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ECO-FRIENDLY SYNTHESIS OF COPPER OXIDE NANOPARTICLES USING PLANT SEED EXTRACT: POTENTIAL ANTIOXIDANT, ANTIFUNGAL AND BETA-HEMATIN INHIBITORS [a]

SÍNTESIS ECOLÓGICA DE NANOPARTÍCULAS DE ÓXIDO DE COBRE UTILIZANDO EXTRACTO DE SEMILLAS DE PLANTAS: POTENCIAL ANTIOXIDANTE, ANTIFÚNGICO E INHIBIDOR DE BETA-HEMATINA

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Research paper

ABSTRACT: Copper oxide nanoparticles (CuO NPs) have emerged as a promising area of research due to their remarkable biological and medical properties. This investigation showcases an environmentally friendly method for synthesising CuO NPs using garden cress seed extract, which serves as both a reducing and stabilising agent. The study highlights the potential applications of CuO NPs as antioxidants, antifungal agents, and beta-hematin inhibitors. The synthesized CuO NPs underwent comprehensive characterization using various techniques, including UV-Vis spectroscopy, X-ray diffraction (XRD), Scanning Electron Microscopy (SEM), and Fourier transform infrared spectroscopy (FTIR). The antioxidant potential was assessed using the DPPH assay, while antifungal activities were investigated against specific pathogens. Additionally, the antimalarial activity was evaluated through beta-hematin inhibition studies. The results revealed that CuO NPs exhibited a distinctive absorption peak at 420 nm, indicative of their surface plasmon resonance property and a spherical shape with sizes ranging from 30 to 50 nm, as observed through SEM. Additionally, the resulting CuO NPs demonstrate significant antioxidant activity comparable to ascorbic acid, promising antifungal properties against Monosporascus cannonballus,and substantial activity against beta-hematin formation. The findings suggest that CuO NPs hold potential as therapeutic agents, warranting further research and development.

KEYWORDS: Green synthesis; nanoparticles; antioxidants; antimicrobial; antimalarial.

RESUMEN: Las nanopartículas de óxido de cobre (NP de CuO) se han convertido en un área de investigación prometedora gracias a sus notables propiedades biológicas y médicas. Esta investigación presenta un método ecológico para sintetizar NP de CuO utilizando extracto de semilla de berro, que actúa como agente reductor y estabilizador. El estudio destaca las posibles aplicaciones de las NP de CuO como antioxidantes, antifúngicos e inhibidores de la

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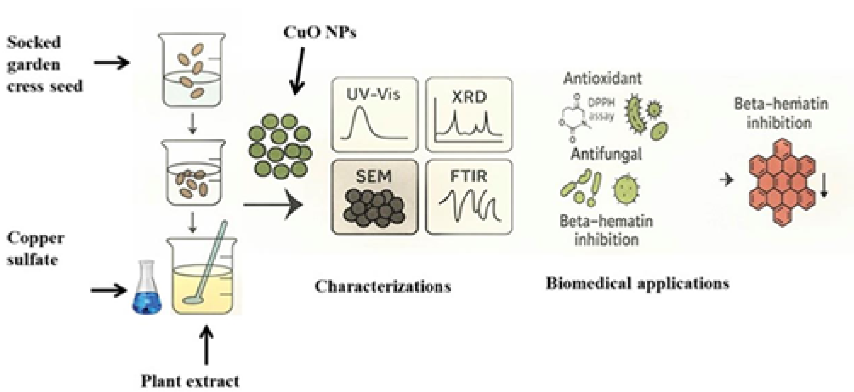
beta-hematina. Las NP de CuO sintetizadas se sometieron a una caracterización exhaustiva mediante diversas técnicas, como espectroscopia UV-Vis, difracción de rayos X (DRX), microscopía electrónica de barrido (MEB) y espectroscopia infrarroja por transformada de Fourier (FTIR). El potencial antioxidante se evaluó mediante el ensayo DPPH, mientras que las actividades antifúngicas se investigaron contra patógenos específicos. Además, la actividad antipalúdica se evaluó mediante estudios de inhibición de beta-hematina. Los resultados revelaron que las nanopartículas de CuO exhibieron un pico de absorción distintivo a 420 nm, indicativo de su propiedad de resonancia plasmónica superficial y una forma esférica con tamaños que oscilaban entre 30 y 50 nm, como se observó mediante SEM. Además, las nanopartículas de CuO resultantes demuestran una actividad antioxidante significativa comparable a la del ácido ascórbico, prometedoras propiedades antifúngicas contra Monosporascus cannonballus y una actividad sustancial contra la formación de beta-hematina. Los hallazgos sugieren que las nanopartículas de CuO tienen potencial como agentes terapéuticos, lo que justifica mayor investigación y desarrollo.

PALABRAS CLAVES: Síntesis verde; nanopartículas; antioxidantes; antimicrobianos; antipalúdicos.

Graphical abstract



1. INTRODUCTION

Metal oxide nanoparticles have garnered significant attention due to their unique properties, which bridge the gap between bulk materials and atomic structures. These nanoparticles exhibit distinct physicochemical characteristics, including high reactivity, large surface area, pore size, and particle shape (Nieto-Maldonado *et al.*, 2022). The combination of novel nanoparticles into various applications has sparked interest in their potential immunological and inflammatory responses (Mitchell *et al.*, 2021). Nanoparticles are being explored for various applications, including information and communication technology, food technology, energy technology, and medical products. In the medical field, nanoparticles are being investigated for their potential use in drug delivery systems, diagnostics, and medical technology. Nanoparticles can be synthesized using physical, chemical, biological, and hybrid methods (Vijayaram *et al.*, 2024; Sharma *et al.*, 2022). However, the use of toxic materials in physical and chemical synthesis methods has raised concerns. As a result, eco-friendly biogenetic methods have gained popularity due to their ease of use and flexibility (Ali *et al.*, 2025; Priya *et al.*, 2023). Nanoparticles were extensively studied in recent years due to their many potential uses in chemistry, drug delivery, biomedical, and other areas (Haleem *et al.*, 2023).

Copper nanoparticles (CuO NPs) have attracted attention due to their potential applications in medicine, catalysis, and electronics. Plant-based synthesis offers a sustainable and cost-effective alternative to traditional chemical methods (Al-Refai'a *et al.*, 2024). Recent studies have shown that metal nanoparticles exhibit enzyme-like antioxidant capabilities, enabling them to scavenge free radicals and lower the levels of reactive oxygen species (Bidan & Al-Ali , 2024).

Metal nanoparticles, including magnetic, silver, and gold nanoparticles, can offer considerable potential in the treatment and prevention of diseases induced by excess ROS generation (Bidan & Al-Ali , 2025; Akhtar *et al.*, 2017). The combination of nanotechnology and material science significantly reduces the formation of free radicals during the production of nanoparticles, thereby producing nano-antioxidants (Lushchak *et al.*, 2018). The antioxidant properties of nanoparticles depend on their chemical composition, properties, stability, surface area-to-volume ratio, size, surface coating, and surface charge (Flieger *et al.*, 2021).

In addition to scavenging reactive nitrogen and oxygen species, some oxide nanoparticles can mimic enzymes or antioxidant molecules because of their essential physicochemical characteristics (Khalil *et al.*, 2020; Kumar *et al.*, 2020). Additionally, oxide nanoparticles' ability to reduce free radicals is determined by their ability to convert alkyl peroxyl radicals into hydro-peroxides (Li *et al.*, 2020).

Moreover, nanoparticles have emerged as a promising solution to combat fungal infections.their unique properties make them effective against various fungal pathogens (Al-Refai'a *et al.*, 2009). The requirement for new antifungal medicines has consistently increased due to the rise of resistant fungal isolates and the necessity for antifungals that exhibit reduced toxicity and lower costs (Lee *et al.*, 2023).

Metallic nanoparticles have been found to be highly effective in malaria therapy, particularly in the early stages of human infection and in attacking the mosquito vector. Based on comprehensive reviews by Rahman *et al.* (2019) and Barabadi *et al.* (2019),silver, gold, and palladium are the most common MNPs studied for these applications. Another study employs copper nanoparticles (Al-Refai'a *et al.*, 2024). Chloroquine (CQ) is a widely used antimalarial drug, but its effectiveness is threatened by the emergence of resistance in Plasmodium falciparum, the most virulent malaria parasite. This resistance has necessitated the development of new antimalarial medications (Al-Refai'a & Alkarimi, 2020). While alternative drugs like mefloquine and halofantrine were introduced in the 1970s, resistance has also developed to these medications over time. CQ works by targeting parasites in the erythrocytic stage, leveraging its small size and lipophilic properties to penetrate both erythrocyte and parasite membranes (Tse *et al.*, 2019; Mishra *et al.*, 2017).

This work critically examines recent advances in the plant-mediated green synthesis of CuO NPs, their characterization, underlying mechanisms of antioxidant and antifungal action, and potential activity as a beta-hematin inhibitors.

2. MATERIALS AND METHODS

2.1. Materials

Copper sulfate, and other chemicals were purchased from Sigma-Aldrich. while, garden cress seeds were provided from Iraqi local market.

2.2. Plant extract preparation

Garden Cress was collected from the Babylon governance market, washed first with tap water then with distilled water, left to dry at room temperature for 2 days. Then, 10 g of plant seed was soaked in 100 mL of deionized water for $24h$. The solution was heated to 80°C for 1 h, filtered, and stored at 4°C until used.

2.3. Synthesis of copper nanoparticles

Copper oxide NPs were prepared according to previous work (Al-Khafaji *et al.*, 2022) with slightly modification. Briefly, a (60 mL) of plant seed extract was added to (200 mL) of (1 mM) copper sulphate solution with constant stirring until the colour changed to flat yellow then to brown. The resulting solution was centrifuged and the precipitate was washed and dried. The producing NPs were purified using a Millipore Filter (0.5 M), collected and finally stored for the next day's work.

2.4. Characterisation

The synthesized CuO NPs were fully characterized. All UV-Vis absorption spectra were obtained using T80 UV/VIS Spectrometer PG Instruments Lt, Fourier transform infrared ($FT-IR$) analysis, X-ray diffraction (XRD), Scanning electron microscope (SEM), and Energy-dispersive X-ray spectroscopy (EDX).

2.5. Biological applications

2.5.1. Antioxidant assay(DPPH assay)

The antioxidant potential of CuO NPs was evaluated using DPPH assay. The activity of the synthesised nanoparticles was measured by scavenging free radicals from DPPH according to Das et al. who described the technique with a little change (Das *et al.*, 2013). The stable radical DPPH (2, 2-diphenyl-1-picrylhydrazyl) was employed to assess the free radical scavenging properties of CuO NPs and vitamin C. Various doses ($10-100$ μg/mL) were mixed with 1mL of freshly prepared DPPH (1 mM in methanol) and extensively vortexing. The solution was then kept at ambient temperature and darkness for 30 minutes.

Finally, absorbance was measured at 517 nm. Equation 1 shows how the free radical scavenging activity was calculated using the percentage of inhibition.

$$\text{Percentage of DPPH Scavenged} = \frac{(\text{Ab of control} - \text{Ab of test})}{(\text{Ab of control})} \times 100 \quad (1)$$

Where *Ab* is the control's absorbance and *Ab* test is the absorption of (*CuO NPs*) or Vitamin *C*.

2.6. Antifungal activity

2.6.1. Pathogenic fungi:

Monosporascus cannonballus Pathogenic fungus was used in these experiments namely soil-borne fungus. The standard culture of this fungus was obtained from laboratory of fungi in department of biology, collage of science in Babylon university, Iraq.

2.6.2. Assessment of Antifungal Assay

Antifungal activity of CuO NPs produced using garden cress seed extract in vitro against M. *cannonballus* was achieved by the agar dilution method (Ali *et al.*, 2015; Ali *et al.*, 2025). The medium agar was supplemented with two different concentrations of CuO NPs and plant extract separately (1, 2 mg/mL). A disc of 1 cm of mycelial growth of the tested fungi, taken from the edge of 6-day-old fungal culture, was placed in the centre of each plate. The plates with the inoculums were then incubated at 25 °C. The treatment efficacy of CuO NPs was calculated after 7 days by measuring the radial growth of fungal colonies.

$$\text{Inhibition rate}(\%) = R - \frac{r}{R} \quad (2)$$

where (R) is the radial growth of fungal hyphae on the control plate and (r) is the radial growth of fungal hyphae on the plate supplements with CuO NPs or plant extract.

2.7. Beta-hematin inhibition

Inhibition of beta-hematin by test samples was conducted using the modified protocol as described elsewhere, Joshi *et al.* (2017).The reaction mixture consisted of hematin chloride and HCl incubation with samples at $2mM$ and $1M$ respectively followed by treatment with sodium acetate buffer (pH 4.5).

Chloroquine, *CQ* was used as a reference control. The reaction mixtures were incubated overnight then centrifuged at 14000 rpm and washed with 2.5% (w/v) *SDS*, 100 mM sodium bicarbonate solutions in sequence. Beta-hematin formed was determined by measuring absorbance at 400 nm accordingly (Al-Refai'a, 2019). All the test samples were evaluated within the concentration range of $10 - 50\mu M$, and % inhibition of heme crystallization $(I\,\%)$ of the test sample was calculated relative to chloroquine using standard equation.

$$I\,\% = \frac{[AC - AS]}{AC} \times 100 \quad (3)$$

AC, the absorbance of CQ and AS, the absorbance of copper oxide NPs.

3. RESULTS AND DISCUSSION

3.1. Characterisations

CuO Nanoparticle formation and synthesis confirmation were investigated using UV-Vis spectroscopy ($T80$ UV/VIS Spectrometer PG Instruments Lt). As illustrated in Figure 1B, the absorption peak of the synthesized $CuO\ NPs$ and plant extract occurred at 420 and 350 nm respectively.

$UV-Vis$ spectroscopy was used to monitor the synthesis of $CuO\ NPs$ with the assistance of a plant extract. The obtained spectra give helpful information on the nanoparticle formation process. From the spectra, a distinct transformation from the plant extract to the final product can be seen. A wide absorption band is seen for the plant extract due to many organic compounds. $UV-Vis$ spectroscopy data serves as the first primary evidence for synthesizing CuO NPs using a plant extract. The results suggest that the size of the nanoparticles is very small and quite stable, with a nearly uniform size distribution.

$FTIR$ was employed for optical characterization, functional group analysis, and identification of functional groups. $FT-IR$ spectra of the plant extract Figure 2A was identified the band at $3387cm^{-1}$ is ascribed to $O-H$ stretching vibrations. The absorption band at $2943cm^{-1}$ was attributed to $C-H$ vibrations, encompassing CH, CH_2, and CH_3 stretching and bending vibrations, both symmetric and asymmetric, and occasionally overlapping with $O-H$. The characteristic bands around 1604 and $1419cm^{-1}$ were attributed to the asymmetrical and the symmetrical COO - stretching vibrations, respectively. The wave values ranging from 1700 to 1600 cm^{-1} and from 1600 to 1500 cm^{-1} were ascribed to Amide I (stretching vibrations of $C-O$ and $C-N$ groups) and Amide II (mostly due to $N-H$ bending), indicating protein presence. The distinctive band at 1072 cm^{-1} was ascribed to the vibrations of $C-O$, $C-O-C$ glycosidic, and $C-O-H$

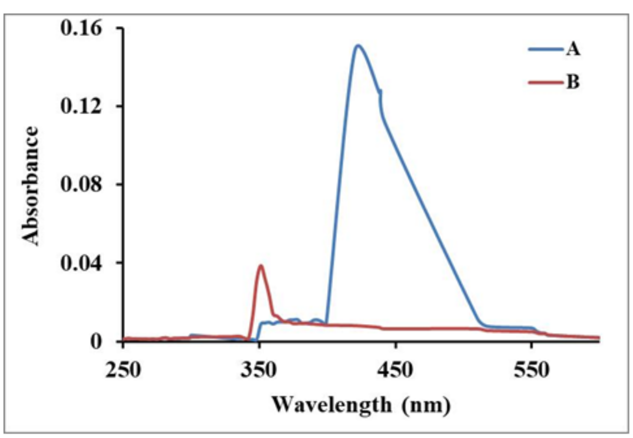


Figure 1: UV-Vis spectra of A: $CuO\ NPs$ and B: plant extract. Author's Own Work

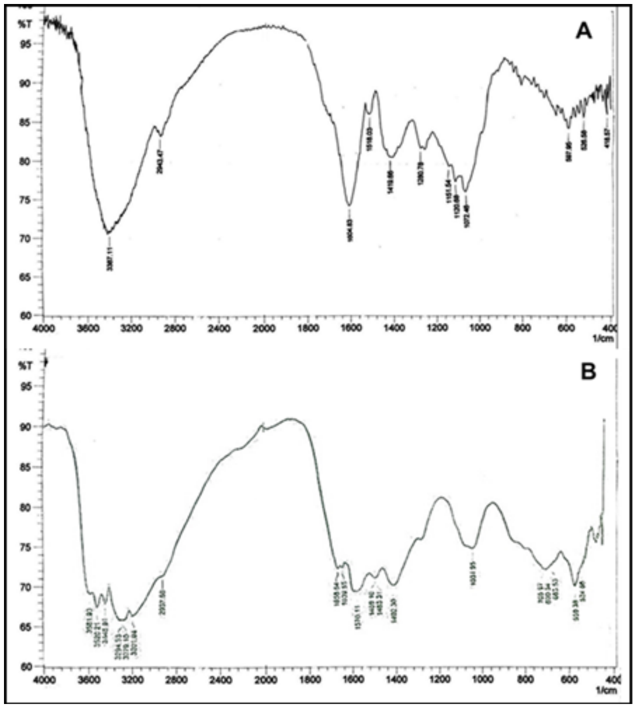


Figure 2: FTIR spectra of, A-garden cress seed extract and B. CuO nanoparticles Author's Own Work

bonds.

The surface functional groups on $CuO\ NPs$ were analyzed via $FT-IR$ spectroscopy shown many absorption peaks, reflecting the diverse functional groups as seen in Figure 2-B. A band between 3201.94 to $3581.93cm^{-1}$ is ascribed to the stretching vibration of the $O-H$ group. The bands at 1658.64, 1638.55 were identified the stretching vibrations of $C=O$ while a bands at 1570.11 and 1489.10 cm^{-1} correspond to the characteristic stretching vibrations of $C-C$ bonds within the aromatic ring anther band was indicated the $C-N$ stretching amide at 1402.30 cm^{-1}. The frequencies are similar to those reported elsewhere. Several additional peaks identified at 1031.95 cm^{-1} corresponding to the stretching vibrations of the $C-O$ bond and the bending vibrations of the $O-H$ bond. A peak at 559.35 cm^{-1} assumed to be a good evidence of copper's interaction plant extract to produce $CuO\ NPs$.

Phase purity and crystal structure formation of copper oxide nanoparticles were successfully analysed using X-ray diffraction (XRD). Figure 3A presents the XRD patents of CuO NPs via garden cress seed extract with many diffraction peaks located at 2θ values = 30:76°, 44.56°, 63.37°,74.32°and 77.55° corresponds to (110),(111), (200), (202),(220),(311) and (222) indicated a spherical structure of CuO NPs, and the diffraction data were in good agreement with previous study (Eslami *et al.*, 2017).

SEM analysis was used to confirm the size and shape of Cu oxide nanoparticles with no homogeneity as

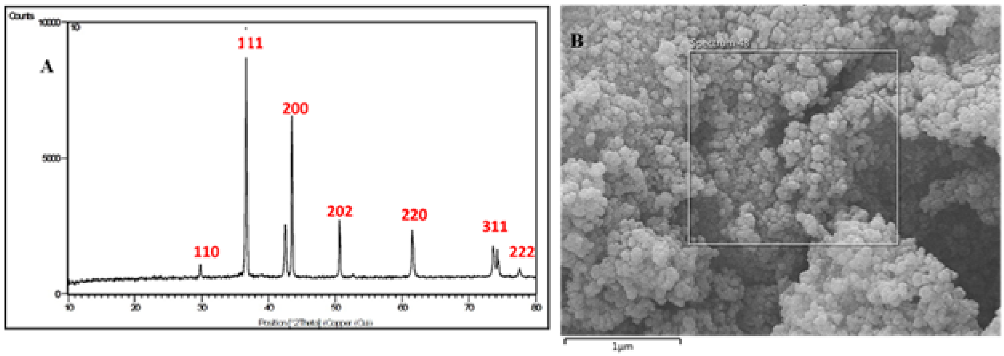


Figure 3: XRD diffraction pattern of CuO NPs, and B. SEM micrographs of green synthesised CuO NPs. Author's Own Work

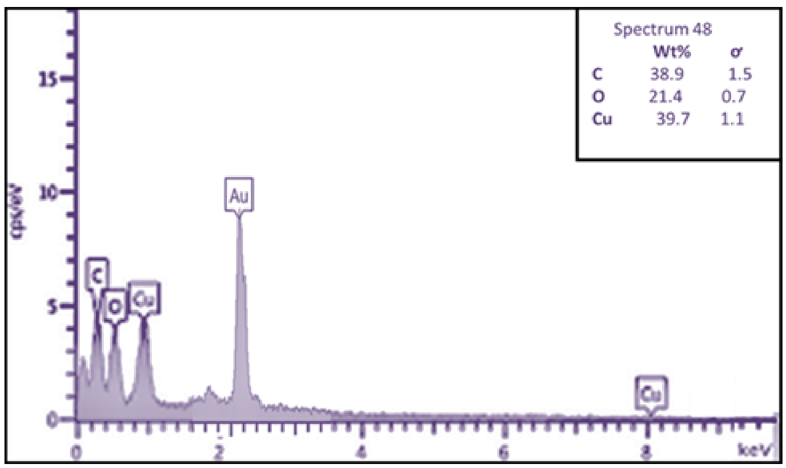


Figure 4: EDX spectrum of green synthesised CuO NPs. Author's Own Work

shown in Figures 3(B). The possible spherical shape of these NPs with varying particle sizes were measured. The nanoparticles size was found to be 35 nm with no agglomeration. The biochemical composition of these NPs was indicated by EDX mapping analysis. Figure 4, displays the EDX spectra of CuO NPs. Three elements were evidently recognized referring to Cu, O, and C.

3.2. Antioxidant activity

NPs' antioxidant properties are determined by their chemical composition, nature, stability, surface-to-volume ratio, size, surface coating, and charge (Ge *et al.*, 2022). The results revealed that both CuO nanoparticles and Vitamin C have an antioxidant properties, but CuO NPs had the maximum radical scavenging activity. Increasing nanoparticle concentration from 10 to $100 \mu g.mL^{-1}$ resulted in a significant increase in antioxidant activity from 45 % to 91 %. Figure 5.

The results showed that CuO NPs have higher antioxidant activity than Vitamin C when DPPH is used. This might be attributed to the change of DPPH on the surface of nano copper oxide to scavenge radicals. Further-

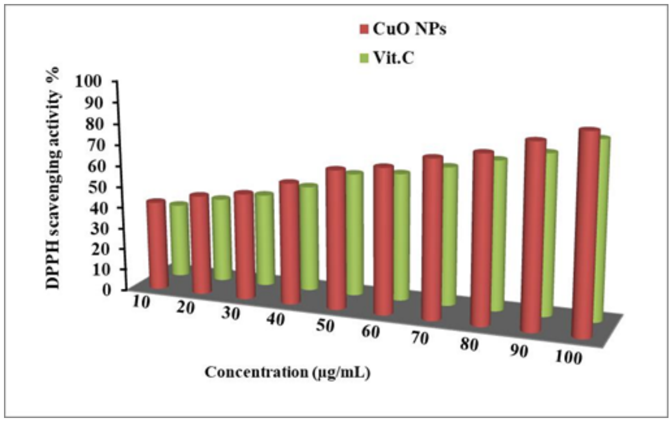


Figure 5: DPPH scavenging activity of CuO NPs in comparison with Vit.C using different range of concentrations. Author's Own Work

more, DPPH-CuO NPs may improve the efficiency of DPPH in scavenging radicals created by the oxidation process same as other metallic nanoparticles. It was reported that conventional coupling of the antioxidant functional moieties or entrapping the functional bio-activates on the surface of the inorganic nanoparticles could be one of the fantastic strategies for combining the surface activities of the nano-scaled particles and the antioxidant effects of incorporated functional moieties (Aardra *et al.*, 2023; Kumar *et al.*, 2020).

The mechanisms of nanoparticle antioxidant action are unknown; however, one theory is based on their intrinsic physicochemical features, which can improve scavenging reactive nitrogen and oxygen species while mimicking antioxidant molecules or antioxidant enzymes (Samrot *et al.*, 2022). Another way nanoparticles reduce free radicals is by converting alkyl peroxyl radicals to hydroperoxides (Ali *et al.*, 2025).

3.3. Antifungal activity

Copper oxide nanoparticles (CuO NPs) occupy a prominent place as potential antifungal agents for clinical use due to their broad spectrum of antimicrobial activity. The agar-well diffusion technique was used to assess the activity of these nanoparticles on M. cannonballus species, and the results showed that synthesised CuO NPs had potential antifungal action against the tested fungal strains. This may be due to their ability to limit fungal development by separating the cell membrane from the cell wall and intruding into the cytoplasm, resulting in the loss of cell wall integrity and homogeneity (Hashem *et al.*, 2022).

Various concentrations (1 and 2mg.mL^{-1}) of garden cress seed extract and CuO NPs were tested against this fungi. The results were significantly inhibited of M. cannonballus growth, resulting in effective fungus growth control. Figure 6; Table 1.

Nano-CuO was shown very good inhibition of M.C. in compassion to plant extract especially at the highest concentration possibly due to the increased permeability of the cell membrane. This leads to the structural

Table 1: The radial growth of fungi pathogen M.C. in different concentration of garden cress seed extract and CuO NPs.

Samples	Concentrations	R (cm)	Inhibition %
	Control	5	0 %
CuO NPs	1 mg.mL^{-1}	2.0	60 %
	2 mg.mL^{-1}	1.2	76 %
Plant extract	1 mg.mL^{-1}	3.9	22 %
	2 mg.mL^{-1}	3.5	30 %

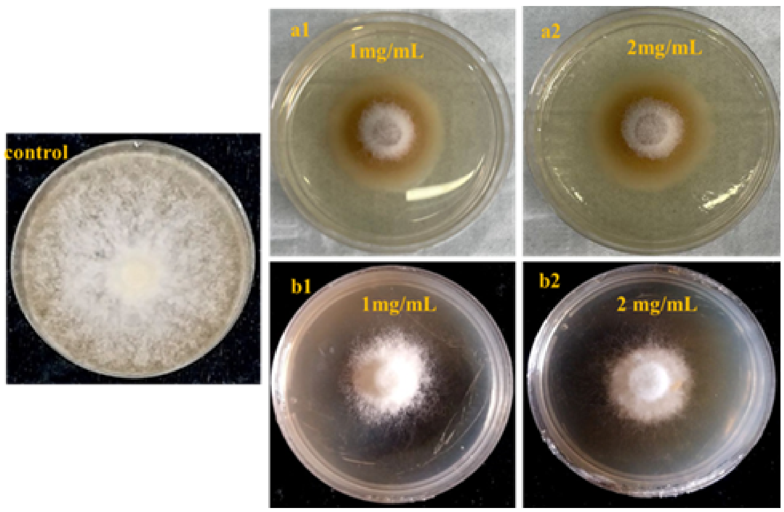


Figure 6: Antifungal activity of CuO NPs in comparison to garden cress seed extract against M.C. growth at two different concentrations (1 and 2 mg.mL^{-1}). $a1$, $a2$ plant extract, while,$b1$,$b2$ *CuO NPs*. Author's Own Work

destruction of cells and organelles (Ali *et al.*, 2025).

3.4. Beta-hematin inhibition

Recent discoveries have pointed out the possibility of using metallic oxide nanoparticles as therapeutic agents against malaria (Tiwari *et al.*, 2024). In this regard, CuO nanoparticles synthesized using garden cress seed extract have shown encouraging antimalarial activities through inhibition of beta-hematin formation, a precursor of hemozoin, which is an important factor for parasite survival. In particular, the heme crystallization inhibition activity was higher compared to the commercially used antimalarial drug chloroquine, suggesting their potential therapeutic implications Figure 7.

MNPs demonstrated promising antimalarial activity against both sensitive and chloroquine-resistant strains of P. falciparum, indicating that these particles could be effective and non-artificial therapeutic agents, such as silver nanoparticles (Avitabile *et al.*, 2020).

It is attributed to its ability to inhibit beta-hematin formation, crucial for parasite survival. MNPs may disrupt the crystallization of hemozoin through several mechanisms: by blocking crystal nucleation, binding to

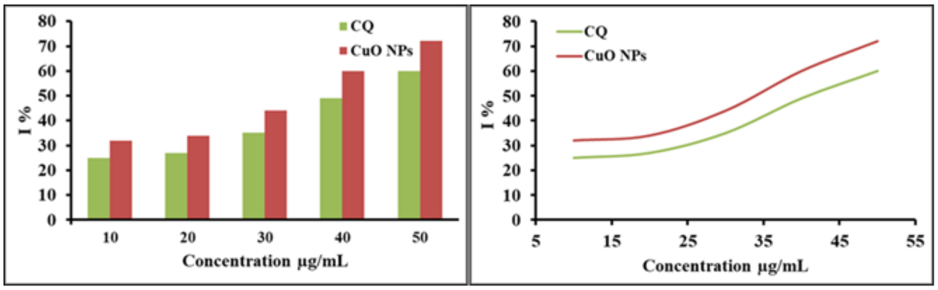


Figure 7: Heme crystallization inhibition (I%) of CuO NPs compared to CQ as a control. Author's Own Work

and destabilizing small existing crystals, or adsorbing onto the surface of growing crystals and preventing further growth and aggregation. The nanoparticle-mediated disruption of hemozoin formation presents a novel avenue in antimalarial therapy (Najer *et al.*, 2024).

4. CONCLUSION

This study demonstrates the eco-friendly synthesis of CuO NPs using plant seed extract. The synthesized CuO NPs exhibited significant antioxidant and anti-fungal activities with excellent inhibitory for BH formation making them potential candidates for therapeutic applications. Additionally, preparing copper oxide nanoparticles from plants could be a solid step forward in creating greener, more powerful drugs against malaria. Nevertheless, we still need careful, in-depth studies to identify how these tiny particles work and to make sure they are safe and effective when used in clinical studies.

Authors' contributions

Khilowd Omran Ali and Rana A.K. Al-Refai'a supervised the work in terms of its design and methodology. They processed the data obtained, analyzed it, and reviewed the manuscript. They also contributed to the preparation of the samples. Besides that, Ali H. Alsadoon designed the literature review section of the work and its draft manuscript. All authors read and benefited from the comments and suggestions of the other authors in the preparation and improvement of the research analysis.

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