

Biosynthesis of Calcium Oxide Nanoparticles Using *Jatropha tanjorensis* Leaf Extract and the Evaluation of its Antibacterial Efficacy Against Multidrug Resistance Uropathogens and Haemocompatibility

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ABSTRACT

The biosynthesis of metal oxide nanoparticles using phytochemicals from plant extracts has been recognized as a sustainable, low-toxicity alternative to chemically synthesized antimicrobials. It provides an environmentally friendly approach to combat the growing burden of multidrug-resistant (MDR) pathogens. This study investigates the biosynthesis of calcium oxide nanoparticles (CaONPs) using aqueous leaf extract of *Jatropha tanjorensis*, an indigenous Nigerian medicinal plant rich in phytochemicals capable of chelating and reducing Ca^{2+} ions, and evaluates their antibacterial and haemocompatibility profiles against pathogens isolated from urine samples. CaONPs were synthesized by combining CaCl_2 solution with the leaf extract under alkaline conditions, followed by centrifugation and calcination at 700 °C. The nanoparticles were characterized using ultraviolet-visible spectrophotometry (UV-Vis), Fourier transform infrared (FTIR), X-ray diffraction (XRD) and scanning electron microscopy (SEM). Antibacterial activity was assessed against four uropathogenic isolates, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Bacillus cereus* and *Staphylococcus aureus*, using agar diffusion and broth microdilution to determine MIC and MBC. Biocompatibility was evaluated using a haemolytic assay on human erythrocytes. Characterization confirmed nanoparticle formation, phytochemical capping, heterogeneous surface morphology and particle dimensions within the nanoparticle size range. All isolates showed concentration-dependent bactericidal activity, with MIC and MBC values ranging from 1.88–3.75 µg/mL for *P. mirabilis* and 7.50–15.00 µg/mL for the remaining isolates, with inhibition zones of 15.20–22.00 mm. Haemolytic assays showed a favorable safety margin, with an estimated LC_{50} of 436.973 µg/mL, indicating minimal haemolytic activity within the effective antibacterial range. These findings position *J. tanjorensis*-mediated CaONPs as promising, biocompatible option for topical or catheter-coating antimicrobial applications.

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INTRODUCTION

Nanobiosynthesis has developed into a growing focus of recent antimicrobial research. It uses biological materials, specifically plant phytochemicals, to create nanoscale metal and metal oxide particles. This approach serves as a sustainable, environmentally friendly and low-toxicity alternative to chemically synthesized antimicrobials and conventional antibiotics. This shift is necessary due to the projected 39 million deaths from bacterial antimicrobial resistance (AMR) between 2025 and 2050, the equivalent of roughly three deaths every minute (Lithi *et al.*, 2025; Nguyen *et al.*, 2022; Shadhiya *et al.*, 2024). In May 2026, the World Health Organization launched an updated Global Action Plan on Antimicrobial Resistance, aiming for a 10% reduction in bacterial AMR-related deaths by 2030, using 2019 as a baseline (WHO, 2026). Nanobiosynthesis, also referred to as green synthesis, is recognized as a promising response to this issue. Unlike conventional antibiotics, metal oxide nanoparticles function through physical and oxidative mechanisms, including membrane disruption, generation of reactive oxygen species (ROS) and interference with intracellular biomolecules, reducing the probability of

bacteria developing cross-resistance (El-Seedi *et al.*, 2024; Archana *et al.*, 2023).

Calcium oxide nanoparticles (CaONPs) are unique antibacterial nanomaterials due to their biological abundance, safety and cost-effectiveness. They exhibit broad-spectrum antibacterial activity against both Gram-positive and Gram-negative bacteria by generating reactive oxygen species and disrupting bacterial membranes (Gedda *et al.*, 2015; Jadhav *et al.*, 2022; Algadi *et al.*, 2025). CaONPs show potentials for diverse biomedical applications, including antibiofilm therapy, drug delivery and dental disinfectants, while their compatibility with calcium hydroxide materials, which is already in clinical use, is a particular advantage (Kumari *et al.*, 2022). This study emphasizes their safety and compatibility for use in human tissue not only antibacterial efficacy. Biological synthesis methods, which uses phytochemicals from plant extracts as reducing and capping agents, offer a cost-effective and environmentally sustainable alternative to hazardous chemical reductants in producing biomedically relevant nanoparticles (Oghenefejiro *et al.*, 2026). Calcium oxide nanoparticles (CaONPs) have been phyto-synthesized from various plants, such as *Moringa oleifera* and *Ficus carica*, where they showed concentration-

dependent antibacterial activity against pathogens (Jadhav *et al.*, 2022; Khan *et al.*, 2023; Oghenefejiro *et al.*, 2026; Khalid *et al.*, 2025).

Jatropha tanjorensis (Euphorbiaceae), a leafy vegetable locally known as “hospital too far,” is widely consumed and used as medicine across Nigerian households for its health benefits (Yusuf *et al.*, 2021; Falodun *et al.*, 2014). Its rich phytochemical profile presents a novel opportunity for CaONP synthesis using its leaf extract, thereby strengthening the integration of phyto-nanotechnology and ethnobotanical studies. CaONPs has shown antibacterial activities against multidrug resistant (MDR) pathogens. Among other infections, there has been a rising concern of urinary tract infections (UTIs) due to multidrug resistant uropathogens, necessitating urgent action; hence, its relevance as a test case in this study. UTIs are prevalent globally, causing strain on health services, with common pathogens that include *Klebsiella pneumonia*, *Proteus mirabilis*, and *Escherichia coli* (Sokhn *et al.*, 2020). Recent findings from Nigerian hospitals indicate that MDR rates exceeds 85% especially among *Klebsiella* isolates, driven by inappropriate antibiotic use, in resource-limited areas like Agbor, Delta State (Mike-Ogburia, Monsi, & Nwokah, 2025). This situation complicates standard UTI treatments which informed the importance of exploring new antibacterial solutions, such as those offered by *J. tanjorensis* synthesized CaONPs.

The compatibility of the nanomaterial with human blood has been proposed for biomedical applications. Since haemolysis, the destruction of red blood cells, is a well-established indicator of nanoparticle-induced membrane damage and a mandatory safety parameter for any therapeutic use (Dobrovolskaia *et al.*, 2008). Nanoparticles can induce haemolysis through electrostatic interactions with erythrocyte membranes or by generating reactive oxygen species. Due to their small size and unique properties, the mechanisms of haemolysis can be substantially different from conventional drugs. Therefore, reporting haemolytic potential alongside

antibacterial activity is essential for determining the biomedical viability of such agents.

Despite extensive research on green synthesis of metal oxide nanoparticles, studies on calcium oxide nanoparticles (CaONPs) have mostly used ornamental or non-indigenous plants. This study focuses on *Jatropha tanjorensis* which is an indigenous plant known for its antimicrobial properties, to address the lack of site-specific evidence on the antibacterial and haemocompatibility profiles of CaONPs. It evaluates the antibacterial activity of the nanoparticle against uropathogenic bacteria and assesses biocompatibility through *in vitro* haemolytic tests. *Jatropha tanjorensis* has so far been exploited for green nanoparticle synthesis utilizing silver nanoparticle exclusively, where its antibacterial performance has been described only as moderate (Awote *et al.*, 2022). No prior report appears to describe *J. tanjorensis*-mediated biosynthesis of calcium oxide nanoparticles, nor their evaluation specifically against uropathogenic bacterial isolates. By utilizing *J. tanjorensis*, the research aims to enhance the therapeutic potential of CaONPs and establish the physicochemical characterization data for future studies.

MATERIALS AND METHODS

Plant Collection and Extract Preparation

Fresh leaves of *Jatropha tanjorensis* were collected from a family residential compound at Ogbe-Isogban community, Agbor, Delta State, Nigeria. The plant was identified and authenticated by a botanist in the Department of Botany, University of Delta, Agbor, and a voucher specimen JLE64. The leaves were washed with distilled water, dried in shade for 14 days and pulverized. Twenty grams of the powdered leaf material was extracted in 100 mL of deionized water and boiled for 5 minutes. A separate 20 g of powdered leaf was extracted in 100 mL of methanol and macerated for 72 hrs with agitation. The aqueous extract was used for nanoparticle synthesis, while the methanolic extract, obtained after double filtration through muslin cloth and Whatman No. 1 filter paper, for phytochemical screening (Marukurti *et al.*, 2025).

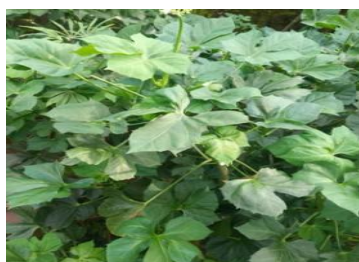


Figure 1: (A) *J. tanjorensis* leaf



(B) Powdered *J. tanjorensis* leaf+ $\text{Ca}(\text{NO}_3)_2$



(C) CaONPs

Qualitative Phytochemical Screening

The methanolic leaf extract was screened for alkaloids, tannins, anthraquinones, phenols, terpenoids, glycosides, saponins, flavonoids, steroids, protein and carbohydrate content using standard colorimetric and precipitation assays, with results shown qualitatively as present (+/++) or absent (–), following the methods of Harborne (1989) and Karthigaivelvi and Rameshwari (2016) with minor modifications the methanolic extract was used for phytochemical screening owing to its broader recovery of polar and semi-polar secondary metabolites compared to aqueous solvents.

Biological Synthesis and Physicochemical Characterization of CaONPs

Calcium oxide nanoparticles were synthesized following the procedure of Mazher *et al.* (2023). 50 mL of 0.2 M

$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ was combined with 50 mL of aqueous *J. tanjorensis* leaf extract and pH was adjusted to 10.5 using dropwise addition of 2.0 M NH_4OH under continuous agitation for 30 minutes until a milky $\text{Ca}(\text{OH})_2$ precipitate was formed. The precipitate was centrifuged at 10,000 rpm for 15 minutes, repeatedly washed with distilled water to remove unreacted starting material, and calcined at 700 °C for 3 hours to yield CaONPs. The synthesized nanoparticles were characterized by UV-Vis (nm), FTIR, XRD and SEM.

UV-VIS Spectrophotometric Analysis

UV-visible spectrophotometric analysis of the synthesized CaONPs was conducted using a UV-visible spectrophotometer (Apel 3000UV) with a slit width of 2 nm and a 10-mm quartz cell at room temperature. Absorbance was recorded over a wavelength range of 200–1100 nm to confirm nanoparticle formation (Jadhav *et al.*, 2022).

Fourier Transform- Infra - Red (FTIR) Spectroscopic Analysis

The Fourier transform infrared (FTIR) spectroscopic analysis was used to determine the functional groups of produced chitosan oil nanocomposites using Buck scientific M530 USA FTIR spectrometer for the analysis. This instrument was equipped with a detector of deuterated triglycine sulphate and beam splitter of potassium bromide. The software of the Gram A1 was used to obtain the spectra and to manipulate them. Zero point five millilitres (0.5 mL) was approximately of 1.0 g of samples, mixed properly and placed on the salt pellet. During measurement, FTIR spectra was obtained at frequency regions of 4,000 – 600 cm^{-1} and co-added at 32 scans and 4 cm^{-1} resolution. FTIR spectra were displayed as transmitter values (Lawrie et al., 2007).

X-ray Diffraction (XRD) Analysis

The phase purity and crystalline nature as well as the X-ray diffraction patterns of the produced chitosan oil nanocomposite was determined using an X-ray diffractometer (Philips X-ray Analytical, Amsterdam, The Netherlands). The samples for analysis were prepared by pressing the powder into the cavity of a sample holder and smoothing with a glass slide. They were scanned from 5° to 70° 2 θ with a Cu anode X-ray operated at 40 kV and 40 mA in combination with a Ni filter to give a monochromatic Cu-K α radiation ($\lambda = 1.5418 \text{ \AA}$). The average nanocomposite sizes were calculated using Equation 3 proposed by Patterson, (1939) where D is average particle size, K is the Scherrer's constant (0.9), λ is the wavelength of X-ray radiation (0.1542 nm), and β and θ are the half of maximum intensity and Bragg's angle, respectively (Patterson, 1939).

Scanning Electron Microscopic (SEM) Analysis

The sizes and morphologies of the produced chitosan oil nanocomposite was determined using scanning electron microscope (SEM) Quanta FEG 450 (FEI) (APOLLO X - EDAX). Zero-point five-gram samples were coated with Au/Pd film and the SEM images were obtained using a secondary electron detector. Point chemical analysis was performed in 2 independently selected particles (Aghayan et al., 2022).

Sample Collection and Identification

Bacterial isolates obtained from urine specimens of female patients attending a hospital in Agbor, Delta State, Nigeria were used for this study and their identity was confirmed using standard colonial, staining and biochemical characterization procedures (Cheesbrough, 2006) and interpreted according to standard biochemical identification

schemes for bacterial identification (Mito et al., 2024; Roy et al., 2023). Confirmed isolates were subcultured onto Nutrient Agar slants and preserved at 4 °C until further use.

Ethical approval for the use of urine isolates obtained from routine diagnostic testing was granted by the Health Research Ethics Committee of General Hospital, Agbor, Delta State, Nigeria. As only residual, de-identified isolates from tests already performed as part of patients' routine clinical care were used, individual patient consent was waived by the approving committee.

Antibiotics Susceptibility Profile and Multiple Antibiotics Resistant Index

Determination of MIC and MBC

Minimum inhibitory and bactericidal concentrations of the CaONPs were determined by broth microdilution (Wiegand et al., 2008). A CaONP stock (60 mg/mL in DMSO) was serially two-fold diluted in Mueller-Hinton broth to yield a concentration range of 0.23–60.00 mg/mL, inoculated with standardized bacterial suspensions (0.5 McFarland, approximately 1×10^8 CFU/mL), and incubated at 37 °C for 24 h. The MIC was recorded as the lowest concentration showing no visible turbidity, while the MBC was determined by subculturing broths onto fresh Mueller-Hinton agar and identifying the lowest concentration yielding no colony growth after further incubation CLSI, (2018).

Haemolytic (Biocompatibility) Assay

The haemocompatibility of the CaONPs was assessed on human erythrocytes following the method of Neun et al. (2018), with informed consent obtained from the blood donor. Erythrocytes, isolated from EDTA-anticoagulated venous blood by centrifugation (3,000 rpm, 15 min) and washed in phosphate-buffered saline (PBS), were exposed to CaONP concentrations of 10–100 $\mu\text{g/mL}$ for 1 h at 37 °C. Triton X-100 (1.5 mL) served as the positive (100% lysis) control. Following centrifugation, supernatant absorbance was read at 540 nm, and percentage haemolysis was calculated as: % Haemolysis = $[(\text{OD}_{\text{sample}} - \text{OD}_{0\% \text{ lysis}}) / (\text{OD}_{100\% \text{ lysis}} - \text{OD}_{0\% \text{ lysis}})] \times 100$. The median lethal concentration (LC50) was estimated from the resulting dose-response curve.

Statistical Analysis

All experiments were conducted in triplicate and results are expressed as mean $\pm$ standard deviation. Zones of inhibition, MIC, MBC values, and haemolysis percentages were compared descriptively across test organisms and treatment groups. Regression analysis was conducted using the Matplotlib library (Hunter, 2007; Bohmert et al., 2019; Dobrovolskaia et al., 2008).

RESULTS AND DISCUSSION

Phytochemical Composition of Methanolic Leaf Extract

Table 1: Phytochemical Component of the Methanolic Leave Extract of *Jatropha tanjorensis*

Component	Observation
Glycoside	+
Cardiac glycoside	+
Flavonoids	+
Tannins	+
Alkaloids	+
Terpenoids	++
Protein and amino acid	+
Carbohydrate	+
Saponins	+
Anthroquinone	-

Key: + =Present, ++ = Stronger, - = Absent

The x-ray diffraction (XRD) analysis *Jatropha tanjorensis* biosynthesized calcium oxide nanoparticles showed several diffraction peaks. These diffraction peaks indicates the presence of crystalline phases in the synthesized nanoparticles confirming the successful formation of the nanomaterial. The relative low-angle reflections and limited diffraction range suggest that the nanoparticles possess a combination of

crystalline armorphous characteristics, which is common in plant-mediated biosynthesis due to the presence of phytochemical capping agents. The observed diffraction therefore demonstrates the biosynthesized nanoparticles exhibit structural organization with varying degree of crystallinity.

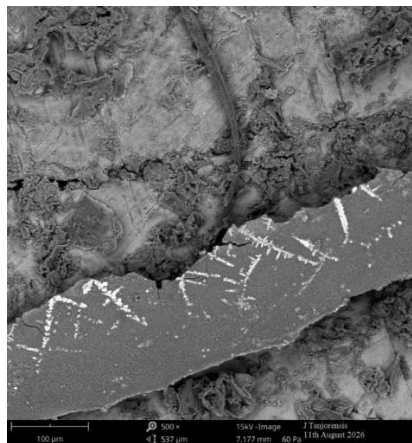


Figure 4: Scanning Electron Micrograph (SEM) of *Jatropha tanjorensis* Biosynthesized Calcium Oxide Nanoparticle

The scanning electron micrograph of the *Jatropha tanjorensis*-biosynthesized calcium oxide nanoparticles in this study shows a heterogeneous and irregular surface morphology characterized by pronounced aggregation with rough surface and compact plate-like structures interspersed with numerous bright particulate and elongated features approximately 4.11nm in size (Figure 4).

Uro-bacterial Isolation and Identification

A total of 33 bacterial isolates were recovered from urine samples and presumptively identified using colonial

morphology and biochemical tests. Antimicrobial susceptibility testing was subsequently carried out to determine the resistance profile of the uro-bacterial isolates and the isolate with the highest multiple antibiotic resistance (MAR) index was selected for further study. The uropathogenic bacterial isolates includes *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Proteus mirabilis* and *Bacillus cereus*.

Table 2: Antibacterial Activity of the *Jatropha tanjorensis* Calcium Oxide Nanoparticles Against Tested Uro-Bacterial Pathogens

S/N	Test strains	<i>Jatropha tanjorensis</i> CaONP (mm)	Ciprofloxacin (mm)	DMSO (mm)
1	<i>Klebsiella pneumoniae</i>	15.20±0.21	18.14±0.20	0.00±0.00
2	<i>Proteus mirabilis</i>	16.80±0.29	20.00±0.15	0.00±0.00
3	<i>Bacillus cereus</i>	18.00±0.00	19.34±0.23	0.00±0.00
4	<i>Staphylococcus aureus</i>	22.00±0.39	25.00±0.50	0.00±0.00

Key: Values Represent Mean Standard Deviation of Triple Determination; DMSO = Dimethylsulphide; CaONP = Calcium Oxide Nanoparticles

Table 2 shows that the CaONPs produced zones of inhibition against all four uropathogenic isolates, the largest zone 22.00±0.39 mm was observed for *Staphylococcus aureus* and the smallest against *Klebsiella pneumoniae* (15.20±0.21 mm). *B. cereus* showed 18.00±0.00mm and *P. mirabilis* showed 16.80±0.29. Although ciprofloxacin produced larger

inhibition zones across all isolates, the DMSO negative control produced no inhibition (0.00±0.00 mm) in any isolate.

Turbidity response of four bacterial isolates across serial two-fold concentration of calcium oxide nanoparticles

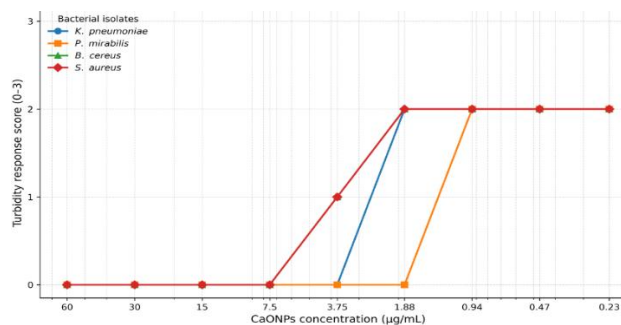


Figure 5: Turbidity Response of Bacterial Isolates to Serial Concentrations of *Jatrophha tanjorensis* CaONPs

Key: Turbidity Response 0= no Visible Turbidity, 1= Slight Turbidity, 2= Moderate Turbidity and 3= Dense Turbidity Observed for 1% Dimethyl Sulfoxide (DMSO) as Control

The CaONPs showed concentration-dependent antibacterial activity against the tested uropathogenic isolates. Complete inhibition of visible growth was observed at 7.50–60.00 µg/mL for all isolates, while *Klebsiella pneumoniae* and *Proteus mirabilis* were inhibited at 3.75 µg/mL but *Bacillus cereus* and *Staphylococcus aureus* showed slight turbidity.

Turbidity increased progressively with further dilution, becoming moderate at 1.88 µg/mL and dense across all isolates below 0.94 µg/mL. The clear minimum inhibitory concentrations (MICs) were 7.50 µg/mL for *K. pneumoniae*, *B. cereus*, and *S. aureus*, and 3.75 µg/mL for *P. mirabilis*. The DMSO control showed dense turbidity in all isolates.

Table 3: The MIC and MBC of *Jatrophha tanjorensis* CaONPs Against Selected Uropathogenic Bacterial Isolates

Bacterial isolate	MIC (µg/ML)	MBC (µg/ML)	MIC/MBC ratio
<i>Klebsiella pneumonia</i>	3.75	7.50	2.0
<i>Proteus mirabilis</i>	1.88	3.75	2.0
<i>Bacillus cereus</i>	7.50	15.00	2.0
<i>Staphylococcus aureus</i>	7.50	15.00	2.0

Key: MIC= Minimum Inhibitory Concentration Defined as the Lowest CaONP Concentration Showing no Visible Turbidity; MBC = Minimum Bactericidal Concentration, Defined as the Lowest CaONP Concentration Yielding no Colony Growth Following Subculture onto Fresh Mueller–Hinton Agar

Table 4: Biocompatibility Profile of *Jatrophha tanjorensis* Calcium Oxide Nanoparticles on Red Blood Cells

S/N	Treatment (RBC+CaONPs)	Observation
1	Control RBC	0.00
2	Triton X-100	100.0±0.00
3	10µg/ mL	1.10±0.01
4	20µg/ mL	2.73±0.02
5	40µg/ mL	4.50±0.04
6	60µg/ mL	7.67±0.06
7	80 µg/ mL	9.34±0.06
8	100 µg/ mL	11.20±0.03
9	LC ₅₀ (µg/ mL)	436.973

Key: RBC= Red Blood Cells

Regression Analysis of CaONPs on Red Blood Cells

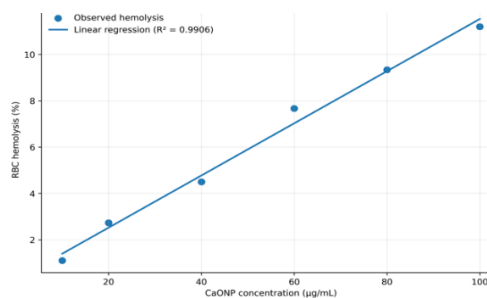


Figure 6: Showing Relationship Between CaONPs and Red Blood Cell Haemolysis

Discussion

This study presents the biosynthesis of calcium oxide nanoparticles (CaONPs) using *Jatrophha tanjorensis* leaf

extract, which functioned as both a reducing and stabilizing agent in the formation of the nanomaterial. The UV-Vis absorption peaks recorded at 280 nm and 380 nm confirms

phytosynthesized CaONPs, is consistent with the reports of Khan *et al.*, (2023); Naik *et al.*, (2023); Jadhav *et al.*, (2022). The SEM micrograph revealed a heterogeneous morphology of the CaONPs and the estimated particle size that falls within the nanoparticle size range. (Khan *et al.*, 2023). The potent bactericidal activity of CaONPs against all four uropathogenic isolates tested is notable aligns with report of Musa and Abubakar (2023). Complete inhibition of visible turbidity occurred at concentrations ranging from 7.50 to 60.00 µg/mL across the isolates, while increased turbidity, showing bacterial growth, was observed with decreasing CaONP concentrations. The corresponding MBC values obtained in this study indicates that the CaONPs showed bactericidal activity against all the isolates tested (Pankey & Sabath, 2004; Yoonus *et al.*, 2021). The observed activity may be attributed to the ability of CaONPs to exert multiple antibacterial effects, potentially involving several cellular targets and mechanisms (Azam *et al.*, 2012). The CaONPs may be considered as a target property of interest with regards to the increasing global burden of multidrug resistant uropathogen (Futoma-Kołoch *et al.*, 2025). Although the present study demonstrated the bactericidal activity of the CaONPs, it did not investigate the specific mechanisms responsible for this activity. Further studies are therefore required to determine how the CaONPs exert their antibacterial effects against the tested isolates. The haemocompatibility data, falls within the non-to-slightly-hemolytic zone, well below concentrations associated with meaningful erythrocyte damage. The calculated LC₅₀ of 436.973 indicates a substantial therapeutic window, distinguishing these CaONPs from nanomaterials whose antibacterial and cytotoxic thresholds align closely. These findings advance existing literature by documenting the evaluation of *J. tanjorensis*-mediated calcium oxide nanoparticles against clinically isolated uropathogens, while integrating antibacterial, MIC/MBC and haemocompatibility assessment within a single green-synthesis approach. This study remains scarcely represented in literature, positioning the present work as a genuine, contribution linking green nanotechnology to a specific area of clinically significant infection. Given that multidrug-resistant Enterobacterales and Gram-positive uropathogens increasingly limit oral antibiotic options in urinary tract infection management (Futoma-Kołoch *et al.*, 2025), a bactericidal, low-cytotoxicity, plant based nanomaterial represent a promising option for further development as a topical, catheter-coating or adjunctive antimicrobial agent.

CONCLUSION

This work revealed the use of *Jatropha tanjorensis* leaf extract as a reducing and stabilizing agent in the synthesis of calcium oxide nanoparticles. This synthesis is biologically safe and environmentally sustainable. Spectroscopic and structural characterization which includes UV-Vis, FTIR, XRD, SEM confirmed nanoparticle formation and retained phytochemical capping. The synthesized CaONPs exhibited strong, consistent bactericidal activity against both Gram-negative and Gram-positive uropathogenic bacterial isolates, with an especially strong effect against the resilient *Proteus mirabilis*, while remaining within safe haemocompatibility margins throughout the entire effective antibacterial range. These results position *J. tanjorensis*-mediated CaONPs as a promising, biocompatible option for further development against multidrug-resistant uropathogens, however future research is needed to clarify the exact mechanism of antibacterial action.

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Conflict of interest: The authors declare no conflict of interest

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