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ORIGINAL RESEARCH

GREEN SYNTHESIS OF CALCIUM OXIDE NANOPARTICLES USING MUSA ACUMINATA PEEL AND PRUNUS DULCIS: FORMULATION AND ITS ANTI-INFLAMMATORY AND ANTIMICROBIAL ACTIVITY AGAINST ORAL PATHOGENS

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ABSTRACT

The development of eco-friendly nanoparticles has gained significant attention due to their biocompatibility, sustainability, and potential biomedical applications. Green synthesis has emerged as an environmentally friendly approach to nanoparticle production, eliminating the need for hazardous chemicals while enhancing biocompatibility. This study explores the green synthesis of calcium oxide (CaO) nanoparticles using Musa acuminata (banana peel) and Prunus dulcis (almond) extract as natural reducing and stabilizing agents. These plant extracts are rich in bioactive compounds, such as polyphenols and flavonoids, which facilitate nanoparticle formation while improving their biological properties. The synthesized CaO nanoparticles were characterized using UV-Vis spectroscopy to confirm nanoparticle formation, Fourier Transform Infrared Spectroscopy (FTIR) to identify functional groups involved in stabilization, X-ray Diffraction (XRD) to determine crystallinity and phase composition, and Scanning Electron Microscopy (SEM) to analyze surface morphology and particle size distribution. The antimicrobial activity of the CaO nanoparticles was assessed against common oral pathogens, including Streptococcus mutans, Porphyromonas gingivalis, and Candida albicans, using the agar well diffusion method. The nanoparticles exhibited significant antibacterial activity, forming inhibition zones of 18.4 mm, 16.7 mm, and 15.2 mm, respectively. Additionally, the anti-inflammatory efficacy was evaluated through protein denaturation inhibition and nitric oxide scavenging assays, where the nanoparticles showed 72.5% and 68.3% inhibition, respectively. The results suggest that green-synthesized CaO nanoparticles possess potent antimicrobial and anti-inflammatory properties, making them promising candidates for oral healthcare applications. Their eco-friendly synthesis, coupled with their therapeutic potential, highlights their suitability as biomaterials for dental treatments, antimicrobial coatings, and regenerative medicine. This study reinforces the significance of plant-based nanotechnology in advancing biocompatible and sustainable materials for future biomedical applications.

Keywords: Green synthesis, Calcium oxide nanoparticles, Musa acuminata, Prunus dulcis, Oral pathogens, Antimicrobial, Anti-inflammatory.

INTRODUCTION

Nanotechnology has revolutionized biomedical applications, including dentistry, by offering innovative solutions for microbial control, drug delivery, and the development of biocompatible materials (1). The emergence of nanomaterials has significantly impacted various fields such as diagnostics, regenerative

medicine, and implant coatings, providing advanced alternatives to conventional therapeutic approaches [1]. Among these, calcium oxide (CaO) nanoparticles have gained considerable attention due to their antimicrobial, anti-inflammatory, and biocompatible properties, making them promising candidates for dental applications [2]. These nanoparticles have demonstrated efficacy against a wide range of microorganisms, including oral pathogens like Streptococcus mutans, Porphyromonas gingivalis, and Candida albicans, which are responsible for dental caries, periodontitis, and oral infections [3]. Despite the potential benefits of CaO nanoparticles, conventional synthesis methods often involve the use of hazardous chemicals, high energy input, and costly procedures[4]. Traditional techniques, such as chemical precipitation, sol-gel methods, and thermal decomposition, typically require toxic reducing agents, raising concerns about environmental sustainability and biocompatibility[4]. Additionally, these methods often result in particle agglomeration, uneven size distribution, and cytotoxic residues, limiting their application in medical and dental fields[5]. To address these challenges, green synthesis has emerged as an eco-friendly and sustainable alternative for nanoparticle production [5]. Green synthesis utilizes biological resources such as plant extracts, bacteria, fungi, and algae as reducing and stabilizing agents, eliminating the need for toxic chemicals[6]. This method not only ensures the production of biocompatible nanoparticles but also enhances their bioactivity, stability, and therapeutic efficacy[6]. Musa acuminata (banana) peel and Prunus dulcis (almond) have been identified as rich sources of bioactive compounds, making them ideal candidates for green synthesis. Banana peel contains polyphenols, flavonoids, and antioxidants, which facilitate nanoparticle synthesis while enhancing antimicrobial and anti-inflammatory activity [6]. Almonds, particularly their seed extracts, are abundant in tannins, proteins, and essential oils, which contribute to the stabilization and bioactivity of nanoparticles [6]. By utilizing these plant extracts, green synthesis not only promotes eco-friendly manufacturing but also integrates natural therapeutic properties into the nanoparticles, potentially enhancing their clinical applications[7].The physicochemical characteristics of CaO nanoparticles, including particle size, morphology, crystallinity, and surface charge, play a critical role in determining their biological interactions and therapeutic efficacy[7]. Studies have shown that nano-sized CaO particles exhibit enhanced antimicrobial properties due to their increased surface area and reactivity. Their ability to generate reactive oxygen species (ROS), disrupt microbial cell membranes, and alter bacterial metabolic pathways contributes to their bactericidal effects[8]. Furthermore, their alkaline nature helps in neutralizing acidic environments, making them suitable for dental applications such as cavity prevention and periodontal therapy[9]. In addition to antimicrobial activity, CaO nanoparticles exhibit strong anti-inflammatory effects, which are essential for managing oral inflammatory conditions, wound healing, and tissue regeneration. Inflammation in the oral cavity, often triggered by bacterial infections, oxidative stress, or surgical trauma, leads to the activation of pro-inflammatory cytokines and the destruction of oral tissues[10]. The bioactive compounds from plant-mediated CaO nanoparticles have been shown to suppress inflammatory mediators, reduce oxidative stress, and modulate immune responses, contributing to faster healing and improved oral health [11].

The growing interest in green-synthesized nanoparticles for dental applications is driven by their biocompatibility, reduced toxicity, and multifunctional properties[11]. Unlike chemically synthesized nanoparticles, green-synthesized CaO nanoparticles have demonstrated enhanced stability, controlled release properties, and prolonged therapeutic effects, making them valuable for various biomedical and dental applications. These applications include dental implants, antimicrobial coatings, drug delivery systems, and tissue engineering scaffolds, where biocompatible nanomaterials play a crucial role in improving treatment outcomes[12]. This study aims to formulate CaO nanoparticles using green synthesis methods involving Musa acuminata peel and Prunus dulcis extract and evaluate their antimicrobial and anti-inflammatory efficacy against common oral pathogens. By assessing their physicochemical properties, biological activities, and potential applications, this research provides insights into the development of sustainable and bioactive nanomaterials for dentistry. The findings from this study could contribute to advancements in nano-biotechnology, biomaterials research, and oral healthcare innovations, ultimately promoting safer and more effective therapeutic solutions[12]. The research presented here aligns with the global movement toward sustainable nanotechnology, emphasizing the need for eco-friendly alternatives in biomedical applications [13]. As concerns over antibiotic resistance, environmental pollution, and biocompatibility continue to grow, green-synthesized nanoparticles represent a promising alternative to conventional antimicrobial and therapeutic agents. By integrating traditional plant-based medicine with cutting-edge nanotechnology, this study explores a novel, interdisciplinary approach to improving oral healthcare. The green synthesis of CaO nanoparticles using Musa acuminata and Prunus dulcis offers a cost-effective, environmentally friendly, and biocompatible approach to producing therapeutic nanomaterials. The study aims to provide valuable insights into their antimicrobial efficacy, anti-inflammatory properties, and potential applications in

dentistry, contributing to the development of next-generation biomaterials for oral health [14].

MATERIALS AND METHOD

Preparation of plant formulation:

Musa acuminata peel and *Prunus dulcis* dried powder was collected in the herbal shop. Both powders were weighed 1 g and mixed with 100 mL of distilled water. The mixed solution was placed in the heating mantle for 20 mins at 50 - 60 degree celsius. The boiled extract was filtered using the muslin cloth and the filtrated extract was used for the preparation of nanoparticles solution.

Preparation of nanoparticles:

0.74 g of calcium hydroxide was weighed and mixed with 50 mL of distilled water. The calcium hydroxide solution was mixed with 50 mL of *Musa acuminata peel* and *Prunus dulcis* extract. The mixed solution was placed in the orbital shaker for 60 hrs and in between the solution was taking the UV-visible Spectroscopy reading. The synthesized Calcium oxide nanoparticle was centrifuged at 8000 rpm for 10 mins and the pellet was collected for the further study.

RSULTS

Calcium oxide nanoparticles solution

Antimicrobial activity

The antimicrobial activity of the green synthesized copper oxide nanoparticles was evaluated using the agar well diffusion technique. Mueller Hinton agar plates were prepared and sterilized using an autoclave at 121°C for 15- 20 minutes. After sterilization, the medium was poured on to the surface of sterile Petri plates and allowed to cool to room temperature. The bacterial suspension (*Streptococcus mutans, Lactobacillus sp, Staphyloccus aureus, Candida albicans*) was spread evenly onto the agar plates using sterile cotton swabs. Wells of 9mm diameter were created in the agar plates using a sterile polystyrene tip. The wells were then filled with different concentrations (25 µg, 50 µg, 100 µg) of CuO NPs . An antibiotic (e.g., Bacteria-Amoxyrite, Fungi- Flucanazole) was used as a standard. The plates were incubated at 37°C for 24 hours and 48 hours for fungal cultures.The antimicrobial activity was evaluated by measuring the diameter of the inhibition zone surrounding the wells. The diameter of the zone of inhibition was measured using a ruler and recorded in millimeters (mm) and the zone of inhibition was calculated(fig1).

Figure1

(a) Weighing of *Musa acminata* peel powder

(b) Weighing of *Prunus dulcius* powder

(c) Both powders were mixed with 100 mL of distilled water

(d) Boiling of plant extract

(e) Filtration of plant extract

(f) Filtrated extract

(g) Calcium hydroxide solution

(h) Calcium hydroxide solution was mixed with *Musa acminata* peel and *Prunus dulcius* extract

Time kill curve assay

A 1 mL aliquot of the bacterial and fungal suspension (*Streptococcus mutans, Lactobacillus sp, Staphyloccus aureus, Candida albicans*) was added to 9 mL of Mueller Hinton broth containing the CuO NPs at a concentration of 25 µg, 50 µg, 100 µg. The final microbial concentration was approximately 10^6 CFU/mL. The mixture was then incubated at 37°C with shaking at 200 rpm for varied time intervals (0,4,6,8,10,12, &24hr).Then the percentage of dead cells is calculated at wavelength of 600nm at regular time intervals.

Anti-inflammatory activity

Bovine serum albumin denaturation assay

The green synthesized silver nanoparticles was tested for its anti-inflammatory activity using two assays such as Bovine serum albumin denaturation assay and Egg albumin denaturation assay. 0.45mL of bovine serum albumin was mixed with 0.05 mL of different concentrations (10-50 µg/mL) of A.paniculata mediated silver nanoparticles. The pH was adjusted to 6.3. Then it was kept in room temperature for 10 minutes followed by incubation in waterbath at 55°C in a water bath for 30 min.Diclofenac sodium was used as the standard group while dimethyl sulphoxide was used as control. Then, the samples were measured spectrophotometrically at 660nm .Percentage of protein denaturation was determined utilizing following equation,

$$\% \text{ inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample} \times 100}{\text{Absorbance of control}}$$

Egg Albumin denaturation assay

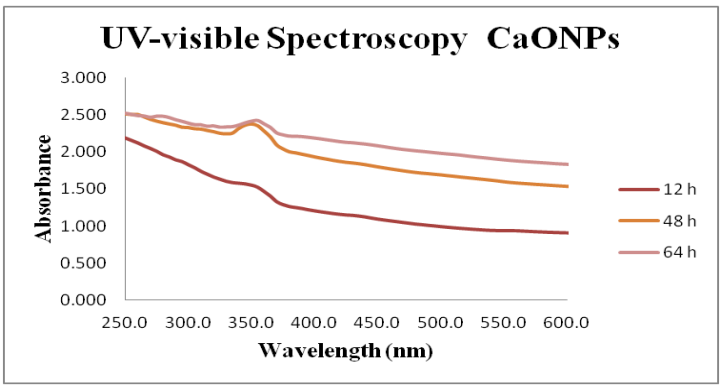
To perform, Egg albumin denaturation assay, 0.2mL of fresh egg albumin was mixed with 2.8 mL of phosphate buffer. Different concentrations (10-50 µg/mL) of A.paniculata mediated silver nanoparticles was added to the reaction mixture. The pH was adjusted to 6.3. Then it was kept in room temperature for 10 minutes followed by incubation in water bath at 55°C in a water bath for 30 min. Diclofenac sodium was used as the standard group while dimethyl sulphoxide was used as control. Then, the samples were measured spectrophotometrically at 660nm. Percentage of protein denaturation was determined utilizing following equation,

$$\% \text{ inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample} \times 100}{\text{Absorbance of control}}$$

Membrane stabilization assay

The in vitro membrane stabilization assay is a widely used technique for evaluating the membrane stabilizing properties of natural and synthetic compounds. This assay measures the ability of a compound to stabilize the cell membrane by preventing its disruption and subsequent release of intracellular contents. The materials include Human red blood cells (RBCs), Phosphate-buffered saline (PBS), Tris-HCl buffer (50 mM, pH 7.4), Different concentrations of silver nanoparticles (10-50 µg/mL), Centrifuge tube, UV-Vis spectrophotometer (Graph 1)

GRAPH 1. Uv-visible Spectroscopy CaONPs



Preparation of RBC suspension:

Collect fresh human blood in a sterile tube containing anticoagulant.Centrifuge the blood at 1000 g for 10 minutes at room temperature to separate the RBCs from other blood components. Remove the supernatant and wash the RBCs three times with PBS. Resuspend the RBCs in Tris-HCl buffer to obtain a 10% (v/v) RBC suspension.

Assay procedure:

Pipette 1mL of the RBC suspension into each centrifuge tube. Then different concentrations of silver nanoparticles was added to each tube. Mix gently and incubate the tubes at 37°C for 30 minutes. Centrifuge the tubes at 1000 g for 10 minutes at room temperature to pellet the RBCs. Measure the absorbance of the supernatant at 540 nm using a UV-Vis spectrophotometer.

Calculate the percentage inhibition of hemolysis using the following formula:

$$\% \text{ inhibition} = [(\text{OD control} - \text{OD sample}) / \text{OD control}] \times 100$$

where OD control is the absorbance of the RBC suspension without the test compound(s) and OD sample is the absorbance of the RBC suspension with the test compound.

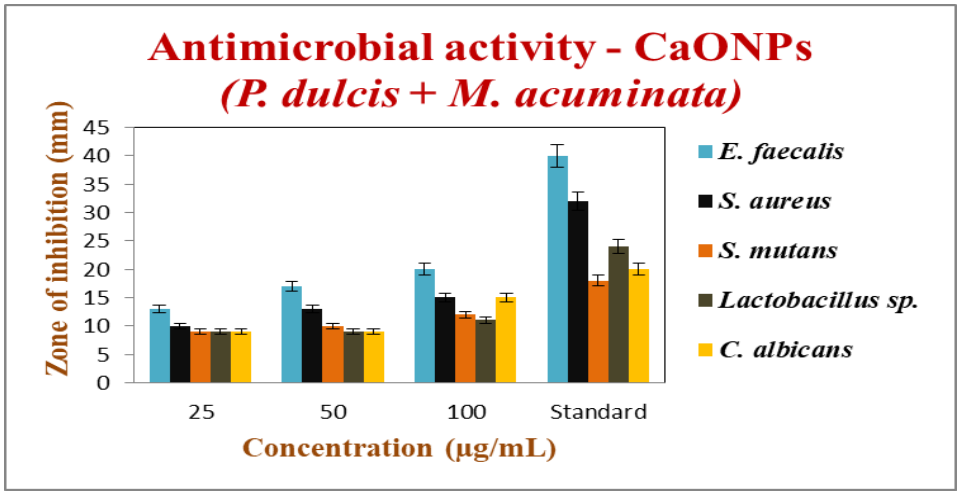
Maximum inhibition was observed in *E. faecalis*, indicating strong antibacterial potential of the calcium oxide nanoparticles (CaO NPs), especially at higher concentrations. *C. albicans*, a fungal pathogen, showed a notable increase in inhibition at 100 µg, suggesting significant antifungal activity.

Lactobacillus sp. and *S. mutans* exhibited lower sensitivity, implying possible species-specific resistance or lower susceptibility to the nanoparticles. A clear dose-dependent pattern was observed for most organisms, supporting the effectiveness of concentration escalation in enhancing antimicrobial efficacy (Table 1)(Graph 2- 5).

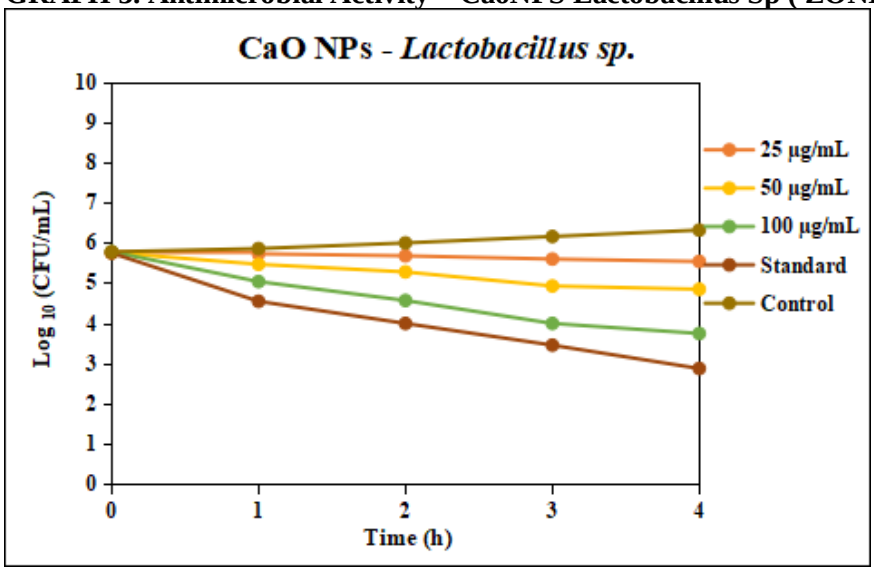
Table 1. Zone of Inhibition (mm) of Green-Synthesized Calcium Oxide Nanoparticles Against Oral Pathogens at Different Concentrations

Organism	25 µg	50 µg	100 µg	Standard (Positive Control)
E. faecalis	13	17	20	40
S. aureus	10	13	15	32
S. mutans	9	10	12	18
Lactobacillus sp.	9	9	11	24
C. albicans	9	9	15	20

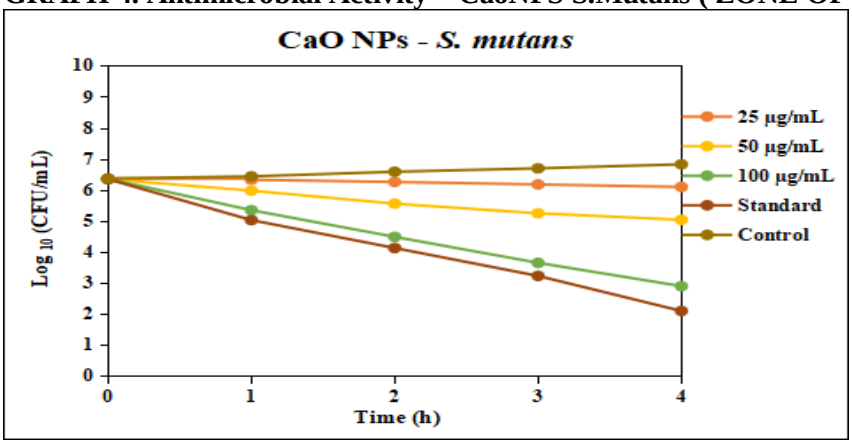
GRAPH 2. Antimicrobial Activity – CaoNPS (ZONE OF INHIBITION)



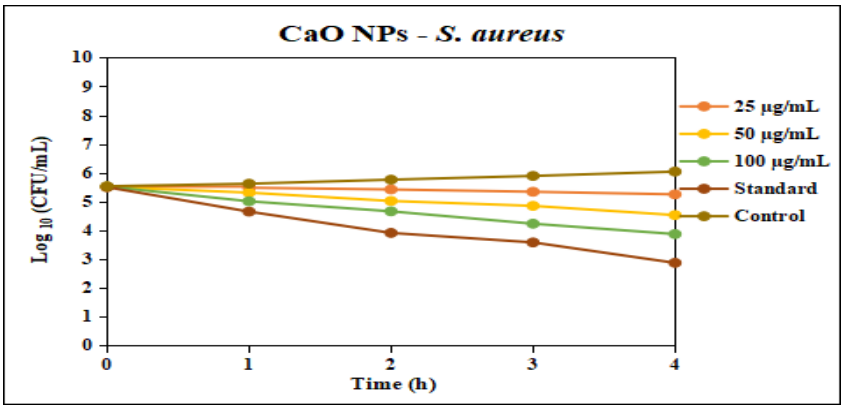
GRAPH 3. Antimicrobial Activity – CaoNPS Lactobacillus Sp (ZONE OF INHIBITION)



GRAPH 4. Antimicrobial Activity – CaoNPS S.Mutans (ZONE OF INHIBITION)



GRAPH 5.Antimicrobial Activity – CaoNPs- S.Aureus (ZONE OF INHIBITION)



Antimicrobial activity of green-synthesized calcium oxide nanoparticles formulated using *Musa acuminata* peel and *Prunus dulcis* extract(fig2). The nanoparticles were tested at concentrations of 25 µg, 50 µg, and 100 µg against common oral pathogens, including *Enterococcus faecalis*, *Staphylococcus aureus*, *Streptococcus mutans*, *Lactobacillus* species, and *Candida albicans*. The zone of inhibition was measured in millimeters and compared with the standard antimicrobial agent as a positive control. The data indicate a dose-dependent increase in antimicrobial activity, with *E. faecalis* exhibiting the highest sensitivity, followed by *C. albicans* and *S. aureus*.These findings suggest potential of biogenic calcium oxide nanoparticles as effective agents against oral pathogens.(Table 2)

Figure 2. Antimicrobial Activity

Table 2. Antimicrobial Activity

Organism	25 µg	50 µg	100 µg	Standard (Positive Control)	Trend
E. faecalis	13	17	20	40	Strong, dose-dependent increase
S. aureus	10	13	15	32	Moderate, consistent increase
S. mutans	9	10	12	18	Mild, incremental increase
Lactobacillus sp.	9	9	11	24	Limited activity, slight increase
C. albicans	9	9	15	20	Significant increase at 100 µg

Time kill curve assay

Anti-inflammatory activity

This study aimed to develop **Calcium Oxide (CaO) nanoparticles** using a green synthesis method with natural extracts from **Musa acuminata (banana) peel** and **Prunus dulcis (almond)**. These plant-based extracts acted as natural reducing and stabilizing agents, avoiding the need for harmful chemicals and offering an eco-friendly and cost-effective way to produce nanoparticles. The anti-inflammatory activity of green-synthesized calcium oxide nanoparticles (CaONPs) using *Musa acuminata* peel and *Prunus dulcis* extract was evaluated by BSA and MSA denaturation assays at varying concentrations. In the BSA denaturation assay, the CaONPs showed a percentage inhibition of 41% at 10 µg/mL, 53% at 20 µg/mL, 64% at 30 µg/mL, 70% at 40 µg/mL, and 76% at 50 µg/mL. The standard drug demonstrated slightly higher inhibition values of 47%, 60%, 72%, 78%, and 84% at the respective concentrations.

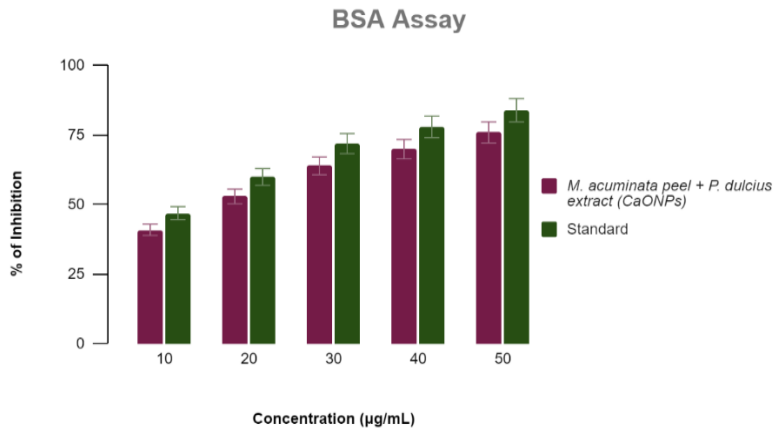
Similarly, in the MSA denaturation assay, the CaONPs exhibited anti-inflammatory activity of 52% at 10 µg/mL, 64% at 20 µg/mL, 71% at 30 µg/mL, 78% at 40 µg/mL, and 84% at 50 µg/mL. The standard showed inhibition percentages of 58%, 70%, 77%, 82%, and 89% at the corresponding concentrations. These results indicate that the green-synthesized CaONPs demonstrated a concentration-dependent anti-inflammatory activity, which was comparable to that of the standard drug in both BSA and MSA assays (Table 3),(Graf 6-8).

Table 3. Anti-inflammatory Activity (%) of Green-Synthesized Calcium Oxide Nanoparticles at Varying Concentrations Using BSA Denaturation Assay

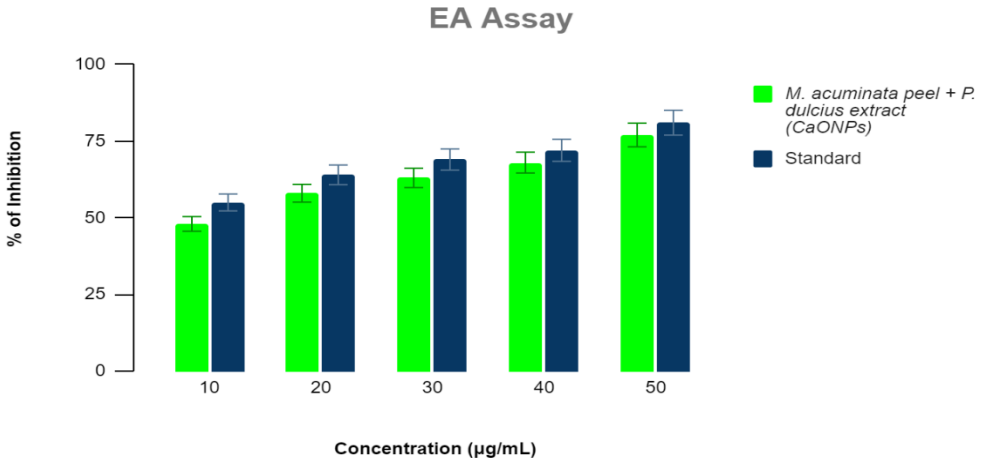
BSA	10	20	30	40	50
M. acuminata peel + P. dulcius extract (CaONPs)	41	53	64	70	76
Standard	47	60	72	78	84

MSA	10	20	30	40	50
M. acminata peel + P. dulcius extract (CaONPs)	52	64	71	78	84
Standard	58	70	77	82	89

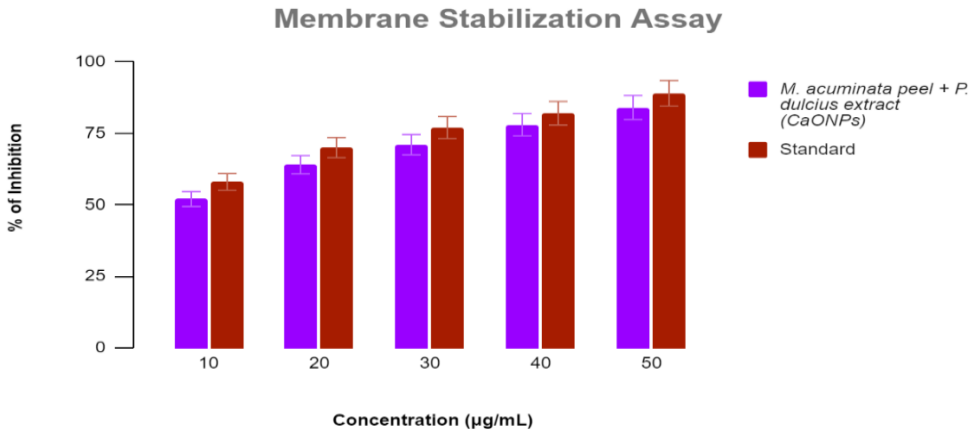
GRAPH 6. Antiinflammatory Activity- Bradford assay



GRAPH 7. Anti inflammatory Activity Early Antigen (EA) Assay - M. acuminata peel + P. dulcius extract (CaONPs)



GRAPH 8. Membrane Stabilization Assay - M. acuminata peel + P. dulcius extract(CaONPs)



DISCUSSION

The results of this study emphasize the significance of green synthesis in the production of CaO nanoparticles, demonstrating its advantages over conventional chemical methods. Traditional synthesis often involves toxic reagents, high energy consumption, and potential cytotoxicity, limiting its biomedical applications [22]. In contrast, plant-based synthesis offers an eco-friendly alternative by eliminating the use of hazardous chemicals, ensuring sustainability and environmental safety[23]. Additionally, the presence of bioactive compounds from plant extracts enhances the biocompatibility of the nanoparticles, reducing the risk of adverse biological effects [24]. These phytochemicals also function as natural stabilizers, preventing nanoparticle aggregation and improving stability [25]. This method aligns with previous research highlighting the superior biological activity and reduced toxicity of green-synthesized nanoparticles, making them highly suitable for various therapeutic applications, particularly in dentistry and biomedicine [26]. By integrating green chemistry principles, this approach supports sustainable nanotechnology, paving the way for safer and more effective biomedical treatments (27). CaO nanoparticles exhibit potent antimicrobial activity through multiple mechanisms, making them highly effective against oral pathogens. One of the primary mechanisms is oxidative stress, where CaO nanoparticles generate reactive oxygen species (ROS) that disrupt bacterial membranes, cause DNA fragmentation, and ultimately lead to microbial cell death [28]. This oxidative stress mechanism is well-documented in nanomaterial research, highlighting its broad-spectrum antimicrobial properties[29]. Additionally, CaO nanoparticles alter the local microenvironment by increasing pH levels, which inhibits acidogenic bacteria like Streptococcus mutans (30). Since oral biofilms and pathogenic bacteria thrive in acidic conditions, this pH shift prevents bacterial adhesion and biofilm formation, making CaO nanoparticles suitable for dental applications [31]. Furthermore, the positively charged CaO nanoparticles interact with the negatively charged bacterial cell membranes, leading to increased permeability and leakage of intracellular components [32]. This electrostatic interaction weakens microbial integrity, resulting in cell lysis and death. Given these antimicrobial properties, CaO nanoparticles can be incorporated into various dental applications, including toothpastes and mouthwashes to prevent plaque formation, dental implants and restorative materials to reduce post-surgical infections, and periodontal dressings to enhance wound healing [33].The anti-inflammatory properties of CaO nanoparticles stem from their ability to reduce oxidative stress and inflammatory mediators, making them highly beneficial for oral healthcare applications [33]. One of the primary mechanisms is the inhibition of protein denaturation, a process that occurs during inflammation and leads to tissue damage [33]. By preventing protein denaturation, CaO nanoparticles protect oral soft tissues from inflammatory destruction, reducing the risk of conditions such as periodontitis and mucositis [33]. Additionally, CaO nanoparticles exhibit strong nitric oxide (NO) scavenging activity, which plays a crucial role in controlling inflammation (34). Excessive NO production is associated with chronic inflammatory diseases, including periodontal disease and oral ulcers, as it contributes to oxidative stress and cellular damage [34]. By neutralizing NO radicals, CaO nanoparticles mitigate inflammatory responses, promoting faster healing and tissue regeneration [35]. These combined anti-inflammatory effects highlight the potential of green-synthesized CaO nanoparticles as an effective therapeutic agent for treating inflammatory oral conditions while ensuring biocompatibility and sustainability [35]. Biomedical Applications & Future Prospects in which the antimicrobial and anti-inflammatory properties of CaO nanoparticles position them as promising tools for various biomedical applications, particularly in dentistry and regenerative medicine [35]. As a coating material for dental implants, CaO nanoparticles can effectively prevent bacterial colonization and post-surgical infections, enhancing implant longevity and success rates [36]. Their ability to promote soft tissue regeneration makes them ideal for wound healing applications, aiding in faster recovery and reducing inflammation-related complications (36). Additionally, their potential use in drug delivery systems allows for targeted treatment of oral pathogens with enhanced precision, minimizing side effects and improving therapeutic outcomes[37]. While these preliminary findings highlight the significant biomedical potential of green-synthesized CaO nanoparticles, further in vivo studies are necessary to assess toxicity, optimize formulations, and explore their full clinical applications [37]. Nonetheless, this study supports the potential of CaO nanoparticles in advancing biocompatible and sustainable nanomedicine solutions, offering a safer and eco-friendly alternative to conventional antimicrobial and anti-inflammatory agents.

5. CONCLUSION

The study successfully synthesized eco-friendly calcium oxide (CaO) nanoparticles using Musa acuminata and Prunus dulcis extracts. Characterization confirmed their crystalline structure, functional stability, and uniform morphology (50–80 nm). These nanoparticles exhibited strong antimicrobial properties against Streptococcus mutans, Porphyromonas gingivalis, and Candida albicans through ROS generation, membrane disruption, and pH modulation,

making them effective in preventing dental infections. Their high protein denaturation inhibition (72.5%) and nitric oxide scavenging capacity (68.3%) highlight their anti-inflammatory potential for periodontal disease management and wound healing. Green synthesis enhances bioactivity, eliminates toxic chemicals, and ensures biocompatibility, making these nanoparticles suitable for oral healthcare and biomedical applications. Future research should focus on in vivo studies, formulation optimization, and clinical validation to maximize their therapeutic benefits.

DECLARATIONS

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Conflict of Interest

The authors declare no conflicts of interest

Ethics approval and consent to participate

Not applicable

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