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Green Synthesis, Characterization, and *In Vivo* Anti-Plasmodial Activity of Copper Oxide Nanoparticles Using *Moringa oleifera* Leaf Extract

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ABSTRACT

Introduction: Green synthesis is a commonly used method in the preparation of metal nanoparticles due to its cost-effectiveness and environmentally friendly nature. Metal nanomaterials have demonstrated superior inhibitory properties compared to their corresponding biological extracts. In this study, *Moringa oleifera*-based copper oxide nanoparticles designated (CuONPMOLE) were synthesized using *Moringa oleifera* (MOLE) leaf extract and tested for their antiplasmodial effects in an *in vivo* model.
Methods: The biosynthesized cuprite nanoparticles were characterized via UV-vis absorption spectroscopy, Fourier transform infrared spectroscopy (FT-IR), scanning electron microscopy (SEM), energy-dispersive spectroscopy (EDX), and X-ray diffraction (XRD). Inhibitory activity of CuONPMOLE on *Plasmodium berghei* was evaluated in rats.
Results: The results from SEM showed the morphology of CuONPMOLE, which appeared predominantly spherical, with some hollow areas. EDX analysis confirmed the presence of Cu atoms at 85.87%. The copper nanoparticles exhibited crystalline properties with an average size of 20.34 nm, and the XRD pattern displayed characteristic peaks corresponding to (111) and (200) reflections of the cubic cuprite crystal. The action CuONPMOLE against *Plasmodium berghei* parasites resulted in inhibitory activity of 43.37 ± 2.46%, demonstrating promising antiplasmodial activity and a notable decrease in malaria parasite growth ($P<0.05$).
Conclusion: The effectiveness of CuONPMOLE could be attributed to the plant phytochemicals present along with copper ions.

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Introduction

Bioactive compounds present in plant and spice extracts play a vital role due to their active functional groups, such as hydroxyl, aldehyde, amine, and carboxyl units, which are instrumental in reducing and stabilizing metals (Patra and El Kurdi, 2021). Conversely, the use of plant extracts for biogenic synthesis, exemplified by *Moringa oleifera*, has garnered attention as an environmentally friendly and cost-effective method with potential applications in drug delivery and antimicrobial treatments (Pagar et al., 2020). *M. oleifera* is renowned for its rich content of bioactive compounds, including flavonoids, polyphenols, alkaloids, and vitamins, making it a noteworthy source for reducing and capping agents in the synthesis of metal nanoparticles (Aaga and Anshebo, 2023). The bioactive elements within *M. oleifera* play a pivotal role in various pharmacological activities, highlighting its promise for sustainable synthetic metal nanoparticles (Bhalla et al., 2023; Ibrahim et al., 2021).

Copper oxide nanoparticles exhibit distinctive antimicrobial and antiparasitic properties, showing promise in combatting malaria parasites (Jayaseelan et al., 2022). However, traditional chemical synthesis approaches for copper oxide nanoparticles often necessitate high-temperature reducing and stabilizing agents, posing environmental hazards (Itodo et al., 2018). Malaria, stemming from Plasmodium parasites transmitted via Anopheles mosquito bites, remains a significant global health issue, particularly affecting tropical regions (Sukkanon et al., 2022). Annually, malaria claims around 409,000 lives, with a notably high mortality rate among infants and pregnant women (Monroe et al., 2022). Beyond the loss of life and health implications, malaria imposes a substantial socioeconomic burden due to healthcare costs, decreased productivity, and barriers to education and development, necessitating innovative interventions (Ahuru et al., 2021).

Therefore, leveraging at the properties of nanoparticles emerges as a promising strategy for diverse medical applications, notably in drug delivery (Qiu et al., 2023), imaging (Guan et al., 2023), and cancer therapy (Ghaffarlou et al., 2023). Nanoparticles offer enhanced drug loading and targeted delivery in malaria treatment, improving therapeutic effectiveness (Ghosh & Banerjee, 2020). Their capacity to enhance anti-malarial drugs ensures sustained efficacy and improved bioavailability (Kannan et al., 2019), underscoring their potential in malaria treatment for enhanced efficiency and delivery. We have previously reported the results of the activities of the nanoparticle of copper oxide with extracts of the roots and stem bark of MO (Emmanuel et al., 2025), this study therefore provides further insight to the properties of the copper oxide nanoparticles utilizing Moringa oleifera leaf extract to evaluate their anti-plasmodial potential in an in vivo model.

Materials and Methods

M. oleifera Leaf Extract (MOLE) Preparation

A fresh extract of *M. oleifera* was prepared using freshly harvested leaves sourced from the *M. oleifera* plantation at the Sheda Science and Technology Complex (SHESTCO) in Abuja, where herbarium voucher was deposited. The leaves were thoroughly cleaned with distilled water to eliminate impurities before being air-dried. To prepare the extract, 6 grams of finely powdered *M. oleifera* leaf were added to 200 mL of deionized water. The mixture was then heated to 100°C between duration of 20 to 30 minutes. Subsequently, the obtained extract was filtered using Whatman No. 1 filter paper and was stored at 4°C for future use.

Synthesis of CuONPMOLE

Initially, 3.63 grams of copper acetate (0.02M) were dissolved in 70 mL of deionized water and agitated using a magnetic stirrer for 10 minutes at room temperature. Subsequently, 30 mL of *M. oleifera* leaf extract (with a pH value of 7.5) were added drop by drop and heated to 60°C with continuous stirring at 250 rpm for 3 hours. The resulting solution underwent centrifugation at 3000 rpm for 20 minutes, followed by washing the precipitates with ethanol to eliminate impurities. The washed precipitates were then dried in an oven at 90°C for 2 hours until the solvent evaporated, and subsequently calcined in a preheated muffle furnace at 400 $\pm$10°C, resulting in the formation of brown CuONPs. (Almisbah and Mohammed, 2023).

Characterization of CuONPMOLE

The synthesis of CuONPMOLE was characterized using the JENWAY 6405 UV-Vis spectrophotometer. The UV-Vis absorption spectrum of copper oxide was characterized by conducting a wavelength scan ranging from 200 to 800 nm. The absorption spectra of the synthesized copper oxide nanoparticles obtained was generated and deduced. Following this, the FT-IR spectra of CuONPMOLE were analysed using an Agilent 630 Cary FT-IR Spectrophotometer. This instrument features an ATR module for liquids and solids, as well as a 'Dial Path' module for absorbance spectra of liquids, films, and gels. The CuONPMOLE were examined on KBr discs with a 4% (w/w) solid/KBr mixture, and the FT-IR spectra were recorded within the range of 400–4,000 cm-1. This analysis facilitated the study of bond details within compounds and the investigation of biomolecules present in plant extracts using 1 mm thick re-crystallized KBr discs.

For X-ray diffraction analysis, the Thermo Scientific ARL 'XTRA X-ray diffractometer with serial number

197492086 was employed at Umaru Musa Yar'adua University in Katsina State, Nigeria. XRD is a non-destructive technique used to characterize crystalline materials, providing valuable insights into structures, phases, texture, lattice parameters, chemical composition, and various structural parameters like average grain size, crystallinity, strain, and crystal defects.

Additionally, the PRO: X: 800-07334 Phenom World EDX Scanning Electron Microscope with serial number MVE01570775 was utilized to examine the surface morphology of materials. This instrument offers detailed information on structures and can conduct pore, fibre metric, and particle size analyses for nanoparticle materials.

Experimental Animals

The experimental Wistar rats were sourced from the College of Veterinary Medicine at Joseph Sarwuan Tarka University in Makurdi. Each group of experimental rats was housed in a 15x30cm cage with metal tops, which underwent regular thorough cleaning. Bedding materials, specifically wood shavings (sawdust), were utilised and replaced every week.

In vivo Parasitaemia Infection Assessment

The effectiveness of synthesized copper oxide nanoparticles was evaluated along with *M. oleifera* leaf extract, serving as the capping agent, following a methodology previously documented by researchers (Gitau et al., 2023).

After acclimating, Wistar rats weighing between 115 – 120 g for a period of 2 weeks, they were randomly segregated into four groups, with two rats in each group. Groups 1 and 2 were allocated as the test groups, while Groups 3 and 4 were assigned as the positive and negative control groups, respectively. Group 1 received treatment with CuONPMOLE, and Group 2 with the *M. oleifera* leaf extract. Group 3 was administered the standard drug Artemether as a positive control, while Group 4, as the negative control, received no treatment.

Approximately 2 mL of blood was freshly drawn from the Wistar rats and combined with 0.6 mL of normal saline. The rats were induced with 0.5 mL of parasitaemia (1 x 107 *Plasmodium berghei*) intraperitoneally to initiate infection. Subsequently, the rats were monitored for 24 hours to allow the Plasmodium parasite to propagate.

Following the 24-hour incubation period, the test groups were treated with the *M. oleifera* leaf extract and copper oxide nanoparticles, as well as the positive control (Artemether), each administered at a single dose of 50 mg/kg body weight and left for 48 hours. After this interval, blood samples were collected from a pricked tail and smeared onto a clean glass slide to create a thin blood film. The sample was air-dried in the laboratory for an hour, mixed with methanol, stained with Giemsa stain for 10 minutes, rinsed, dried, and examined under a microscope using a x100 objective lens. The percentage parasitemia and percentage inhibition on the red blood cells (RBC) were calculated using the equations (1) and (2) as follows:

(1)

$$\%\ \text{Parasitaemia} = \frac{Number\ of\ Parasitized\ Cells\ x\ 100}{Number\ of\ Red\ Blood\ Cells\ (RBC)}$$

(2)

$$\%\ \text{Inhibition} = \frac{\%\ Parasitaemia_{control} - \%\ Parasitaemia_{test\ group}\ x\ 100}{\%\ Parasitaemia_{control}}$$

Statistical Analysis

The data were analysed utilizing OriginPro Software® 2017. The study results were presented as the mean ± standard error of the mean (M±SEM). One-way analysis of variance (ANOVA) was employed to compare the treatment groups, supplemented by Tukey and Levene post-hoc analyses. Statistical significance was determined at a threshold of $p < 0.05$.

Results

UV–Vis analysis

The result of the UV-Vis analysis, depicted graphically in Figure 1, confirm the synthesis of CuONPMOLE.

FT-IR Analysis

The FTIR spectrum analysis of copper oxide nanoparticles further supports with the appearance of some distinct peaks (Figure 2), notably at 813 cm-1, attributed to metal-oxygen (Cu-O) stretching vibrations, aligning with the anticipated copper oxide composition.

SEM Analysis of CuONPMOLE

The surface morphology and size of CuONPMOLE was scrutinized through SEM analysis. The SEM illustrations depicting the images showcased intriguing visual representations, as shown in Figures 3(a) and (b) at magnifications of 5 μm and 1 μm, respectively.

EDX Analysis of CuONPMOLE

To verify the presence of the metals in CuONPMOLE, an EDX analysis was conducted, revealing distinctive peaks displayed in Figure 4 and the constituents in Table 1.

XRD Pattern of CuONPMOLE

The powder X-ray diffraction (XRD) pattern of CuONPMOLE is illustrated in Table 2 and Figure 5, displaying diffraction peaks identifiable with the cubic phase of Copper (I) oxide crystals (JCPDS card No. 01-078-2076).

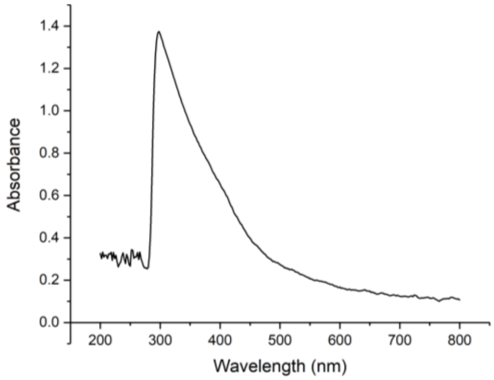

Figure 1: UV-visible spectra of CuONPMOLE

CuONPMOLE*: Moringa oleifera*-based copper oxide nanoparticles designated (CuONPMOLE) synthesized using *Moringa oleifera* (MOLE) leaf extract

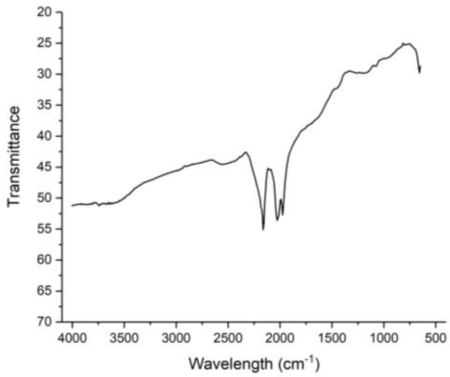

Figure 2: FTIR spectrum analysis of CuONPMOLE

CuONPMOLE*: Moringa oleifera*-based copper oxide nanoparticles designated (CuONPMOLE) synthesized using *Moringa oleifera* (MOLE) leaf extract

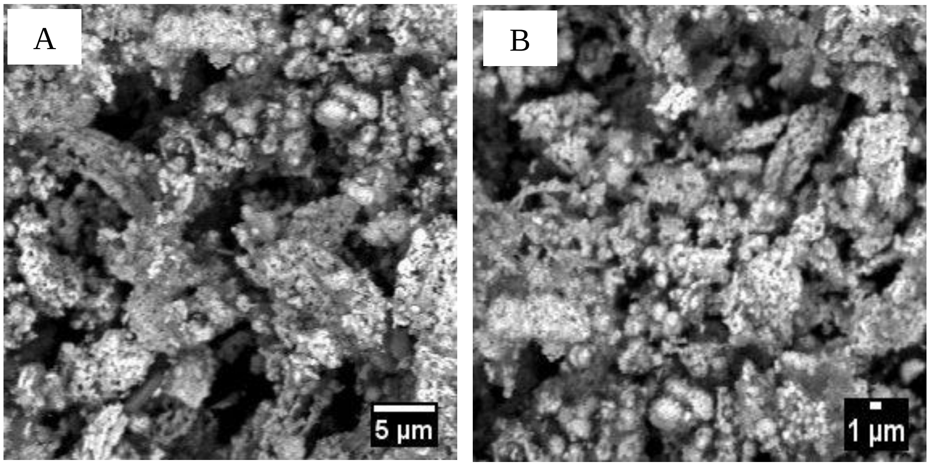

Figure 3: SEM of CuONPMOLE.

CuONPMOLE*: Moringa oleifera*-based copper oxide nanoparticles designated (CuONPMOLE) synthesized using *Moringa oleifera* (MOLE) leaf extract; A: 5 µm; B: 1 µm.

Table 1. EDX weight ratio of electrospun cuonps derived from *Moringa oleifera* leaf extract

Element Number	Element Symbol	Element Name	Atomic Concentration	Weight Concentration
29	Cu	Copper	85.87	93.59
11	Na	Sodium	8.90	3.51
19	K	Potassium	0.98	0.66
13	Al	Aluminum	1.18	0.55

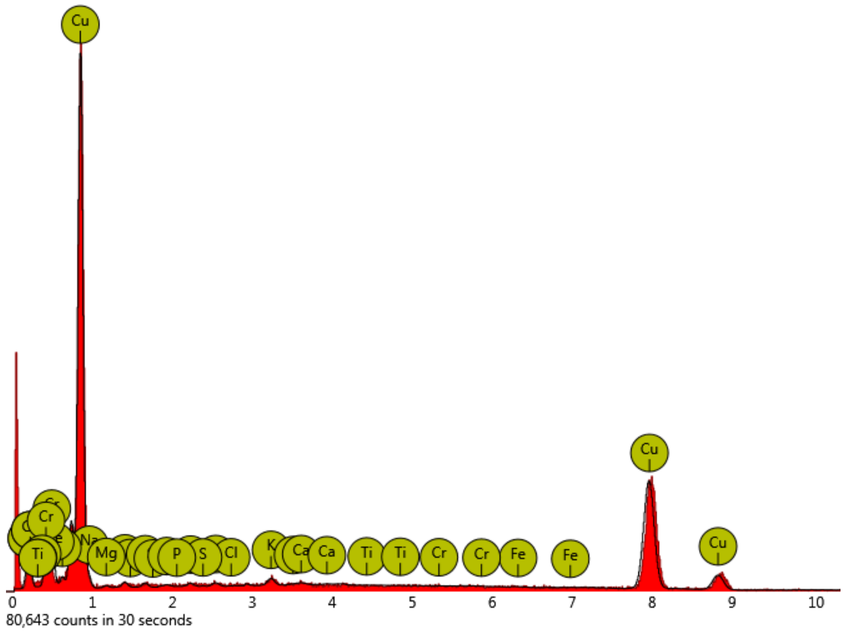

Figure 4: EDX spectrum analysis of CuONPMOLE.

CuONPMOLE*: Moringa oleifera*-based copper oxide nanoparticles designated (CuONPMOLE) synthesized using *Moringa oleifera* (MOLE) leaf extract.

Table 2. Some crystallographic parameters of the synthesized CuONPMOLE based on the x-ray diffraction pattern

S/N	Peak Position (2θ)	FWHW (β) (degree)	Crystallite size D (nm)	D (Average) (nm)
1	29.74	0.5925	13.88	20.34
2	32.88	0.6000	13.81	
3	36.54	0.3937	21.25	
4	39.06	0.3402	24.78	
5	42.38	0.5745	14.84	
6	43.40	0.2874	29.76	
7	45.30	0.3349	25.71	
8	50.58	0.3374	26.04	
9	61.44	0.7122	12.98	

CuONPMOLE*: Moringa oleifera*-based copper oxide nanoparticles designated (CuONPMOLE) synthesized using *Moringa oleifera* (MOLE) leaf extract.

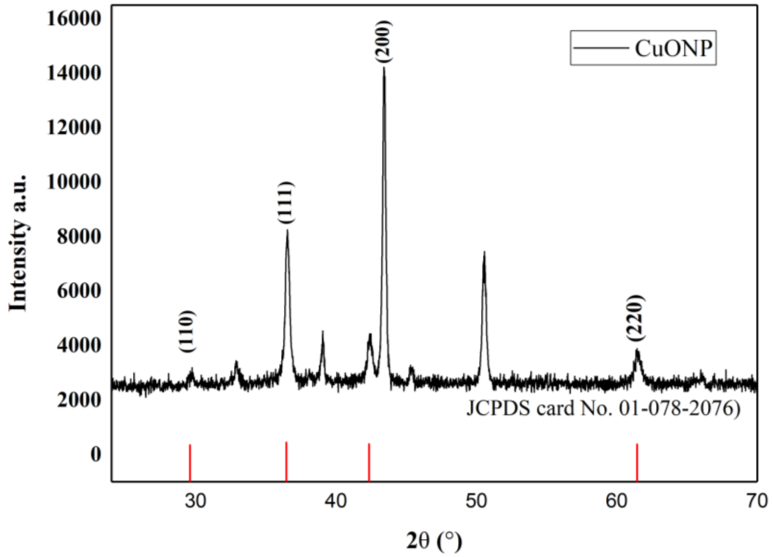

Figure 5: XRD patterns for synthesized CuONPMOLE.

CuONPMOLE*: Moringa oleifera*-based copper oxide nanoparticles designated (CuONPMOLE) synthesized using *Moringa oleifera* (MOLE) leaf extract; Different peak positions at 2θ along with the JCPDS card number indicating the miller indices of the synthesized nanoparticles.

Antiplasmodial Evaluation of CuONPMOLE

The efficacy of CuONPMOLE was compared with the crude MOLE and a positive control (Artemether) in combatting *Plasmodium* parasites. Figure 6 illustrates the average parasitized cells in the negative control and test groups among the experimental rats, with values presented as mean and standard error of the mean, with significance set at $p<0.05$ for comparison.

The in vivo anti-plasmodial potential, gauged by percentage inhibition, showed that the test materials (CuONPMOLE and MOLE) exhibited moderate activity against the growth of *Plasmodium berghei*. CuONPMOLE demonstrated significant inhibition of parasitaemia at 43.37±2.46%, while the MOLE showed inhibition at 13.33±7.88%. Artemether exhibited a percentage inhibition of 70.45±0.01%.

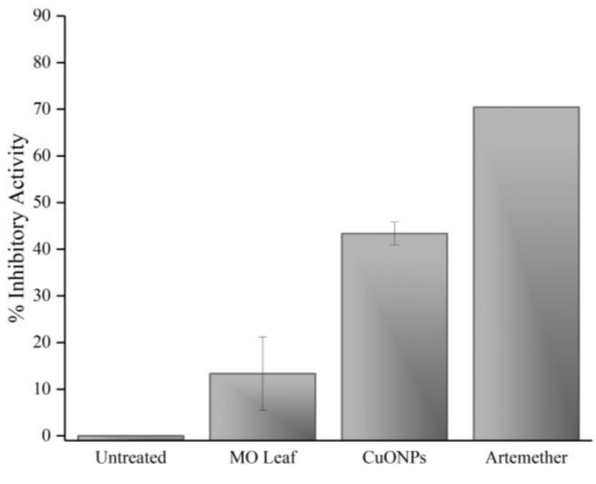

Figure 6: *In vivo* anti-plasmodial activity of CuONPMOLE

CuONPMOLE*: Moringa oleifera*-based copper oxide nanoparticles designated (CuONPMOLE) synthesized using *Moringa oleifera* (MOLE) leaf extract; Artemether (positive control); Values are presented as mean±SEM; $p<0.05$ was considered significant for comparison.

DISCUSSION

Previous reports of similar works underscored the potential of air-dried plants in acting as a stabilizing and reducing agent for generating metal nanoparticles (Teklu et al., 2023) as well as highlights on the initial color alteration observed during synthesis which could be attributed to the reaction between a plant extract and a metal solution. Moreover, distinct spectra indicative of shifts in electronic energy levels were reported.

CuONPMOLE was analyzed using UV–vis spectrophotometry ranging between 200 to 800 nm. The result of the UV-Vis analysis evidenced by an absorption band present around 300 nm wavelength, aligning with recent research outcomes (Nzilu et al., 2023).

The absorption bands from the FTIR spectra in Figure 2 further showed, the peak at 1334 cm-1 as stretching vibrations associated with alcohol deformation. Additionally, the peak observed at 2117 cm-1 indicates stretching in C≡N, potentially linked to nitrile functionality (Junejo et al., 2023). The peak at 2325 cm-1 likely signifies a metal-carbonyl (C=O) stretching vibration, suggesting the presence of carbonyl ligands on the nanoparticles' surface. Moreover, the peak at 2653 cm-1 is attributed to aryl C-H stretching vibrations. Notably, a prominent broad band at 3779 cm-1 indicates O-H stretching vibrations from surface hydroxyl groups or adsorbed water molecules (Akpomie and Conradie, 2023). These findings collectively offer valuable insights into the composition and surface properties of copper oxide nanoparticles, crucial for understanding their potential biological applications. Moreover, qualitative phytochemical screenings of the leaf extract in the previous works have indicated various phytochemical constituents such as phenols, aromatic compounds, carboxylic acids, alcohols, saponins, alkaloids, and flavonoids (Oladeji et. al, 2019; Walid et.al, 2023; Obialo et.al, 2024).

The presence of shifting peaks in the CuONPs following reduction with $Cu(COOCH3)_2$ suggests interactions between functional groups present in the plant extract and the copper salt precursor. This interaction plays a pivotal role in the reduction and stabilization of CuONPs.

The SEM images in both figures 3 (a) and (b) clearly showed rough surfaces of the CuONPMOLE exhibiting a predominantly spherical shape with dispersed hollows of varying sizes (Singh et al., 2019). This observation suggests the encapsulation of the metal with bioactive compounds sourced from plants, encompassing carotenoids, fatty acids, carbohydrates, polyphenols, amino acids, and vitamins (Waris et al., 2021).

The EDX spectrum derived along with the SEM images confirms the synthesis of copper nanostructures. Specifically, the copper content was quantified at 85.87 within the spectrum. Furthermore, Table 2 provides details on the full width at half maximum (FWHM, β), crystallite size (D), and associated parameters. The crystallite size, which represents the coherent diffraction volume of a material, is a critical parameter. For powdered materials, it may correspond to grain size, while in polycrystalline thin films, it can reflect thickness.

Peak broadening in the XRD pattern, observed at different 2θ positions along with the JCPDS card number, indicates the Miller indices of the synthesized nanoparticles. This broadening suggests the presence of small nanocrystals (Bin et al., 2022). As detailed in Table 2, the crystallite sizes range from 12.98 nm to 29.76 nm, with an average size of 20.34 nm. The peak positions at 2θ values of 29.74°, 36.54°, 42.38°, and 61.44° correspond to the (110), (111), (200), and (220) crystal planes, respectively (Kirfel & Eichhorn, 1990).

The in vivo antiplasmodial potential aligns with recent research findings, supporting the inhibitory effects of metal nanomaterials on malarial parasite proliferation (Veeragoni et al., 2023; Adeyemi et al., 2018).

We can further state that the anti-plasmodial efficacy of CuONPMOLE nanoparticles is linked to the phytochemicals present in *M. oleifera* leaf extract, known for enhancing immune function. Once introduced into infected red blood cells, CuONPMOLE nanoparticles can liberate copper (I) ions, inducing heightened oxidative stress within the cell, ultimately leading to parasitic nucleus damage. This evaluation of cuprite nanoparticles derived from plant sources signifies a promising avenue for potential antimalarial agents (Gitau et al., 2023).

Conclusion

The investigation outlines a cost-effective and environmentally friendly approach for synthesizing the copper oxide nanoparticles CuONPMOLE. Various characterization techniques, including UV-VIS, FTIR, SEM, EDX, and XRD, were utilized to confirm the properties of the metal nanoparticles. CuONPMOLE exhibited significant antiplasmodial efficacy against *P. berghei* in in vivo models, surpassing the effects of *M. oleifera* leaf extract. This enhanced antiplasmodial activity of CuONPMOLE is attributed to the presence of metabolites within the *M. oleifera* leaf extract. Furthermore, CuONPMOLE were observed to inhibit parasitaemia growth in an in vivo rat model infected with *P. berghei.*

Declaration

Conflict of interest

There is no conflict of interest among the authors.

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Consent for publications

The authors gave approval for the publication of the manuscript.

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None.

Authors' contributions

Conceptualization, data curation, formal analysis, funding acquisition, investigation , methodology, project administration, resources, software, supervision, validation, visualization, writing–original draft: Olajide Olutayo Olawumi
Writing–review & editing, conceptualization, funding acquisition, methodology, supervision, validation, and resources.
Fayomi Omotola Michael: Conceptualization, visualization, writing-original draft, data curation, software, investigation, methodology, supervision, and resources : Stella Adedunni Emmanuel:

Ethical considerations

All ethical issues (including plagiarism, misconduct, data fabrication, falsification, double publication, or redundancy) have been thoroughly observed by the author.

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