



RESEARCH ARTICLE

Antibacterial activity and toxicological insights of *Cissampelos pareira* L.-mediated zinc oxide targeting urinary tract infection pathogens

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Abstract

Eco-friendly methods for creating nanoparticles have gained attention due to the rising incidence of antibiotic resistance. Zinc oxide nanoparticles (ZnONPs) can be produced sustainably using *Cissampelos pareira* L., a medicinal plant high in bioactive phytochemicals. In this study, ZnONPs were synthesised using *C. pareira* extract and their antibacterial efficacy and initial toxicity profile were assessed. During ZnONP synthesis, the aqueous extract of *C. pareira* performed as a stabilising and reducing agent. The UV-visible spectroscopy confirmed the creation of nanoparticles, revealing a distinctive absorption peak at 350 nm that remained consistent for up to 48 hr. Agar well diffusion and time-kill experiments against *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella* sp. and *Candida albicans* were used to determine antimicrobial activity. The brine shrimp lethality test (BSLA) was used to assess preliminary toxicity at dosages between 5 and 80 µg/mL. The ZnONPs showed a species-specific susceptibility pattern and inhibitory effects that were dependent on concentration. The most susceptible species was *E. faecalis*, with inhibition zones measuring 23.8 ± 1.1 mm and reduced in viable counts of about $2.5 \log^{-1}$ after 4 hr. While *Klebsiella* sp. exhibited a minimal susceptibility, moderate inhibitory effects were noted against *S. aureus* and *E. coli*. At the highest measured concentration, ZnONPs reduced *C. albicans* by about $2 \log_1$. Under the tested conditions, BSLA results showed low acute toxicity, with ≥ 90 % survival at all concentrations. In the BSLA model, *C. pareira*-mediated ZnONPs showed low acute toxicity and concentration-dependent antimicrobial inhibitory action. Although these results demonstrate the potential of plant-mediated ZnONPs as antimicrobial agents, further characterisation of the nanoparticles, longer stability studies, suitable control experiments and thorough biocompatibility evaluations are needed to confirm their biomedical applicability.

Keywords: antimicrobial activity; brine shrimp lethality assay; *Cissampelos pareira*; green synthesis; time-kill assay; zinc oxide nanoparticles

Introduction

The rising costs of antimicrobial resistance (AMR) have become one of the most critical health problems in the 21st century (1). The World Health Organization reports that AMR has a potential to reverse decades of medical gains and is estimated that by 2050, AMR will report up to 10 million deaths per year in humans unless effective alternative measures are implemented (2). Traditional antibiotics are losing their effectiveness because of poor use as well as misuse, incorrect prescription and bacterial resistance because of efflux pumps, enzyme deactivation and development of biofilms (3). This precarious situation reveals the necessity of new antimicrobial methods that can be used, sustainable and less resistant to generation (4). Nanotechnology has become a potential new

development area in the battle against infectious diseases and is likely to provide materials with physicochemical properties that differ radically to those of the bulk material (5). At nanoscale, the materials are highly reactive, show greater surface area-to-volume ratio and interact well with biological system (6). There has also been a growing interest in metal and metal oxide nanoparticles because of their antimicrobial versatility as well as the versatile surface chemistry and ability to circumvent traditional resistance (7). Nanoparticles can degrade the membrane of bacteria, have the capability to produce reactive oxygen species (ROS) and can disturb metabolic processes causing the death of bacteria even against the resistant strain (8).

Zinc oxide nanoparticles (ZnONPs) are one type of

nanomaterial that has garnered a lot of interest because of their alleged antibacterial and wound-healing qualities. However, little is known about the production and biological assessment of ZnONPs mediated by *Cissampelos pareira* L., specifically their antibacterial activity and initial toxicity profile, which served as the foundation for the current study (9). The ZnONPs are recognised as generally safe by the U.S. Food and Drug Administration (FDA), making them more attractive for biomedical applications compared to other metal nanoparticles such as silver or copper, which may carry higher toxicity risks (10). The ZnONPs' antibacterial activity is mediated by a variety of processes, including as the production of ROS, rupture of membranes and release of metal ions, offering a more scientifically sophisticated explanation. One of the key pathways involves the generation of ROS such as hydroxyl radicals, superoxide anions and hydrogen peroxide, which cause oxidative stress and damage to microbial DNA, proteins and membranes. Another mechanism is the release of Zn^{2+} ions, which interfere with enzymatic activity, disrupt protein structure and impair metabolic processes essential for bacterial survival (11–14). Furthermore, the physical interaction of ZnONPs with microbial membranes can cause structural deformation, leakage of cytoplasmic contents and eventual cell lysis (11). In addition to physicochemical characteristics like size, morphology, surface charge and concentration, ZnONPs' biological activity is also impacted by phytochemical capping agents derived from *C. pareira*, which may have an impact on surface interactions, nanoparticle stability and antimicrobial effectiveness. Smaller nanoparticles with greater surface-to-volume ratios exhibit enhanced antimicrobial activity because of improved penetration and reactivity (12).

The ZnONPs' physicochemical characteristics, such as their antibacterial, antioxidant, UV-protective and wound-healing qualities, have sparked a significant amount of research in the fields of biomedicine, food and cosmetics. The ZnONPs have been investigated in medicine for tissue engineering, medication administration, wound care and surface modification of implants to lessen microbial colonisation (15, 16). These have also been used into topical formulations, oral healthcare medicines and antifungal systems due to their antibacterial activity (17). Besides applications in the medical field, ZnONPs are utilised in food packaging materials to improve microbiological safety and extend shelf life and their antioxidant and UV-blocking qualities facilitate employment in cosmetic formulations (18). The safety of traditionally manufactured ZnONPs is still a problem, though, because leftover chemical reagents and synthesis byproducts may contribute to cytotoxicity and genotoxicity, potentially restricting their biological translation (19).

High temperatures, pressures, or reducing agents like hydrazine and sodium borohydride are frequently needed in conventional methods of nanoparticle synthesis, including physical and chemical procedures (20). These methods can give fine control over the size, shape and crystallinity of nanoparticles, but they are often energy-intensive and may include dangerous chemicals that raise issues with biocompatibility and the environment (21). As a result, green synthesis methods are becoming more popular as ecologically friendly substitutes. These techniques use biological resources, such as plant extracts, algae and microbes, as stabilising and reducing agents. Plant-mediated synthesis makes use of naturally occurring phytochemicals that can aid in the production and stability of nanoparticles, including alkaloids, flavonoids, phenolics, tannins, terpenoids and saponins (22). Furthermore, the

resultant nanoparticles' surface characteristics and biological activity may be impacted by phytochemical capping (23). However, there are drawbacks to plant-mediated synthesis as well, such as inconsistent phytochemical composition from batch to batch, little control over nanoparticle properties and issues with repeatability. Despite these drawbacks, a variety of medicinal plants, such as *Terminalia chebula* Retz., *Zingiber officinale* Roscoe and *Ocimum tenuiflorum* L., have been effectively used for ZnONP synthesis, showing the promise of green nanotechnology while emphasising the necessity of meticulous characterisation and standardisation (24).

Alkaloids, flavonoids, tannins, phenolic compounds and terpenoids are all present in the phytochemically rich medicinal plant *C. pareira*, many of which possess potent redox and metal-chelating qualities (25). During the formation of nanoparticles, these bioactive components can operate as natural reducing and stabilising agents, contributing to transform metal precursors into nanoparticles and promoting colloidal stability through surface capping. Furthermore, phytochemical capping may affect biological interactions, antibacterial activity and surface properties of nanoparticles (26). The antibacterial and antioxidant properties of *C. pareira* extracts have been shown in earlier research, indicating that its phytochemical profile may be beneficial for the creation of green nanoparticles (27). The application of *C. pareira* for ZnONP production is currently mainly unexplored despite these qualities. Thus, studying *C. pareira*-mediated ZnONPs may offer a sustainable substitute for traditional synthesis techniques and shed light on how plant-derived phytochemicals affect nanoparticle formation, stability and biological activity (28).

The primary objective of the current work was to create ZnONPs using an aqueous extract of *C. pareira* and investigate their initial toxicity profile and antibacterial efficacy. Five clinically relevant bacteria were used to investigate the antibiotic activity of these green-synthesized ZnONPs: *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella* sp. and *Candida albicans*. While toxicity was evaluated using the brine shrimp lethality assay (BSLA), a commonly utilised preliminary screening model for biological safety, both agar well diffusion and time-kill curve assays were performed to offer complementing information on antimicrobial activity (29). The future potential of plant-mediated synthesis as an environmentally benign method of creating physiologically active ZnONPs is highlighted in this study. To the best of current information, this is one of the first reports detailing the synthesis of ZnONPs using *C. pareira* extract and assessing their activity against a panel of clinically relevant microorganisms. As a result, it serves as a foundation for further research that will include thorough nanoparticle characterisation, sophisticated biocompatibility testing and assessment against strains resistant to antibiotics.

Materials and Methods

Preparation of *Cissampelos pareira* extract mediated zinc oxide nanoparticles

To obtain the aqueous extract, *C. pareira* powder (1 g) was suspended in 100 mL of distilled water. The mixture was subjected to controlled heating at 55 °C for 15–20 min using a heating mantle. The moderate temperature ensured the release of bioactive phytochemicals while preventing their thermal degradation, which can occur at higher temperatures. After boiling, the extract was

cooled to room temperature to preserve heat-sensitive metabolites and to avoid denaturation of active compounds. The cooled solution was filtered through Whatman No. 1 filter paper, which provides an optimal pore size (11 μm) to remove coarse plant debris while retaining soluble bioactive constituents. The clear filtrate obtained was collected and stored under sterile conditions at 4 °C until further use. This aqueous extract served as a reducing and stabilising agent in the subsequent synthesis of nanoparticles. The ZnONPs were synthesised using a green approach with *C. pareira* extract as both the reducing and stabilising agent. The stepwise preparation of the *C. pareira* extract and ZnONPs is shown in Fig. 1.

Briefly, 30 mM zinc sulfate (ZnSO_4) solution was prepared by dissolving the appropriate amount of salt in 50 mL of distilled water under constant stirring to ensure complete solubilisation. Subsequently, 50 mL of the pre-filtered *C. pareira* extract was added dropwise to the ZnSO_4 solution in a 1 : 1 ratio. The reaction mixture was incubated in an orbital shaker at ambient temperature for 48 hr to promote the reduction of zinc ions and nucleation of nanoparticles. Continuous shaking was employed to maintain homogeneity and to facilitate efficient interaction between phytochemicals and zinc ions. After the incubation period, the nanoparticle suspension was subjected to centrifugation at 8000 rpm for 15 min. This step enabled the separation of ZnONP pellets from the unreacted biomolecules and soluble impurities present in the supernatant, which was subsequently discarded. The obtained pellets were thoroughly washed with double-distilled water to

remove residual impurities and were collected for further drying and characterisation.

Results

The formation and progression of *C. pareira*-mediated ZnONPs were monitored using UV-visible spectroscopy over a period of 48 hr. A distinct absorption band was consistently observed in the range of 340–360 nm, which is characteristic of zinc oxide nanoparticles. At 3 hr, a sharp and intense absorption peak was recorded with an absorbance value of approximately 3.0, indicating rapid nucleation and the onset of nanoparticle formation which was depicted in (Fig. 2). This strong signal reflects the high concentration of ZnONPs generated in the early stages of the reaction. With an increase in incubation time to 6 hr and 15 hr, a gradual decline in absorbance intensity was observed, suggesting the progressive reduction of free zinc ions and their conversion into stabilised nanoparticles. At 24 hr and 36 hr, the absorption peak was retained within the same wavelength range, although the overall absorbance intensity further decreased compared to earlier time points. This indicated that the reaction was approaching equilibrium, with most zinc precursors already transformed into ZnONPs. By 48 hr, the spectral profile remained stable with no significant red or blue shift of the absorption maxima, demonstrating the successful stabilisation and uniformity of the synthesised nanoparticles. Importantly, the absence of major peak shifts throughout the incubation period suggests that the

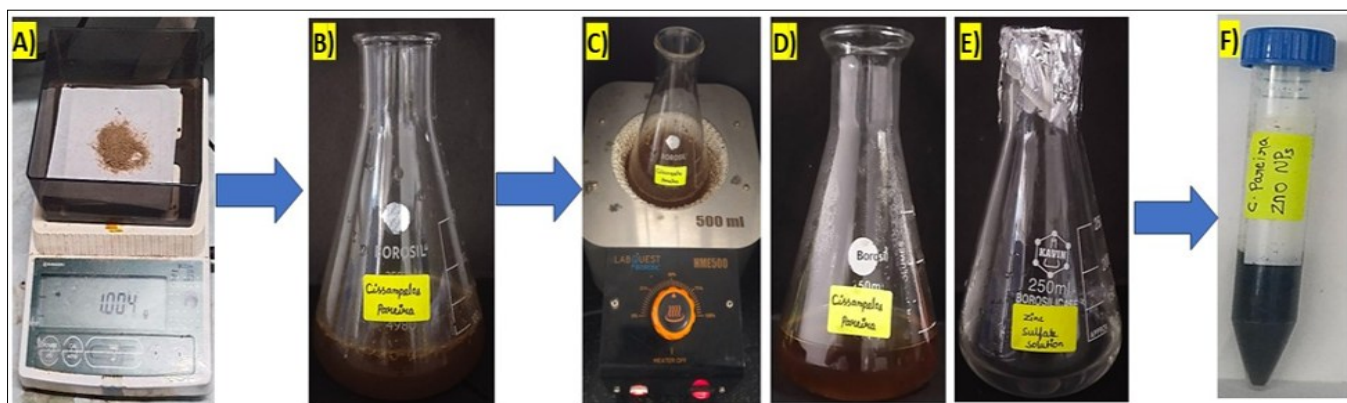


Fig. 1. Stepwise preparation of *C. pareira*-mediated ZnONPs. A: Weighing of *C. pareira* powder (1 g); B: suspension of powder in 100 mL distilled water; C: heating of the mixture at 55 °C for 15–20 min using a heating mantle; D: filtration of the extract; E: mixing of extract with 30 mM zinc sulfate solution; F: collection of ZnONPs pellet after centrifugation.

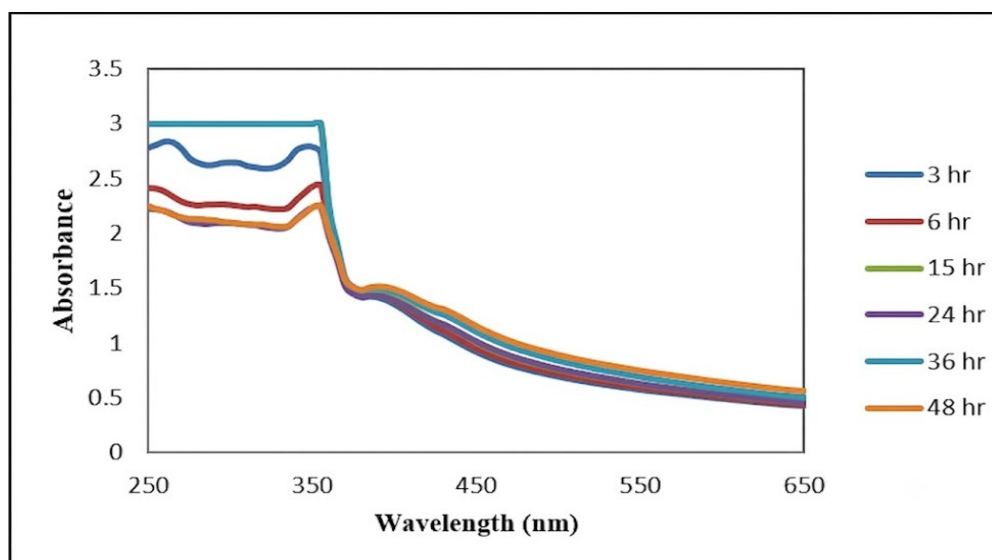


Fig. 2. UV-Visible spectra of green synthesised zinc oxide nanoparticles (ZnONPs).

nanoparticles maintained structural stability without undergoing agglomeration or significant size variation during synthesis. The spectral pattern confirmed that the synthesis was complete by 48 hr, yielding stable ZnONPs with consistent optical properties.

The biogenesis of ZnONPs was characterised by the functional group analysis of the NPs (Fig. 3), the functional group of the NPs and the absorbance peak at 3221, 2931, 1626, 1423, 1141, 1075, 1023, 758, 701 and 610 cm^{-1} . Corresponding peak of the NPs at O-H stretching (hydroxy group), C-H stretching (alkane), C=O Stretching (amide), C-N Stretching, C-O-C stretching, C-O stretching, C-H aromatic bending and Zn-O. it was confirmed the synthesis of

ZnONPs and the plants was utilised the reducing agent.

The antimicrobial potential of *C. pareira*-mediated ZnONPs was evaluated against five clinically relevant pathogens such as *S. aureus*, *E. faecalis*, *E. coli*, *Klebsiella sp.* and *C. albicans* using the agar well diffusion method. Representative inhibition zones are shown in Fig. 4 (A–E), while the quantitative data are presented in Fig. 4A clear concentration-dependent increase in antimicrobial activity was observed. At 25 $\mu\text{g/mL}$, ZnONPs exhibited moderate inhibition against *E. faecalis* (12.7 ± 0.5 mm) and comparatively lower inhibition zones (8.5–9.3 mm) against *S. aureus*, *E. coli*, *Klebsiella sp.* and *C. albicans*. Increasing the concentration to 50 $\mu\text{g/mL}$ enhanced

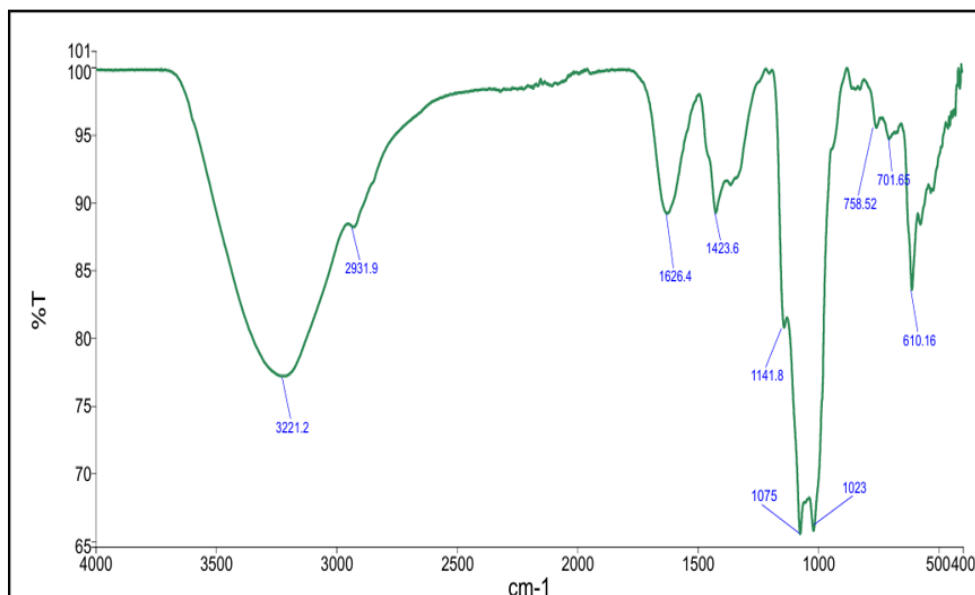


Fig. 3. Fourier-transform infrared spectroscopy analysis of zinc oxide nanoparticles (ZnONPs).

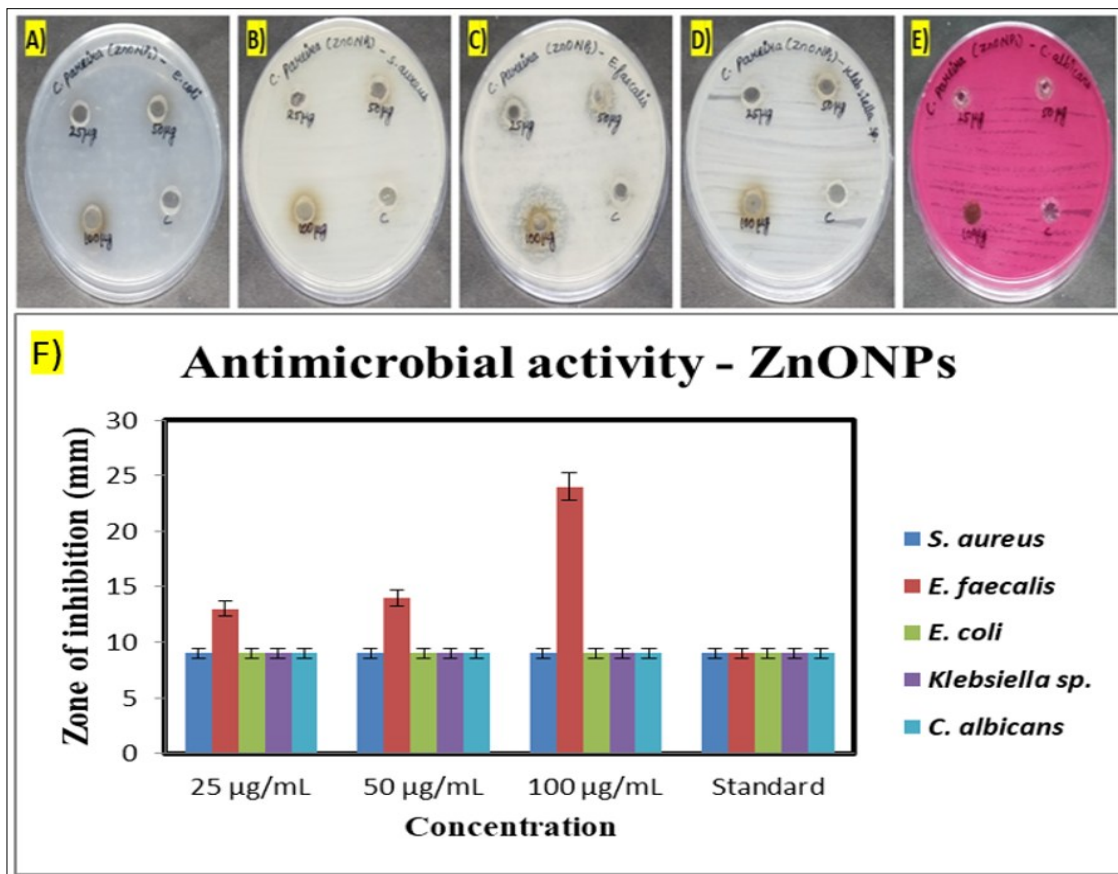


Fig. 4. *Cissampelos pareira*-mediated zinc oxide nanoparticles (ZnONPs) against test pathogens by agar well diffusion assay. A: *Escherichia coli*; B: *Staphylococcus aureus*; C: *Enterococcus faecalis*; D: *Klebsiella sp.*; E: *Candida albicans*; F: Graph representing the inhibition zones of antimicrobial activity.

the inhibition zone of *E. faecalis* to 14.2 ± 0.6 mm, while other test organisms showed inhibition zones of approximately 9–10 mm. The highest concentration tested (100 $\mu\text{g/mL}$) demonstrated a pronounced antimicrobial effect, particularly against *E. faecalis* (23.8 ± 1.1 mm), which was significantly greater compared to other organisms (9.1–9.5 mm). Interestingly, *S. aureus*, *E. coli*, *Klebsiella* sp. and *C. albicans* showed only marginal differences in inhibition zones across concentrations, suggesting a pathogen-specific response to ZnONPs. The standard antimicrobial agent exhibited inhibition zones in the range of 9–10 mm across all organisms, comparable to the lower concentrations of ZnONPs but markedly lower than the effect observed against *E. faecalis* at 100 $\mu\text{g/mL}$. These findings highlight that ZnONPs synthesised using *C. pareira* extract possess broad-spectrum antimicrobial activity, with the most significant inhibitory effect observed against *E. faecalis* in a concentration-dependent manner.

The antimicrobial activity of ZnONPs was further assessed through time-kill kinetics against *S. aureus* over a 4 hr incubation period (Fig. 5A). A clear concentration-dependent reduction in viable cell counts was observed. At 25 $\mu\text{g/mL}$, ZnONPs exhibited only marginal inhibitory effects, with colony-forming unit (CFU) counts remaining close to baseline throughout the experiment. Increasing the concentration to 50 $\mu\text{g/mL}$ resulted in a gradual decline in bacterial viability, reaching approximately 1 log₁₀ reduction at 4 hr. The ZnONPs at 100 $\mu\text{g/mL}$ demonstrated the most pronounced antimicrobial activity, causing a steady reduction in bacterial growth from the 1 hr onward and achieving nearly a 2 log₁₀ reduction by 4 hr. In contrast, the standard antibiotic displayed rapid killing, reducing bacterial counts by more than 2 log₁₀ within 4 hr. The untreated control showed a slight increase in bacterial population, confirming normal growth kinetics in the absence of treatment.

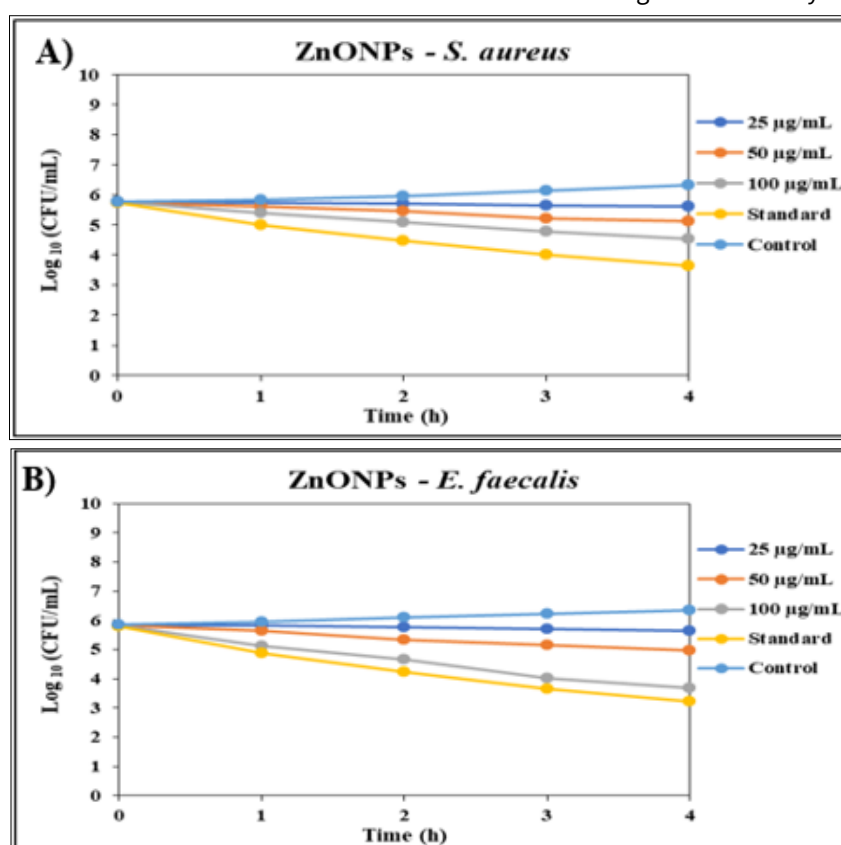
A time-kill experiment was used to determine ZnONPs' antibacterial effectiveness against *E. faecalis* (Fig. 5B). There was a definite inhibitory influence that was concentration dependent. The

ZnONPs caused a negligible decrease in bacterial viability at 25 $\mu\text{g/mL}$, while after 4 hr, 50 $\mu\text{g/mL}$ caused about a 1 log₁₀ reduction. Nearly a 2.5 log₁₀ reduction was obtained at the highest concentration (100 $\mu\text{g/mL}$), indicating significant but insufficient inhibition of bacterial growth. This result did not fulfill the ≥ 3 log₁₀ threshold, which is usually used to indicate bactericidal activity, even though it was close to that of the standard antibiotic. Therefore, rather than bactericidal activity, a substantial inhibitory reaction effect would be a better description of the observed response.

A time-kill curve test was used to determine the ZnONPs' antibacterial efficacy against *E. coli* (Fig. 5C). The ZnONPs showed minimal inhibitory effects at 25 $\mu\text{g/mL}$ and after 4 hr, only a slight reduction of about 1 log₁₀ CFU/mL was achieved at 50 $\mu\text{g/mL}$. Under the tested conditions, these results demonstrate partial suppression of bacterial growth rather than bactericidal activity.

In contrast, the 100 $\mu\text{g/mL}$ concentration displayed more pronounced bactericidal activity, with bacterial counts steadily decreasing from the first hour onward, resulting in nearly a 1.5–2 log₁₀ reduction at 4 hr. The standard antibiotic showed the strongest effect, reducing bacterial viability by almost 3 log₁₀ within the same duration. The control group exhibited a gradual increase in CFU/mL, confirming normal growth in the absence of treatment.

A 4 hr time-kill analysis was used for evaluating ZnONPs' antifungal efficacy against *C. albicans* (Fig. 5E). The ZnONPs showed negligible inhibitory effects at 25 $\mu\text{g/mL}$, with fungal counts being mostly constant over the incubation period, similar to the control; nevertheless, the instructions incubation period limits the possibility of establishing strong inferences on antifungal efficacy. At 50 $\mu\text{g/mL}$, a modest reduction was observed, resulting in approximately a 1 log₁₀ decrease in CFU/mL after 4 hr. At 100 $\mu\text{g/mL}$, ZnONPs exhibited significant antifungal activity, producing a steady reduction in viable cell counts from the 1 hr onward and achieving nearly a 2 log₁₀ decrease by 4 hr. The standard antifungal agent demonstrated the greatest inhibitory effect, with more than 2.5



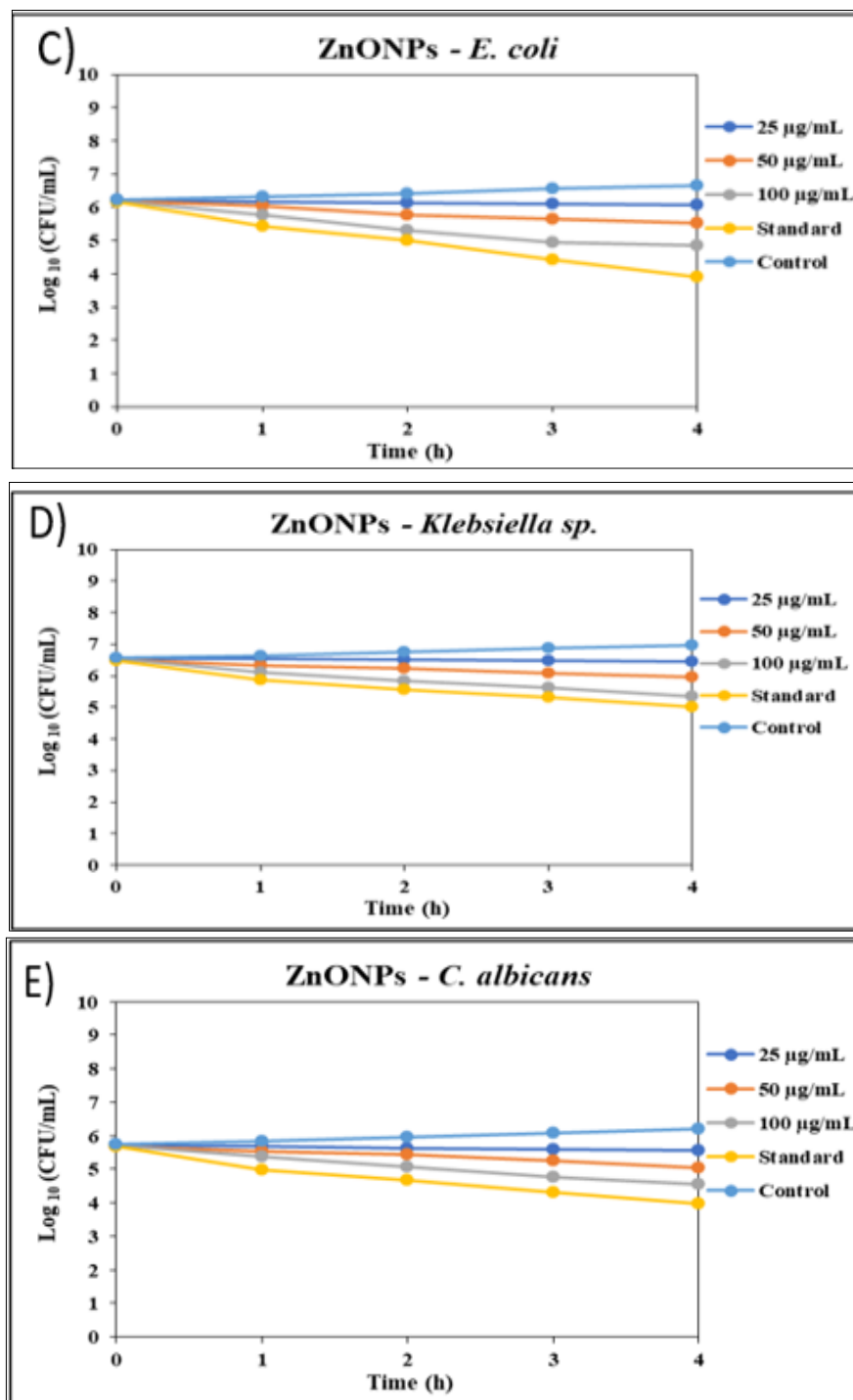


Fig. 5. Time-kill curve assay of zinc oxide nanoparticles (ZnONPs) against urinary tract infection (UTI) pathogens.

log₁₀ reduction within the same duration. The untreated control displayed progressive growth, confirming the normal proliferation of *C. albicans* in the absence of treatment.

Comparative analysis

The time-kill tests indicated that ZnONPs had distinct antimicrobial activities against the assessed pathogens (*S. aureus*, *E. faecalis*, *E. coli*, *Klebsiella sp.* and *C. albicans*) that were both species-specific and concentration-dependent (Fig. 5). The *E. faecalis* was the most susceptible of the bacteria and at 4 hr, ZnONPs at 100 µg/mL produced a roughly 2.5 log₁₀ drop in viable counts, which was very similar to the impact of the conventional antibiotic. The *S. aureus* and *E. coli*, on the other hand, showed intermediate sensitivity, with roughly 1.5–2 log₁₀ reductions at the maximum concentration, while the inhibitory effects were relatively minimal at lower

concentrations (25 and 50 µg/mL). *Klebsiella sp.* was relatively less affected; after 4 hr, 100 µg/mL only produced a 1–1.5 log₁₀ reduction, indicating lesser susceptibility under the analysed limiting conditions. While the most common antifungal was more effective, ZnONPs demonstrated an inhibitory activity against *C. albicans*, where 100 µg/mL caused approximately a 2 log₁₀ reduction. Overall, the time-kill kinetics show a species-specific susceptibility pattern and concentration-dependent inhibitory effects, with *Klebsiella sp.* exhibiting the lowest susceptibility and *E. faecalis* being the most susceptible organism, followed by *C. albicans*, *S. aureus* and *E. coli*. These results demonstrate the concentration-dependent and pathogen-specific action of ZnONPs and are in correspondence with the agar well diffusion observations.

The brine shrimp lethality test was used to assess the toxicity

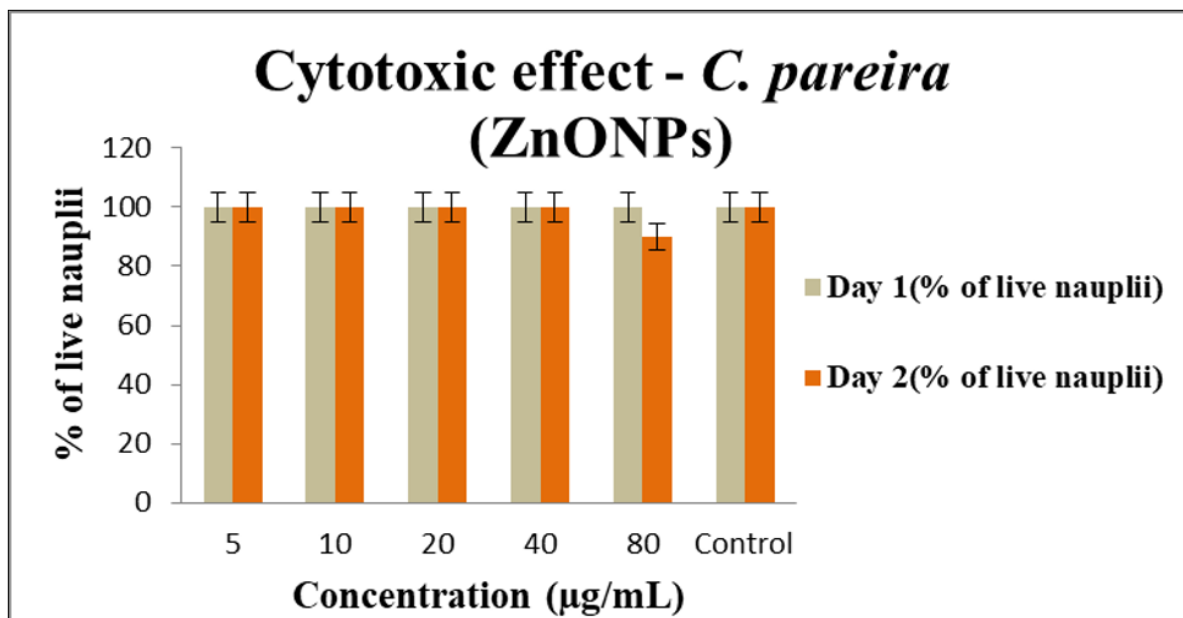


Fig. 6. *Cissampelos pareira*-mediated zinc oxide nanoparticles (ZnONPs) against brine shrimp nauplii at different concentrations (5–80 µg/mL).

of ZnONPs mediated by *C. pareira* (Fig. 6). Nauplii survival rates were measured at various nanoparticle concentrations (5–80 µg/mL) after 24 hr (Day 1) and 48 hr (Day 2), in addition to a control group. All investigated concentrations showed 100 % survival on Day 1, demonstrating that the experimental circumstances did not cause any acute adverse side effects. On Day 2, doses between 5 and 40 µg/mL preserved 100 % survival, whereas the maximum concentration of 80 µg/mL showed a minor decrease in viability (90 %). At both time points, the control group regularly indicated 100 % survival. These results indicate a preliminary indication of low toxicity under the investigated conditions and show minimal acute toxicity of the produced ZnONPs in the brine shrimp lethality assay. Nevertheless, mammalian cytotoxicity, biocompatibility and clinical safety cannot be accurately predicted by the brine shrimp model; it is simple a preliminary toxicity screening technique. Therefore, additional *in vitro* and *in vivo* toxicological research is needed to thoroughly assess the produced ZnONPs' safety profile.

Discussion

The present study explored the antimicrobial efficacy and cytotoxic profile of *C. pareira*-mediated ZnONPs. The findings demonstrate that these green-synthesized ZnONPs exhibit broad-spectrum antimicrobial activity with a concentration- and species-dependent response, while maintaining minimal cytotoxicity in the brine shrimp lethality assay. The UV-visible spectral profile obtained for *C. pareira*-mediated ZnONPs exhibited a well-defined absorption band within the range of 340–360 nm, which agrees with the characteristic surface plasmon resonance (SPR) of ZnO nanoparticles.

The strong absorption observed at 3 hr indicates rapid nucleation, while the gradual decline in absorbance intensity over extended incubation (6–48 hr) suggests the progressive utilisation of zinc ions and stabilisation of nanoparticles. The absence of a significant shift in the absorption maxima across different time points confirms the uniformity of particle size and indicates that no agglomeration occurred during the synthesis process. These results corroborate earlier findings where plant-mediated ZnONPs demonstrated absorption peaks in the 330–370 nm range (8, 30, 31). The stability of the absorption band up to 48 h highlights the efficiency of phytochemicals in *C. pareira* extract, which act as both

reducing and capping agents, thereby preventing particle aggregation. In contrast to chemically synthesised ZnONPs, which often display broader or shifted peaks due to polydispersity, the sharp and stable peaks observed here indicate the formation of homogenous nanoparticles (32).

Mangifera indica L. seed extract mediated ZnONPs and it was estimated the Fourier-transform infrared (FTIR) analysis. The absorbance peak of the peak at 3336.85 cm⁻¹ as (-OH groups) to 416.62 cm⁻¹ (C=O stretching group) (33).

The ZnONPs have quantifiable, concentration-dependent inhibitory effects against *S. aureus*, *E. faecalis*, *E. coli*, *Klebsiella* sp. and *C. albicans*, according to the agar well diffusion assay. With inhibition zones rising from 12.7 ± 0.5 mm at 25 µg/mL to 23.8 ± 1.1 mm at 100 µg/mL, *E. faecalis* showed the highest susceptibility (34, 35). Inhibitory activity was also shown by the conventional antibacterial drugs in the experimental setup. However, as variations in concentration, diffusion properties and modes of action may affect inhibition zone measurements, direct comparisons between ZnONPs and conventional antibacterial drugs should be evaluated cautiously. Consequently, rather than a clear superiority of ZnONPs over the traditional approaches, the obtained results demonstrate a variable antibacterial response (36, 37). Dose-dependent antibacterial actions were further shown by time-kill kinetics. The ZnONPs inhibited *E. faecalis* by at least 2.5 log₁⁻¹ in 4 hr at 100 µg/mL, whereas *S. aureus* and *E. coli* showed intermediate sensitivity with 1.5–2 log₁⁻¹ reductions and *Klebsiella* sp. showed the lowest susceptibility with about 1–1.5 log₁⁻¹ reduction after 4 hr (38). Additionally, *C. albicans* was suppressed in a dose-dependent manner; at the highest concentration, there was about a 2 log₂⁻¹ reduction. These results show ZnONPs' short-term antimicrobial activity under the tested conditions; however, more research using minimum inhibitory concentration (MIC)/minimum bactericidal concentration (MBC) calculations, extended time-kill analyses and standardised dose comparisons is needed to determine relative efficacy and long-term antimicrobial effects.

The results obtained are in confirmation of previous investigation showing ZnONPs' antibacterial efficacy against a variety of microbes (39, 40). The present investigation's antimicrobial effects could be associated with the mechanisms previously

described for ZnONPs, such as interference with cellular metabolic activities, production of ROS and disruption of cell membrane integrity (36, 41). Although this was not particularly examined in the current investigation, variations in cell wall design may be the cause of Gram-positive bacteria's comparatively increased susceptibility compared to Gram-negative bacteria (42). Antimicrobial efficacy has additionally been demonstrated to be influenced by variables such as particle size, surface properties and nanoparticle concentration. Although the strong action against *Enterococcus faecalis* is outstanding, the exact mechanisms underlying this increased susceptibility-including prospective effects on biofilm formation-remain hypothetical and need additional experimental validation (37, 43, 44).

Under the examined experimental circumstances, the brine shrimp lethality assay for toxicity evaluation revealed low toxicity of ZnONPs mediated by *C. pareira*. Up to 40 µg/mL, survival was 100 % and after 48 hr, there was only a minor decrease in viability (90 %) at 80 µg/mL. The brine shrimp model may have restricted acute toxicity because the computed LC₂₀ value was greater than the maximum concentration tested (32). These results are in line with research that suggest phytochemical components used in green production may affect the surface properties of nanoparticles by stabilising and capping them (45). However, the brine shrimp mortality assay is unable to accurately predict mammalian cytotoxicity, genotoxicity, immunocompatibility, or clinical safety; alternatively, it is merely a preliminary toxicity screening approach. Therefore, thorough *in vitro* and *in vivo* toxicological studies are necessary before judgments regarding biocompatibility or biomedical application can be made, even though research results show low toxicity in this model system (46, 47).

The combination of potent antimicrobial efficacy and minimal toxicity positions *C. pareira*-mediated ZnONPs as promising candidates for biomedical applications. Their activity against *E. faecalis* suggests potential for endodontic therapy and dental care, while their antifungal effect against *C. albicans* indicates utility in managing oral candidiasis.

Recognising that of their antibacterial qualities, zinc oxide nanoparticles have been studied for usage in polymeric films for surface coatings and food packaging uses outside of medicine (48, 49). The sustainable manufacturing of ZnONPs may be assisted by the green synthesis strategy, which uses *C. pareira* extract as an eco-friendly substitute for traditional chemical processes (50). However, additional evaluation is needed before the synthesised nanoparticles can be used practically in biomedical, food packaging, or industrial settings. This evaluation should include material compatibility studies, stability evaluations, migration analyses and thorough safety investigations under specific situations.

Conclusion

The present research indicates that zinc oxide nanoparticles (ZnONPs) mediated by *Cissampelos pareira* have broad-spectrum antibacterial activity with unique species-specific sensitivities. ZnONP fabrication was verified by UV-visible spectroscopy using the absorption peak seen at 340–360 nm. While *Klebsiella* sp. showed relatively lower sensitivity, *Enterococcus faecalis* showed the highest susceptibility among the studied bacteria, exhibiting bigger inhibition zones and quick drops in viable cell counts during time-kill

investigations. Additionally, *Candida albicans* was successfully suppressed, demonstrating the produced ZnONPs' antifungal activity. Low toxicity under the studied experimental settings was found by the brine shrimp lethality determination, indicating a positive preliminary safety profile. Nevertheless, this assay does not accurately predict mammalian cytotoxicity, genotoxicity, or *in vivo* safety; rather, it is merely an initial toxicity screening. Therefore, more toxicological research is needed to thoroughly assess the nanoparticles' biocompatibility. Overall, the results show that ZnONPs mediated by *C. pareira* have encouraging antibacterial qualities and could be good individuals for more industrial and biological investigation. However, before practical or translational applications can be established, more research is required, including thorough physicochemical characterisation, mechanistic studies, formulation optimisation, long-term stability assessments, *in vitro* and *in vivo* toxicity evaluations, pharmacokinetic analyses and application-specific validation studies.

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Authors' contributions

PV, AR, RS, EM and NJ formulated the study and conceptual frameworks. PV, AR, RS, EM and NJ performed data collection, documentation, evaluation and validation. RS and PV wrote the preliminary manuscript. All authors have read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflicts of interest to declare.

Ethical issues: None

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