°C for 2-3 h, during which a gradual color change from light blue to dark green was observed, indicating the reduction of Cu^{2+} ions to copper oxide nanoparticles (Ot-CuO NPs). Following the reaction, the solution was centrifuged at 10,000 rpm for 20 min to collect the copper oxide nanoparticles. The precipitate was then washed multiple times with deionized water and ethanol to remove any unreacted plant material and impurities. The washed nanoparticles were dried overnight at 80°C in a hot air oven. Finally, the dried nanoparticles were calcined at 400°C for 2 h to enhance their crystallinity.

Several factors can influence the synthesis and characteristics of CuO NPs such as pH, concentration of precursor salts, reaction kinetics affects the availability of reactive species to combine with the plant extract, directly impacting the nanoparticle size and distribution. The pH of the reaction medium is crucial in maintaining the oxidation state of copper ions, thereby influencing the morphology and stability of the resulting nanoparticles. Lower pH levels can lead to the formation of copper hydroxide species, which may hinder nanoparticle synthesis, whereas an optimal pH range (typically 7–10) promotes the efficient reduction of Cu^{2+} ions to CuO NPs *via* phytochemical interactions. Additionally, reaction kinetics—encompassing stirring speed, temperature, and reaction duration—play a vital role in defining nanoparticle size and crystallinity. Elevated temperatures (*e.g*, 70°C) accelerate the reduction process, improving nanoparticle yield, while extended reaction times may cause particle agglomeration.

Fabrication of PVC polymer with synthesized Ot-CuO NPs

To fabricate a polyvinyl chloride (PVC) polymer composite reinforced with synthesized Ot-CuO nanoparticles (NPs), the process begins with the synthesis of Ot-CuO NPs. Typically, a precursor such as copper nitrate is dissolved in deionized water, followed by the addition of a reducing agent like sodium hydroxide under continuous stirring. The reaction mixture is maintained at an elevated temperature (e.g, 60–80°C) for several h to facilitate nanoparticle formation. The resulting precipitate is collected by centrifugation, washed multiple times with ethanol and water to remove impurities, and dried in a vacuum oven to obtain the Ot-CuO NPs.

For PVC polymer composite preparation, the PVC resin is dissolved in a suitable solvent such as tetrahydrofuran (THF) under continuous stirring until a homogeneous solution is achieved. The synthesized Ot-CuO NPs are dispersed in the PVC solution at various weight percentages, depending on the desired composite properties. To ensure uniform dispersion of the nanoparticles, ultrasonication is applied for 30–60 min. Once the nanoparticles are thoroughly integrated, the mixture is poured into a flat mold and allowed to dry at room temperature or in an oven at a controlled temperature to evaporate the solvent. After complete solvent evaporation, the dried PVC/Ot-CuO composite film is carefully peeled from the mold. The

film is subjected to post-curing at a slightly elevated temperature to enhance interfacial bonding between the PVC matrix and Ot-CuO NPs.

Characterization of Copper Oxide Nanoparticles

X-ray Diffraction (XRD)

The crystalline structure of the synthesized nanoparticles was analyzed using XRD. The XRD patterns were compared to standard CuO diffraction patterns to confirm the presence of copper oxide nanoparticles.

Fourier-Transform Infrared Spectroscopy (FTIR)

FTIR was used to identify the functional groups in the *O. tenuiflorum* extract responsible for reducing and stabilizing the nanoparticles. The spectra were recorded from 4000–400 cm^{-1}.

Transmission Electron Microscopy (TEM)

The morphology and size of the nanoparticles were examined using TEM. The average particle size was estimated based on multiple TEM images.

Antioxidant assessment of synthesized Ot-CuO NPs loaded PVC membrane

DPPH Free Radical Scavenging Assay (Antioxidant Activity)

Different concentrations of synthesized copper oxide nanoparticles (Control, 10, and 20 µg/mL) were prepared in methanol. To 2 mL of DPPH solution, 1 mL of the nanoparticle sample was added and mixed thoroughly. The reaction mixtures were incubated in the dark at room temperature for 30 min. After incubation, the decrease in absorbance was measured at 517 nm using a UV-Vis spectrophotometer.

Antimicrobial Activity

The antimicrobial activity of copper oxide nanoparticles was tested against common bacterial strains, including *Staphylococcus aureus*&*Klebsiella pneumoniae*. Mueller-Hinton agar plates were prepared and inoculated with the bacterial and fungal strains using sterile swabs. Wells of 6 mm diameter were punched into the agar using a sterile cork borer. Different concentrations of the Ot-CuO NPs loaded PVC membrane solution (10, and 20 µg/mL) were loaded into the wells. A standard antibiotic (Erythromycin & Penicillin) for bacteria is used as a positive control, while deionized water is the negative control. The plates were incubated at 37°C for 24 h for bacterial strains. After incubation, the zones of inhibition (clear areas around the wells) were measured in millimeters, indicating the antimicrobial effectiveness of the nanoparticles.

Anti-cancer analysis (MTT)

The MTT assay was utilized to calculate the MCF-7 cell viability percentage. For the experiment, the cells were seeded onto 96-well plates at a cell ratio of 2 × 105 mL^{-1}. A volume of 100 µL of radioimmun o-precipitation buffer was added to each plate, and the cells were left to grow in 5% CO_2 for a whole day. Following the incubation period, the suspension sample was moved to individual plates and the various ratios of Ot-CuO NPs loaded PVC membrane that were obtained (Control, 10, and 20 µg/mL) were mixed with DMSO solution. Following a 48 h incubation period, 100 µl of a 0.5 mg/ml MTT solution in DMEM medium was added to each plate, and the plates were left to incubate for a further three h. The capacity of live cells to convert the MTT yellow dye into formazan allowed for the estimation of the proportion of cell proliferation. Once more, 100 µl of DMSO was added to each plate, and the culture was controlled to destroy metabolic chemicals. After that, each plate was shaken at 150 rpm for 20 min, and the optical density was measured at 570 nm.

Statistical analysis

Three duplicates of each experiment were run, and Microsoft Office Excel 2013 was used to determine the mean. Every test was statistically analyzed using the statistical package for social science software (Version 16.0: SPSS Inc, USA). One-way analysis of variance (ANOVA) was used to examine the variation between the control and test samples. Tukey's post hoc test was then used to do multiple group comparisons at the $P<0.05$ level as statistically significant. Plotting the error bar was done for the important figures.

Results

XRD analysis

The X-ray diffraction (XRD) analysis of our sample reveals prominent peaks, representing distinct crystalline planes, along with the relative percentages of crystalline and amorphous phases. The predominant crystalline nature of the material is evident with 84.1% crystallinity, while 15.9% of the material exhibits an amorphous phase, as indicated by the baseline noise or broad humps in the XRD spectrum (shown in Fig. 1). The well-defined peaks in the XRD pattern correspond to the crystalline regions,

with each peak indicating diffraction from different crystal planes (Miller indices) of the material. Notably, the prominent peak at 2θ = 29.40° signifies a highly ordered structure, possibly indicative of the prevalence of a specific crystallographic plane within the material. Based on peak spacing and intensities, these peaks may correspond to common crystallographic planes of copper oxide (CuO), if the material was indeed synthesized as CuO nanoparticles, possibly including specific planes such as (002) and (111). The XRD data underlines the predominantly crystalline nature (84.1%) of the material and the presence of a smaller amorphous phase (15.9%).

Furthermore, the distinct peaks at specific 2θ values point to well-defined crystallographic planes, indicating a high level of crystalline order. Additional minor peaks present at higher angles (2θ values of 36°, 48°, 53°, 66°, *etc*.) suggest the existence of secondary crystal planes contributing to the overall crystallinity of the material, indicative of further crystalline phases present.The surface chemistry of CuO nanoparticles (NPs) plays a crucial role in their reactivity, stability, and functional applications. Various synthesis methods and post-treatment processes influence the surface composition, oxidation states, and interaction with the surrounding environment. The presence of hydroxyl groups, oxygen vacancies, and adsorbed species can significantly impact catalytic and electronic properties. To confirm the crystalline structure and phase purity of CuO NPs, X-ray diffraction (XRD) analysis was performed. The XRD patterns reveal distinct diffraction peaks corresponding to monoclinic CuO, with sharp and well-defined peaks indicating a high degree of crystallinity.

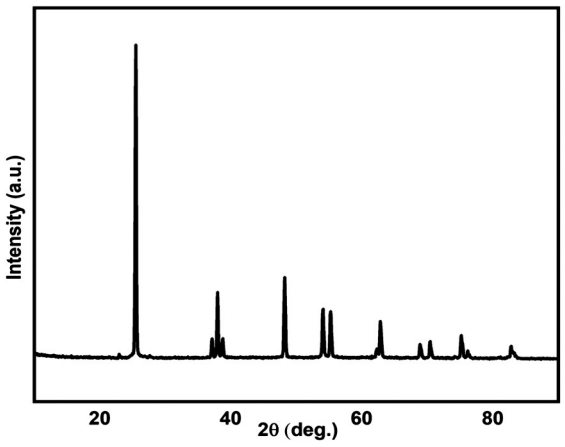

Fig. 1 — XRD pattern of the samples (Ot-CuO NPs loaded PVC membrane) showing distinct peaks corresponding to different crystalline planes

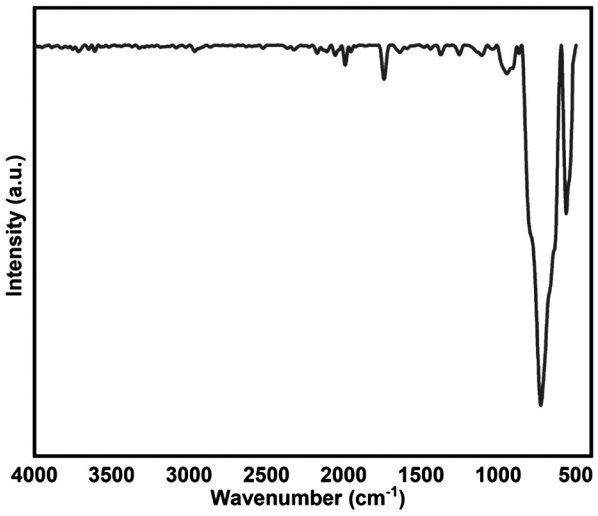

Fig. 2 — FTIR pattern of synthesized Ot-CuO-NPs using *O. tenuiflorum* plant extract

FTIR Analysis

The FTIR spectrum provides insights into the material's functional groups and bonds by measuring the absorption of infrared radiation at different wavenumbers. The broad absorption band at 3400 cm^{-1} is indicative of hydrogen-bonded O-H groups present in alcohols.

The band at 1650 cm^{-1} is likely associated with C=O groups, suggesting the presence of organic compounds, possibly derived from the plant extract (*O. tenuiflorum*) used in the green synthesis (shown in Fig. 2). Additionally, the FTIR spectrum demonstrates the successful formation of copper oxide (CuO) nanoparticles, as evidenced by strong absorption bands in the 500-600 cm^{-1} region, corresponding to Cu-O bonds. This analysis supports the characterization of CuO nanoparticles synthesized using a green method, revealing the metal oxide framework and potential organic functionalization from the synthesis medium. Moreover, this surface functionalization may contribute to the biological activity observed in subsequent assays, such as antibacterial or antioxidant properties.

TEM Analysis

The TEM images depict distinct characteristics of nanoparticle assemblies. Figure 3A shows a dense aggregation of nanoparticles, demonstrating a crystalline structure with discernible lattice fringes, indicating organized atomic planes. These particles exhibit diameters within the 10-30 nm range, consistent with the scale bar. Their irregular morphology and limited

boundaries suggest highly aggregated nanoparticles or larger agglomerates. Conversely, Figure 3B portrays a more dispersed assembly of nanoparticles at 100 nm, with individual particles, clusters, and network-like agglomerates visible. The particles in this image appear smaller, ranging from 5-20 nm, with additional larger clusters around 100 nm. The irregular shapes and network-like agglomeration indicate possible weak interparticle interactions. While both images display a tendency for nanoparticle agglomeration, Figure 3A portrays tighter agglomeration, resulting in less defined particle boundaries, whereas Figure 3B exhibits more dispersed particles but still shows aggregation (shown in Fig. 3). The observed variation in particle sizes between the two images highlights larger particles or agglomerates in (Fig. 3A), as opposed to a wider distribution of smaller, loosely packed particles in Figure 3B. Both sets of images suggest the propensity of nanoparticles to agglomerate, revealing different degrees of aggregation and dispersion.

Antioxidant activity

The copper oxide nanoparticles exhibit antioxidant activity that increases with concentration, demonstrating a dose-dependent response. At 20 µg/mL, the nanoparticles show significantly higher antioxidant activity compared to the lower concentration (10 µg/mL) (shown in Fig. 4). Although the nanoparticles show promising antioxidant potential, their activity is still lower than that of the standard antioxidant (ascorbic acid). However, the data suggests that the nanoparticles may be useful as antioxidants in biomedical applications, especially with further optimization of their concentration. The nanoparticles synthesized from *O. tenuiflorum* (Ot-CuO NPs loaded PVC membrane) have demonstrated antioxidant activity, which could contribute to their potential therapeutic applications, such as reducing oxidative stress in biological systems.

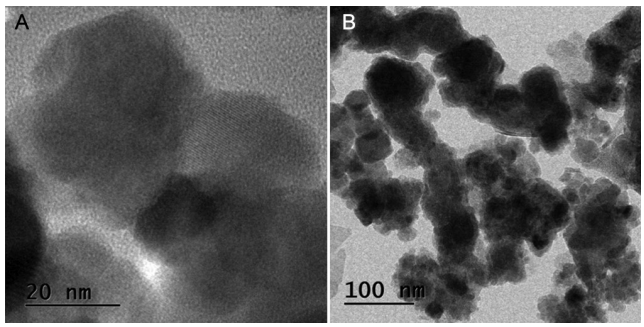

Fig. 3 — Morphological analysis using TEM imaging of Ot-CuO NPs loaded PVC membrane using *O. Tenuiflorum* plant extract

Antibacterial activity

Antibacterial activity was carried out on two different pathogenic strains – *Staphylococcus aureus &Klebsiella pneumoniae*. The positive control used is Erythromycin & Penicillin in 1:1 concentration& the negative control used is dimethyl sulfoxide. The nanoparticles demonstrate concentration-dependent antibacterial activity against both bacteria. The higher concentration sample (40 µg) shows the strongest inhibition, followed by the low concentration (20 µg) (shown in Fig. 5). The positive control (B) also shows significant antibacterial activity, while the negative

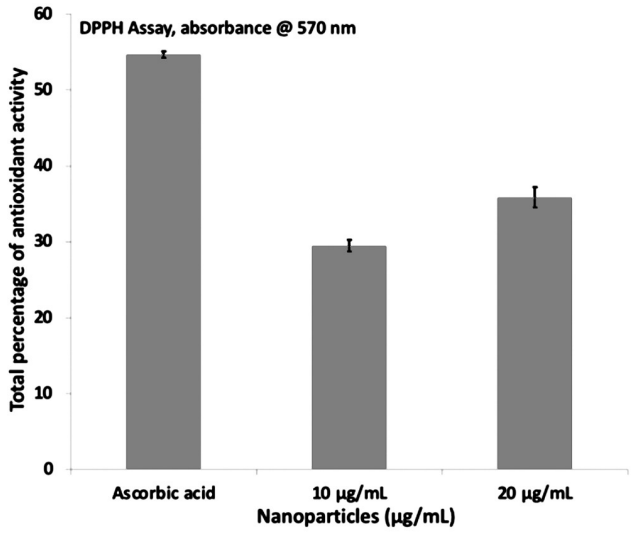

Fig. 4 — Free radical scavenging activity (%) of synthesized Ot-CuO NPs loaded PVC membrane at various concentrations compared with ascorbic acid

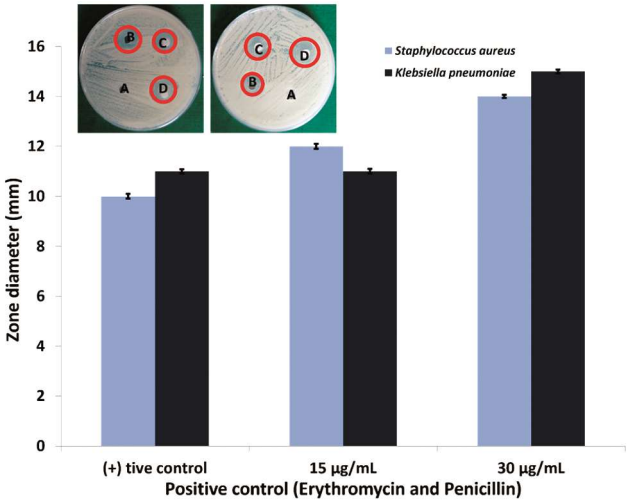

Fig. 5 — Antibacterial activity of Ot-CuO NPs loaded PVC membrane plant extract against *Staphylococcus aureus* and *Klebsiella pneumoniae* compared with standard antibiotics

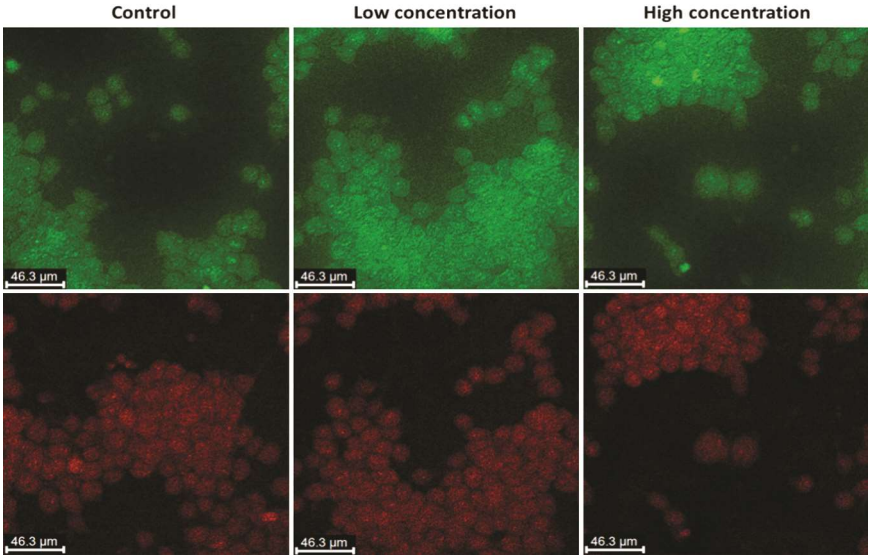

Fig. 6 — Con-focal imaging of breast cancer cell line (MCF-7) after the treatment with Ot-CuO NPs loaded PVC membrane

control (A) does not exhibit any inhibition, as expected. These findings suggest that the nanoparticles are effective in inhibiting bacterial growth, with greater potency at higher concentrations.

Anti-cancer activity of synthesized Ot-CuO NPs loaded PVC membrane through MTT assay

The MTT assay was used to measure the cytotoxicity of the human breast cancer cell line MCF-7 by looking at mitochondrial-mediated activity. MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetra azolium bromide) salt was taken up by cells, reduced by the enzyme succinate in the mitochondria, hydrogenated, and then employed in a colorimetric manner to assess the vitality of the cells. When plant-mediated Ot-CuO NPs loaded PVC membrane are exposed to cancer cell lines, they cause mitochondrial breakage or cellular mediated mortality due to the Ot-CuO NPs loaded PVC membrane reduced size and increased ratios that directly penetrate the cancer cells, developing an increased ratio of oxidative stress that ultimately results in cell apoptosis. The generated copper oxide NPs mediated by *O. tenuiflorum* leaf extracts show a higher rate of 81.46% of MTT salt reduction in 21 h at an increased ratio (20 µg/mL) sample (shown in Figs 6 & 7). At a low ratio (10 µg/ml), the sample discloses 66.02% of MTT salt reduction. The outcome shows time- and dose-based action. The acquired results effectively concluded that in human breast cancer cell lines, Ot-CuO-NPs derived through plant-mediated procurement promoted cellular-mediated death. Confirmation comes from comparing the plant-mediated synthesized Ot-CuO-NPs results with the data from earlier publications in the literature, which shows that the obtained Ot-CuO-NPs significantly suppress the growth of cancer cells.

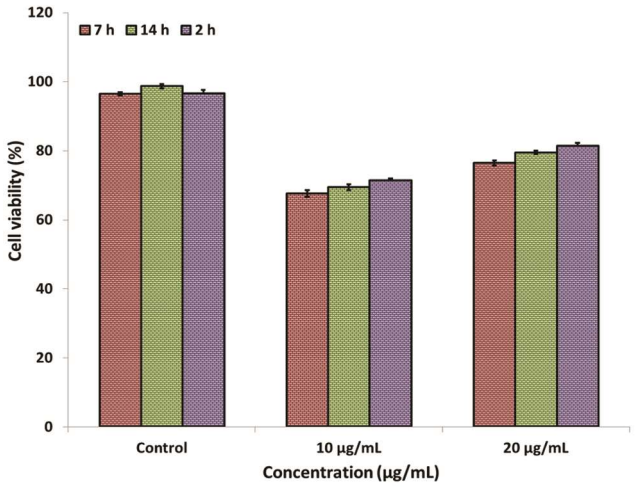

Fig. 7 — Cell viability percentage calculation of MCF-7 cell line after treating with Ot-CuO NPs loaded PVC membrane

Discussion

The synthesized copper oxide nanoparticles (CuO-NPs) using *O. tenuiflorum* extract were characterized extensively to determine their structural, morphological, and functional properties. The X-ray diffraction (XRD) analysis confirmed the crystalline nature of the CuO-NPs. The diffraction peaks observed in the XRD pattern were well-matched with the standard CuO phase, indicating the successful formation of CuO with high purity. The sharp and

intense peaks suggested that the synthesized nanoparticles were highly crystalline, which is crucial for the enhanced catalytic and antibacterial properties. The average crystallite size calculated using the Debye-Scherrer equation was 20-30 nm, consistent with the nanostructured form of CuO[15]. These findings support the formation of well-ordered CuO-NPs, contributing to their high biological activity.

The functional groups responsible for the synthesis of CuO-NPs were identified using Fourier-transform infrared spectroscopy (FTIR). The FTIR spectrum revealed prominent peaks corresponding to Cu-O bond stretching, confirming CuO nanoparticles' formation. Additionally, the presence of functional groups such as hydroxyl (-OH), carbonyl (C=O), and amine (N-H) bands were also observed, indicating the role of *O. tenuiflorum* phytochemicals in reducing and capping the nanoparticles[16,17]. These bioactive compounds likely played a dual role by facilitating the reduction of copper ions and stabilizing the nanoparticles, preventing agglomeration. The phytochemical analysis of *O. tenuiflorum* revealed the presence of anthocyanins, which have strong NP-reducing and stabilizing potential, further supporting the role of these functional groups in nanoparticle synthesis[18]. The presence of these functional groups also suggests that the Ot-CuONPs loaded membrane might interact with bacterial cell membranes through hydrogen bonding or electrostatic interactions, contributing to their antibacterial efficacy. Ot-CuO NPs loaded membrane have shown significant reductions in biofilm formation, indicating their capacity to interact with bacterial surfaces effectively[19].

Transmission electron microscopy (TEM) analysis provided detailed information regarding the size, shape, and morphology of the synthesized Ot-CuO NPs loaded membrane. The TEM images showed that the nanoparticles were predominantly spherical with a uniform distribution. The particle size analysis indicated that the average particle size was around 20-25 nm, which was in good agreement with the XRD results[20,21]. The small particle size and uniform shape of the nanoparticles contribute significantly to their large surface area-to-volume ratio, which is critical for their biological applications. The nanoparticles' small size is particularly beneficial for enhancing their interaction with microbial cells, leading to better antibacterial and antioxidant activity[22,23].

The antibacterial and antioxidant activities exhibited by the synthesized Ot-CuO NPs loaded membrane can be attributed to their nanoscale properties and multiple mechanisms, primarily involving reactive oxygen species (ROS) generation, membrane disruption, and metal ion release[24]. The small particle size, coupled with the bioactive compounds from *O. tenuiflorum*, likely enhances the nanoparticles' ability to penetrate bacterial cell walls, generating reactive oxygen species (ROS) and disrupting cellular processes. Ot-CuO NPs loaded membrane produce ROS, which disrupt bacterial cell membranes and lead to cell death. Once inside or in close proximity to bacterial cells, CuO NPs undergo redox reactions, producing ROS such as hydroxyl radicals (-OH), superoxide anions (O_2^-), and hydrogen peroxide (H_2O_2). These ROS induce oxidative stress, damaging bacterial DNA, proteins, and lipids, ultimately leading to cell death. This mechanism is crucial for their effectiveness against both gram-positive and gram-negative bacteria[25-27]. The bioactive phytochemicals from *O. tenuiflorum* may further enhance antibacterial action by synergistically affecting bacterial metabolic pathways. Additionally, Cu^{2+} ions released from CuO NPs can interact with intracellular components, interfering with enzymatic functions and protein structures.

Supporting literature suggests that CuO NPs exhibit potent antibacterial properties due to their ROS-mediated toxicity and membrane interactions[28]. Studies have shown that ROS generation by metal oxide nanoparticles can effectively inhibit both Gram-positive and Gram-negative bacteria, with variations in efficacy depending on bacterial membrane composition and oxidative stress tolerance[29,30]. Moreover, the antioxidant activity of the nanoparticles suggests their potential in scavenging free radicals, which can be beneficial in medical applications, such as in anti-inflammatory or anticancer therapies. The combination of physical properties, such as high crystallinity and small particle size, along with the biological interactions facilitated by the plant extract, contributed to the overall efficacy of the synthesized Ot-CuO NPs loaded membrane.

Copper oxide nanoparticles (CuO NPs) may undergo structural and chemical transformations when subjected to varying environmental conditions, such as changes in pH, temperature, and ionic strength. Research has demonstrated that in aqueous solutions exhibiting high pH, CuO NPs are prone to aggregation due to van der Waals forces, which subsequently reduces their bioavailability and

activity[31]. Additionally, the presence of salts, organic matter, and biomolecules in environmental contexts can either provide stabilization or lead to the destabilization of the nanoparticles. Moreover, it has been indicated that CuO NPs may experience partial dissolution under acidic conditions, resulting in the release of Cu^{2+} ions, which can influence cytotoxicity and therapeutic efficacy. Studies have suggested that the functionalization of CuO NPs with biopolymers like chitosan and alginate or surfactants such as cationic thiol polyurethane, polyvinyl chloride can enhance colloidal stability, thereby preventing aggregation and improving dispersion across diverse media[32,33]. The encapsulation of CuO NPs within biocompatible polymers or their incorporation into matrices such as polyvinyl chloride (PVC) has been proposed as a strategy to enhance stability and extend functional performance. Additionally, Doping CuO NPs with elements such as silver (Ag), zinc (Zn), iron (Fe), or cerium (Ce) can significantly enhance their antimicrobial and anticancer properties.

The study is limited by the absence of comprehensive *in vivo* evaluations needed to fully assess the biosafety and efficacy of the synthesized Ot-CuO NPs loaded membrane. Although the antibacterial and antioxidant activities were demonstrated *in vitro*, the potential toxicity to human cells and long-term environmental impact were not investigated. Furthermore, the precise mechanisms underlying the antibacterial action and antioxidant properties of the Ot-CuO NPs loaded membrane require further molecular-level investigations. The study also did not address the scalability of the synthesis process, which may present challenges for large-scale production. Variability in the concentration of bioactive compounds in *O.tenuiflorum* extract could impact the reproducibility and consistency of the properties of the Ot-CuO NPs loaded membrane.

Conclusion

Our study shows that copper oxide nanoparticles made using environmentally friendly methods have strong antibacterial and antioxidant effects. These effects depend on the concentration of the nanoparticles. Our findings support previous research showing that these nanoparticles are effective in medical uses. Making the nanoparticles using green methods not only makes them safer for the body but also helps the environment. The results from MTT verified that, depending on the dose and reaction time, the percentage of cell growth decreased as the concentration increased. These nanoparticles have the potential for use in coatings that fight bacteria, antioxidant treatments, and other medical purposes, especially for dealing with antibiotic resistance and diseases caused by oxidative stress.

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Conflict of interest

All authors declare no conflicts of interest.

References

1 Baig N, Kammakakam I & Falath W, Nanomaterials: a review of synthesis methods, properties, recent progress, and challenges. *Mater Adv*, 2 (2021) 1821.

2 Chen Y, Lai Z, Zhang X, Fan Z, He Q, Tan C & Zhang, H. Phase engineering of nanomaterials. *Nat Rev Chem,* 4 (2020) 243.

3 Gandhimathi KA, Francis AP, Rengasamy G, Veeraraghavan VP & Sankaran K, Quercetin-coated biogenic titanium oxide nanoparticles: Synthesis, characterization, and *in vitro* biological studies. *J Part Sci Technol*, 43 (2024) 198.

4 Chinnasamy R, Chinnaperumal K, Arumugam P, Natarajan M, Govindasamy B, Jogikalmat K, Cherian T, Kannupaiyan J, Sigamani S & Willie P, Phyto-fabrication of AgNPs using leaf extract of *Vitex trifolia*: potential to antibacterial, antioxidant, dye degradation, and their evaluation of non-toxicity to *Chlorella vulgaris*. *Biomass Conv Bioref,* 14 (2024) 14903.

5 Kumar N, Verma A, Aggarwal R, Chauhan S, Jain VK & Sengupta S, Integration of ZnO-Ag Nanocomposites into PMMA Thin Film for Enhanced Antibacterial Applications. *Chem Select,* 9 (2024) e202402678.

6 Gebreslassie YT & Gebremeskel FG, Green and cost-effective biofabrication of copper oxide nanoparticles: Exploring antimicrobial and anticancer applications. *Biotechnol Rep (Amst)*, 41 (2024) e00828.

7 Sakthivel A, Sankaran K, Rengasamy G, Priya VV & Sathishkumar P, Formulation of Mouthwash Using Combined Herbal Extracts to Control the Predominant Oral Pathogens and Biofilm. *J Herb Med*, 46 (2024) 100905.

8 Soni J, Panneer Selvam S, Shanmugam R, Ramadoss R. Sundar S, Quantification of the Bioactivity of Ethanolic Extract from Phoenix dactylifera. *Cureus,* 16 (2024) e56391.

9 Kattookaran C, Govindasamy R, Selvam SP & Viswanathan D, Biosynthesis of Ag doped MgO-Zno nanocomposite with Cassia alata plant leaves extract and assessment of their biological applications. *Next Mater*, 8 (2024) 100820.

10 Osman AI, Zhang Y, Farghali M, Rashwan AK, Eltaweil AS, El-Monaem EM, Mohammed IMA, Badr MM, Ihara I, Rooney DW & Yap PS, Synthesis of green nanoparticles for energy, biomedical, environmental, agricultural, and food applications: A review. *Environ Chem Lett*, 22 (2024) 841.

11 Suresh N & Kaarthikeyan G, Green Synthesis and the Evaluation of Osteogenic Potential of Novel Europium-Doped-Monetite Calcium Phosphate by *Cissus quadrangularis*. *Cureus,* 16 (2024) e59202.

12 Ogwuegbu MC, Ayangbenro AS, Mthiyane MN, Babalola OO & Onwudiwe DC, Green synthesis of CuO nanoparticles using Ligustrum lucidum extract, and the antioxidant and antifungal evaluation. *Mater Res Express,* 11 (2024) 055010.

13 Abhimanyu P, Arvind M & Kishor N, Biosynthesis of CuO Nanoparticles Using Plant Extract as a Precursor: Characterization, Antibacterial, and Antioxidant Activity. *Nano Biomed Eng*, 15 (2023) 369.

14 Santana BM, Armentano GM, Ferreira DAS, de Freitas CS, Carneiro-Ramos MS, Seabra AB & Christodoulides M, *In vitro* Bactericidal Activity of Biogenic Copper Oxide Nanoparticles for *Neisseria gonorrhoeae* with Enhanced Compatibility for Human Cells. *ACS Appl Mater Interfaces*, 16 (2021) 21633.

15 Mobarak BM, Hossain MS, Chowdhury F, Ahmed S. Synthesis and characterization of CuO nanoparticles utilizing waste fish scale and exploitation of XRD peak profile analysis for approximating the structural parameters. *Arab J Chem*, 15 (2020) 104117

16 Olawale F, Ariatti M & Singh M, *Ocimum tenuiflorum* L mediated green synthesis of silver and selenium nanoparticles: antioxidant activity, cytotoxicity and density functional theory studies. *Adv Nat Sci: Nanosci Nanotechnol,* 13 (2022) 015015

17 Hosseinzadeh E, Foroumadi A & Firoozpour L, What is the role of phytochemical compounds as capping agents for the inhibition of aggregation in the green synthesis of metal oxide nanoparticles? A DFT molecular level response. *Inorg Chem. Commun*, 147 (2023) 110243.

18 Cherian T, Ragavendran C, Remesh RKV, Jacob J, Jamal W, Kamaraj C & Nakouti I, Assessment of the biological functionalities of hyaluronate macromolecules stabilized copper oxide nanoparticles. *J Environ Chem Eng*, 11 (2023).

19 Ansari M, Al Dhneem H, Ali S, Jamous Y, Alomary M, Atwah B, Alhumaidi M, Hani U, Haider N, Asiri S & Khan F, Facile, polyherbal drug-mediated green synthesis of CuO nanoparticles and their potent biological applications. *Green Process Synth*, 13 (2024) 20230174.

20 Ontiveros-Robles JA, Villanueva-Flores F, Juarez-Moreno K, Simakov A & Vazquez-Duhalt R, Antibody-Functionalized Copper Oxide Nanoparticles with Targeted Antibacterial Activity. *Chem Open*, 12 (2023) e202200241.

21 Santana BM, Armentano GM, Ferreira DAS, Freitas CS, Carneiro-Ramos MS, Seabra AB & Christodoulides M, *In vitro* Bactericidal Activity of Biogenic Copper Oxide Nanoparticles for Neisseria gonorrhoeae with Enhanced Compatibility for Human Cells. *ACS Appl Mater Interfaces,* 16-17 (2024) 21633.

22 Kolahalam LA, Prasad KRS, Krishna PM, Supraja N & Shanmugan S, The exploration of bio-inspired copper oxide nanoparticles: synthesis, characterization and *in vitro* biological investigations. *Heliyon*, 8 (2022) e09726.

23 Pandiyan I, Arumugham MI, Doraikannan SS, Rathinavelu PK, Prabakar J & Rajeshkumar S, Antimicrobial and Cytotoxic Activity of *Ocimum tenuiflorum* and *Stevia rebaudiana*-Mediated Silver Nanoparticles – An *in vitro* study. *Contemp Clin Dent,* 14 (2023) 109.

24 Gudkov SV, Burmistrov DE, Fomina PA, Validov SZ & Kozlov VA, Antibacterial Properties of Copper Oxide Nanoparticles (Review). *Int J Mol Sci,* 25 (2024) 11563.

25 Kadhim FJ & Bedoui MH, Copper Oxide Nanoparticles (CuO NPs) prepared from Chitosan extract for antibacterial activity. 11 March 2024, PREPRINT (Version 1); 10.21203/rs.3.rs-4005861/v1.

26 Abbas SF, Haider AJ, Al-Musawi S & Selman MK, Antibacterial Effect of Copper Oxide Nanoparticles Prepared by Laser Production in Water Against *Staphylococcus aureus* and *Escherichia coli*. *Plasmonics*, 19 (2023) 2401.

27 Charlena C, Sugiarti S & Ardiansyah D, Synthesis and Characterization of Copper(II) Oxide (CuO-NP) Nanoparticles using Chemical Precipitation Method. *J Kim Mulawarman*, 21 (2024) 84.

28 Khairy T, Amin DH, Salama HM, Elkholy IMA, Elnakib M, Gebreel HM & Sayed HAE, Antibacterial activity of green synthesized copper oxide nanoparticles against multidrug-resistant bacteria. *Sci Rep*, 14 (2024) 25020.

29 Havryliuk O, Rathee G, Blair J, Hovorukha V, Tashyrev O, Morató J, Pérez LM & Tzanov T, Unveiling the Potential of CuO and Cu_2O Nanoparticles against Novel Copper-Resistant *Pseudomonas* Strains: An In-Depth Comparison. *Nanomaterials (Basel)*, 14 (2024) 1644.

30 Hall J, Mekapothula S, Coxhill R, Craske D, Varney AM, Cave GWV & McLean S, Surface-Functionalised Copper Oxide Nanoparticles: A Pathway to Multidrug-Resistant Pathogen Control in Medical Devices. *Nanomaterials (Basel)*, 14 (2024) 1899.

31 Sousa SV & Teixeira MR, Aggregation kinetics and surface charge of CuO nanoparticles: the influence of pH, ionic strength and humic acids. *Environ Chem,* 10 (2013) 313.

32 Sabbah. IA, Zaky MF, Hendawy M & Negm N, Synthesis, characterization and antimicrobial activity of colloidal copper nanoparticles stabilized by cationic thiol polyurethane surfactants. *J Polym Res,* 25 (2018) 252.

33 Letchumanan D, Sok SPM, Ibrahim S, Nagoor NH & Arshad NM, Plant-Based Biosynthesis of Copper/Copper Oxide Nanoparticles: An Update on Their Applications in Biomedicine, Mechanisms, and Toxicity. *Biomolecules*, 11 (2021) 564.