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Antimicrobial Activity of Calcium Oxide Nanoparticles Synthesized using CommelinaBenghalensis against Clinical Pathogens

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Clinical pathogens are continually evolving newer drug resistant mechanisms in this antibiotic-dominated medical field. The need for more potent therapeutic methods is on the rise. And green synthesized nanoparticles have shown a way to overcome the new drug-resistant pathogens. The study aimed to evaluate the antimicrobial effects of calcium oxide nanoparticles synthesized using Commelinabenghalensis. The calcium oxide nanoparticles were synthesized through Commelinabenghalensis mediation using a heating mantle, magnetic stirrer and a high speed centrifuge. Their antimicrobial potency was analyzed through agar-well diffusion assay and time-kill kinetics against a fungal strain of C. albicans and the bacterial strains of Escherichia coli, Pseudomonas sp, and Staphylococcus aureus. The green synthesized calcium oxide nanoparticles showed significant antimicrobial activity against the clinical pathogens. Their comparison with existing standards to evaluate their potency showed promising results indicating their potency. This study has proven the antimicrobial properties of the unique combination of Commelinabenghalensis and calcium oxide nanoparticles. Non-toxicity of the nanoparticles makes further research and testing of these nanoparticles relatively easier as this study aims to serve as a stepping stone for the development of a potential side-effect free antimicrobial intervention.

Keywords: Antimicrobial activity, calcium oxide nanoparticles, clinical pathogens, Commelinabenghalensis, green synthesis.

1. Introduction

Nanotechnology is a field of biomedicine which is concerned with the activity of atoms smaller than 100nm. The products of nanotechnology are known to be smaller, durable and have a higher surface area with unique physical and chemical properties (Ramola& Joshi, 2019). Their applications are widespread in fields such as medicine, pharmaceuticals, crop growth amongst others (Hernández-Díaz et al., 2020). Nanoparticles (NPs) have been successfully used for enhancing the activity of catalysts through immobilization. They have also been tested for their microbial activity inhibition, treating wounds, healing burns and preventing bacterial growth [3]. Among all the NPs, inorganic compound nanoparticles are known to have a greater effect on resistant bacterial strains (Roy et al., 2013). Nanoparticles in medicines are an important and active way of tackling antibiotic resistance (Yefimova et al., 2022). Nanoparticles when in close vicinity of a cell, result in a generation of forces which enhances the cellular uptake leading to accurate delivery of compounds (Zhang et al., 2015).

Calcium is the 5th most abundant element by mass. It is an important component of living organisms which has no known alternative (Gandhi et al., 2021). Calcium oxide nanoparticles (CaO NPs) are most importantly safe and biocompatible for all living organisms which eliminates the possibility of calcium intolerance. These nanoparticles have found extensive usage as drug delivery agents in various therapies (Ramola& Joshi, 2019). Being biocompatible, calcium oxide nanoparticles are allowed to penetrate cellular barriers easily making the drug delivery more effective (Harris et al., 2023). CaO NPs are found to have inhibitory effect on microbial growth in the biofilm thereby giving a new aspect for the applications (Yefimova et al., 2022). The CaO NPs are also proven to have high optical activity when green synthesized (Bano&Pillai, 2020).

The diverse antibacterial properties of CaO nanoparticles suggest that they might be standard alternatives to antimicrobial drugs in the future (Khan et al., 2023). CaO NPs have higher antibacterial activity against various organisms compared to existing standard CaO compounds. Plant mediated biological synthesis is highly simple and eco-friendly which has given it much importance in recent years (Marquis et al., 2016). Organic synthesis is highly cost-effective, eco-friendly and can be done on a relatively larger scale compared to other chemical methods.

Commelinabenghalensis, the plant used in this study for nanoparticle synthesis, is proven to have multiple medicinal and therapeutic uses. The plant possesses anti-inflammatory, diuretic and wound healing properties which helps enhance the activity of the nanoparticles. The plant is commonly available in the tropical regions of Asia and Africa making it an effective nanoparticle synthesizing mediator (Roy Orni et al., 2018).In this study, the aim was to further evaluate the existing antimicrobial properties of green synthesized calcium oxide nanoparticles and then study their effect on clinical pathogens which are known to have a higher resistance and prevalence period. The antimicrobial activity evaluation was done by the agar-well diffusion method and then tested on strains of C. albicans, E.coli, Staphylococcus aureus, Pseudomonas sp. Time-kill assays for each species were conducted to assess the bactericidal and bacteriostatic potential of nanoparticles over time.

2. Materials and Methods:

2.1. Preparation of plant extract

Fresh Commelinabenghalensisleaves, collected from the herbal garden of Saveetha medical college, were shade dried and powdered. 1 gram of Commelinabenghalensisplant powder was added to 100 mL of distilled water. The plant extract was then boiled at 50-60°C using a heating mantle for 10 minutes. Later, the boiled extract was filtered using a muslin cloth. 50 mL of the filtered plant extract was then added to 50 mL of precursor solution to form 100 mL of resultant solution.

2.2. Preparation of Nanoparticles

30 millimolar calcium hydroxide [Ca(OH)2] is mixed in 50 mL of distilled water. C. benghalensis acts as the reducing and stabilizing agent. Calcium hydroxide in distilled water was the precursor solution. The resultant 100 mL solution was kept in a magnetic stirrer for 48 hours for synthesis of nanoparticles to occur. The magnetic stirrer was checked after the first 24 hours to confirm a change in color of the solution which is the preliminary confirmation for nanoparticle synthesis. After 48 hours, the nanoparticle solution was centrifuged at 8000 rpm(rotation per minute) for 10 minutes. The nanoparticles were deposited at the bottom and walls of the centrifuge tube. After this procedure, the pellet was collected and kept in the refrigerator for further use.

2.3. Antimicrobial activity

The antimicrobial effectiveness of the calcium oxide nanoparticles synthesized through biosynthesis was assessed using the agar well diffusion technique. To begin, Mueller Hinton agar plates were prepared and sterilized using an autoclave at a temperature of 121°C for duration of 15-20 minutes. Once sterilized, the medium was poured onto sterile Petri plates and allowed to cool down to room temperature. Subsequently, a bacterial suspension consisting of C. albicans, Pseudomonas sp, Staphylococcus aureus, and E.coli was evenly spread onto the agar plates using sterile cotton swabs. Wells with a diameter of 9 mm were then created in the agar plates using a sterile polystyrene tip. These wells were subsequently filled with varying concentrations (25, 50, 100 µg/mL) of CaO NPs. As a standard, antibiotics for Amoxyrite(for bacteria) and Fluconazole(for fungi) were utilized. The plates were then incubated at a temperature of 37°C for a period of 24 hours. The antimicrobial activity was assessed by measuring the diameter of the zone of inhibition surrounding the wells. This measurement was conducted using a ruler and recorded in millimeters (mm), allowing for the calculation of the zone of inhibition.

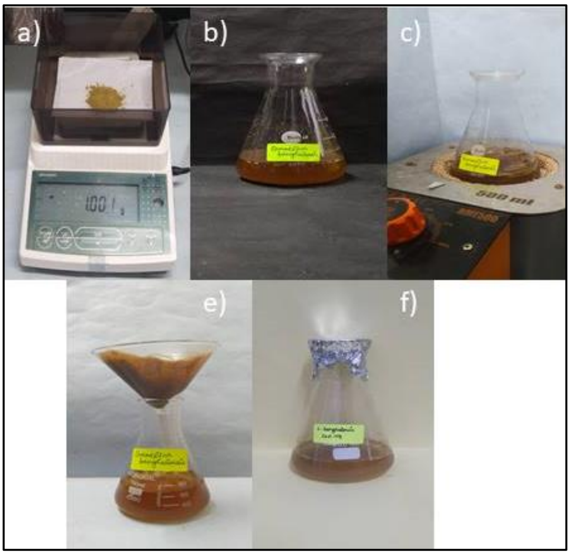
2.4. Time-kill kinetic analysis

A time-kill kinetics analysis was carried out to evaluate the bactericidal effects and concentration-dependent correlation between C.benghalensis-mediated Calcium oxide nanoparticles and the growth rate of C. albicans, Escherichia coli, Pseudomonas sp, and Staphylococcus aureus. The study involved culturing three wound pathogens in Mueller Hinton Broth with varying concentrations of calcium oxide nanoparticles (5, 25, 50, and 100 µg/ml), followed by time-kill curve analysis. Standard antibiotics (Amoxyrite for bacteria; Fluconazole for fungi) were used. Prior to the test, a four-hour pre-incubation period in an antimicrobial-free medium was conducted to ensure that all pathogens had reached a stable early-to-mid log phase. Inoculum was prepared using 0.5 McFarland of each pathogen in sterile phosphate-buffered saline collected from cultures grown on Mueller Hinton agar plates at 37 °C for 18–20 hours. The inoculum was then diluted in antimicrobial-free Mueller Hinton Broth and distributed evenly in a 96-well ELISA plate. C.benghalensis-mediated Calcium oxide nanoparticles at different concentrations were added to each well along with the untreated control.

3. Results

3.1. Preparation of calcium oxide nanoparticles

Figure 1: Preparation of Commelinabenghalensis extract mediated calcium oxide nanoparticles (a) Measuring weight of the powdered Commelinabenghalensis leaves; (b) Mixing the powder with 100 mL of distilled water; (c) Heating the extract at 50-60°C using a heating mantle; (d) Filtration of boiled extract using a muslin cloth e) Synthesis of nanoparticles solution



3.2. Antimicrobial activity

Antimicrobial activity of Commelinabenghalensis-mediated calcium oxide nanoparticles was demonstrated by the zone of inhibition of bacterial cultures. Figure 2 and 3 illustrates the increase in zone of inhibition with increasing concentration of CaO NPs. At 100 µg/ml, the zone of inhibition was highest for all bacterial cultures except for S. aureus which remained relatively constant at all concentrations. The increasing ZOI with increasing concentration in other bacterial cultures can be attributed to the CaO NPs which provide high surface area for the antimicrobial activity.

Figure 2: Antimicrobial activity of C. benghalensis mediated calcium oxide nanoparticles against clinical pathogens a) Escherichia coli, b) Staphylococcus aureus c) Pseudomonas aeruginosa d) Candida albicans

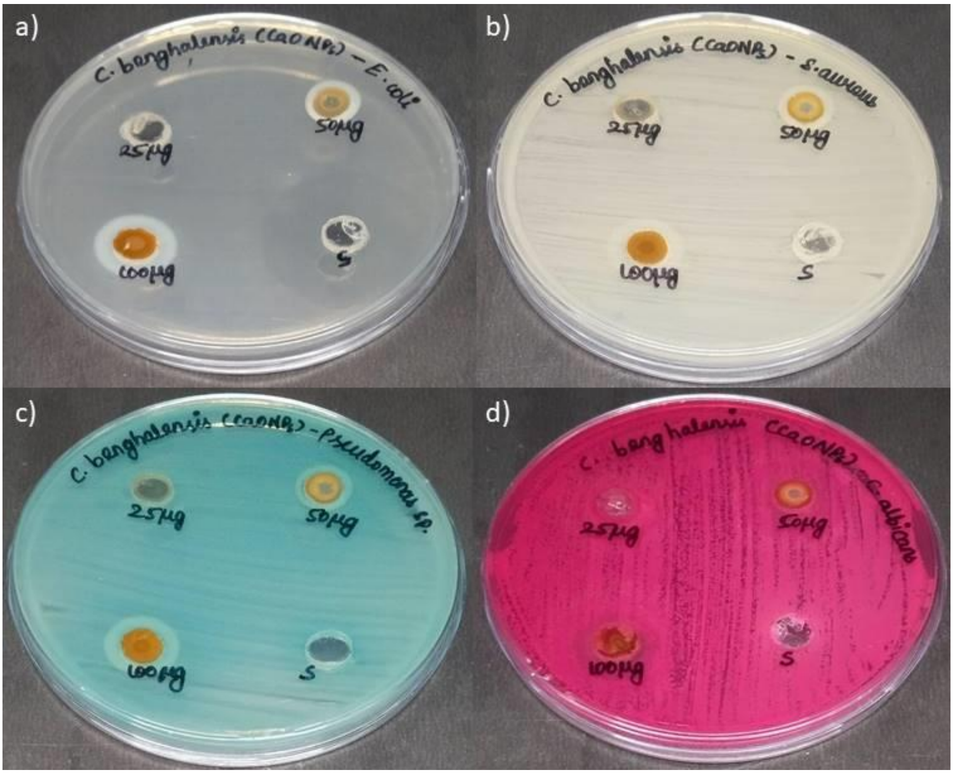
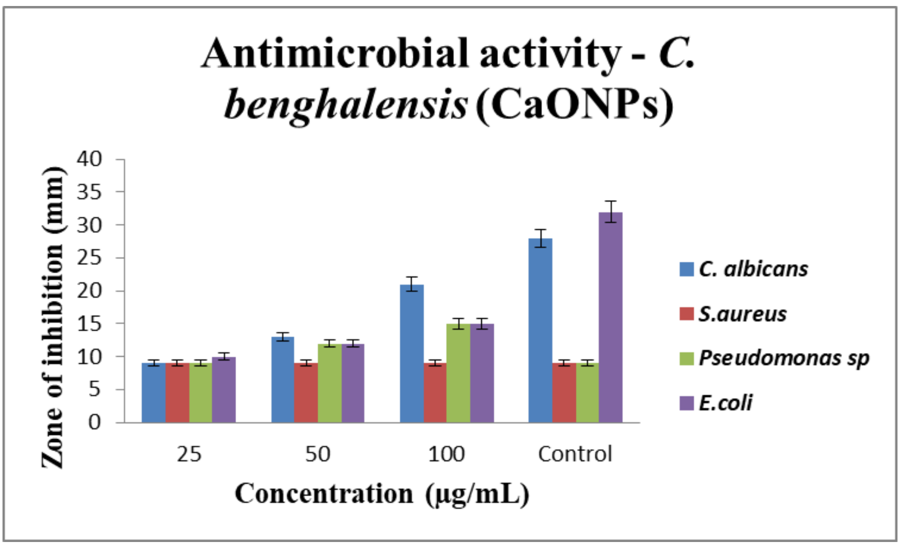


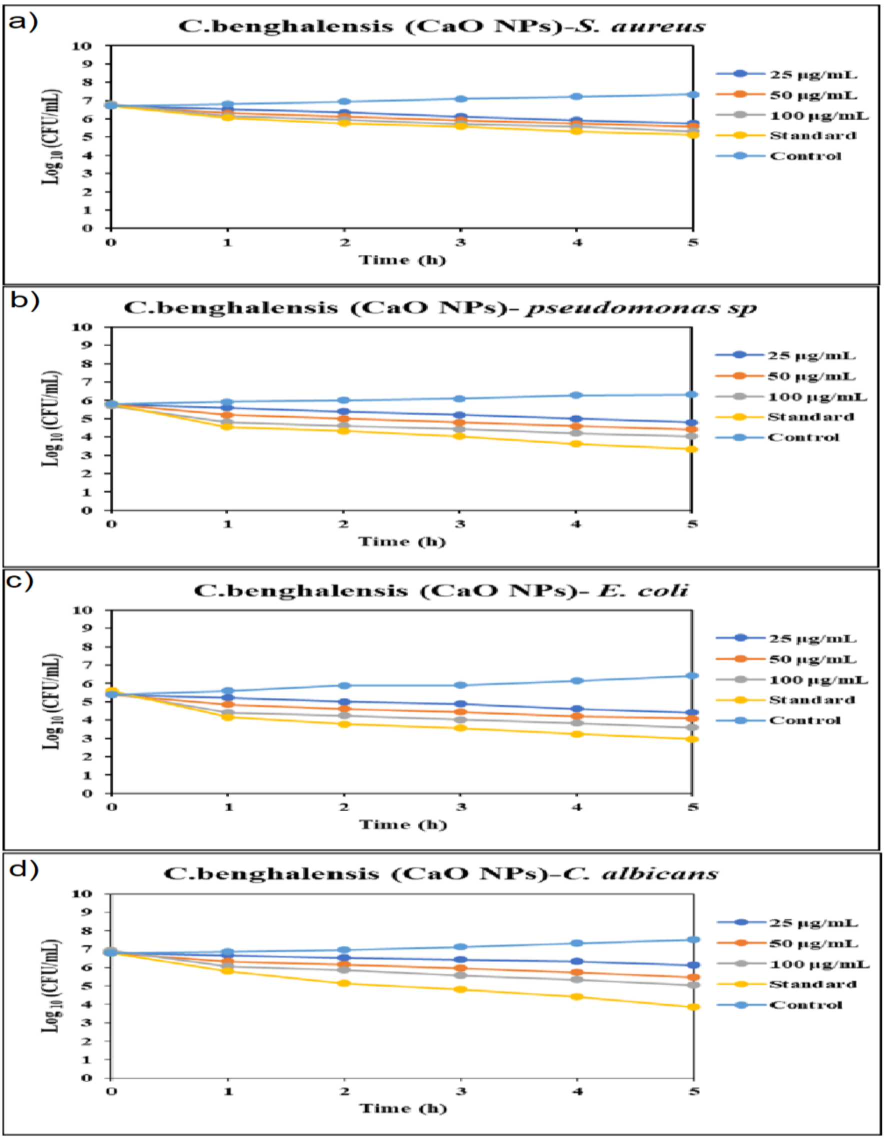
Figure 3: Graph displaying the antimicrobial activity of Commelinabenghalensis-mediated calcium oxide nanoparticles against clinical pathogens



3.3. Time-kill kinetics

Time-kill analysis was conducted for the Commelinabenghalensis-mediated calcium oxide nanoparticles to determine their bactericidal and bacteriostatic activity. As displayed in figure 4, the nanoparticles showed significant bacteriostatic activity against S. aureus and moderate bacteriostatic activity against Pseudomonas sp.and E. coli. Bactericidal effect increased as the concentrations increased with the 100 μg/ml sample showing values comparable to the standard. It possesses significant fungistatic effect against C. albicans at higher concentrations of 50 and 100 μg/ml.

Figure 4: Time-kill kinetics analysis of C. benghalensis mediated calcium oxide nanoparticles a) S. aureus b) P. aeruginosa c) E. coli and d) C. albicans



4. Discussion

Over the past decade, there has been increasing demands for application of nanotechnology in the field of medicine (Rajeshkumar et al., 2019). With pathogens becoming increasingly resistant to existing standard interventions, the need for advanced alternatives arises (Rifaath et al., 2023, Sankar et al., 2024). The method of synthesis has a role in determining the properties of the compound. For this study, we have green synthesized calcium oxide nanoparticles with C. benghalensis mediation. The plant is commonly called Tropical spiderwort and is proven to have antimicrobial, antibacterial and anti-inflammatory properties. The properties are attributed to its rich phytochemical content.

Calcium oxide nanoparticles were successfully synthesized utilizing Commelinabenghalensis. According to a similar study where Ocimumtenuiflorumwas used for CaO NPs synthesis, green synthesis enhances the natural properties of the nanoparticles while also imparting beneficial attributes of the plant to them (6). As seen in another study where calcium oxide nanoparticles were synthesized using Moringaoleiferaand antibacterial evaluation was done on Escherichia coli and Staphylococcus aureus, CaO nanoparticles of this study have shown substantial antimicrobial activity (Jadhav et al., 2022).

Agar-well diffusion method and time-kill kinetics were utilized in this study to evaluate the antimicrobial activity of Commelinabenghalensis-mediated Calcium oxide nanoparticles. The agar-wells displayed the zone of inhibition and the time-kill kinetics indicated the bacteriostatic and bactericidal activity. Similar agar-well diffusion results were obtained in another study where calcium oxide nanoparticles were combined with neem and clove along with melatonin (Harris et al., 2023).With further development and research, these nanoparticles can serve as potential alternatives for existing standards which are yet to be made side-effect free.

5. Conclusion

Green synthesized nanoparticles have a high scope of development and utilization against clincal pathogens in the near future. The study has successfully proven the antimicrobial properties of Commelinabenghalensis-mediated Calcium oxide nanoparticles against clinical pathogens. Antimicrobial potency was evaluated through agar-well diffusion before conducting time-kill kinetics against each pathogen. Throughout the analysis, the nanoparticles showed values within the standard ranges indicating their potential strength. This intervention has high potential for utilization in the future considering the ease of nanoparticle manufacturing, availability of the plant, economic viability and non-toxic nature of calcium oxide. This study serves as a foundation for further research and analysis of the antimicrobial properties of the nanoparticles and the plant. Calcium oxide nanoparticles can also be utilized as high-accuracy drug delivery agents.

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Disclosure Statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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