Research Article

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Evaluation of Antimicrobial and Cytotoxic Effects of *Eclipta prostrata*-mediated Calcium Oxide Nanoparticle-incorporated Mouth Rinse

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ABSTRACT

The study evaluates the antimicrobial and cytotoxic properties of a mouth rinse formulated with *Eclipta prostrata*-mediated calcium oxide nanoparticles (CaO NPs). The agar well diffusion method was used to evaluate the antimicrobial activity against oral pathogens, including Enterococcus faecalis, Streptococcus mutans, Candida albicans, and Lactobacillus. Results demonstrated a dose-dependent antimicrobial effect, with Enterococcus faecalis exhibiting the highest zone of inhibition (13 mm at 100 µg/ml), followed by moderate sensitivity in Streptococcus mutans and lower susceptibility in Candida albicans and Lactobacillus. Cytotoxicity, measured using the Brine shrimp lethality assay, revealed the mouth rinse to be less toxic than commercial alternatives. Statistical analysis, performed through one-way ANOVA and SPSS version 23, confirmed significant differences in microbial inhibition. These findings suggest that *Eclipta prostrata*-mediated CaO NPs have the potential to be an effective antimicrobial mouth rinse with low toxicity, though further research is needed to confirm clinical safety and efficacy.

Keywords: Antimicrobial; Cytotoxicity; *Eclipta prostrata*; Oral pathogens.

1. INTRODUCTION

Eclipta prostrata, also known as False Daisy or Bhringraj, is a notable medicinal herb with deep roots in traditional medicine systems (Timalsina and Devkota, 2021). This herb, a member of the Asteraceae family, was native to tropical and subtropical regions across Asia, South America, and Africa. Rich in bioactive compounds such as wedelolactone, ecliptine, luteolin, and apigenin, *Eclipta prostrata* has garnered significant attention for its therapeutic potential (Mohanta *et al*. 2023). Historically, *Eclipta prostrata* has been employed for its hepatoprotective, anti-inflammatory, and antimicrobial properties. Recent scientific research has substantiated many of these traditional uses while also revealing new biomedical applications. For example, its hepatoprotective properties are linked to its ability to regulate liver enzymes and combat oxidative stress, suggesting its utility in treating liver conditions like hepatitis and cirrhosis. Additionally, its anti-inflammatory effects make it a valuable candidate for managing inflammatory disorders such as arthritis and asthma. The herb's antimicrobial efficacy, confirmed through various studies, highlights its potential in addressing infections caused by bacterial, viral, and fungal pathogens (Li *et al.* 2015). Notably, the cytotoxic effects of *Eclipta prostrata*, mediated primarily through its compound wedelolactone, involve disruption of cell cycle regulation and induction of oxidative stress in cancer cells, leading to apoptosis (Sarveswaran *et al.* 2012).

In recent years, nanoparticles have emerged as powerful tools in biomedical applications due to their enhanced bioavailability and targeted delivery capabilities. Among them, calcium oxide nanoparticles (CaO NPs) have gained attention for their biocompatibility, biodegradability, and multifunctionality. The green synthesis of CaO NPs using *Eclipta prostrata* was a promising approach, leveraging the plant's aqueous extract as both are reducing and stabilizing agents. This eco-friendly synthesis involves combining the plant extract with a calcium precursor, followed by heating and calcination (Al-Maula *et al.* 2021). This method not only reduces environmental impact but also enhances the nanoparticles' bioactivity through the plant's phytoconstituents. CaO NPs exhibit antimicrobial activity by interacting with bacterial cell membranes, increasing permeability, and inducing cell lysis. They release calcium ions that disrupt the bacterial cell wall's integrity and metabolic functions, as well as reactive oxygen species (ROS) that harm cellular components (Kumari *et al.* 2023).

The combined use of *Eclipta prostrata* and CaO NPs provides a synergistic antimicrobial effect, making it effective against various oral pathogens. Both the plant extract and the nanoparticles are biocompatible, minimizing adverse effects associated with traditional mouthwashes. Furthermore, the calcium ions released from the nanoparticles aid in re-mineralizing tooth enamel, offering an additional benefit in preventing dental caries (Ahmadian *et al.* 2018). The antioxidant potential of CaO NPs synthesized with *Eclipta prostrata* has been demonstrated, suggesting their ability to reduce oxidative stress in oral tissues (Yang *et al.* 2023). The synthesis of CaO nanoparticles mediated by *Eclipta prostrata* exhibits a notable lack of standardization, despite these encouraging findings. Variations in extract preparation, synthesis conditions, and characterization methods contribute to inconsistent results regarding their properties and efficacy. Furthermore, the limited number of studies on this subject lack a comprehensive understanding of their full potential, efficacy, and safety, restricting their practical application and advancement in this promising field. This study aims to evaluate the antimicrobial and cytotoxic effects of a mouth rinse formulated with *Eclipta prostrata*-mediated calcium oxide nanoparticles.

2. MATERIALS AND METHODS

2.1 Preparation of *Eclipta prostrata* Plant Extract

Eclipta prostrata in 1-gram powdered form was mixed with 100 ml of distilled water. The extract was boiled at 65 °C until the extract was condensed. Then, CaO NPs were added and further condensed until the color changed to dark yellow.

2.2 Antimicrobial Activity

The antimicrobial activity of calcium oxide nanoparticles was examined using agar well diffusion methods in 2019, which is comparable to the earlier research conducted by Rajesh Kumar et al. Mueller Hinton agar plates were made and autoclaved at 121°C for 15–20 minutes to sterilize them. The medium was sterilized, put on sterile Petri plates, and allowed to cool to room temperature. Using sterile cotton swabs, the bacterial suspension (Enterococcus faecalis, Lactobacillus, Candida albicans, and Streptococcus mutans) was distributed uniformly across the agar plates. The agar plates were made with a sterile polystyrene tip to form nine-millimeter-diameter wells. Different concentrations (25, 50, and 100 µg/ml) of CaO nanoparticles were then added to the wells. An antibiotic, such as Fluconazole for fungi or Amoxyrite for bacteria, was commonly used as a standard. For 24 and 48 hours, the plates were kept at 37 °C to support the growth of fungus. A measurement of the inhibitory zone's diameter around the wells was used to determine the antibacterial activity. The zone of inhibition diameter was measured using a ruler in millimeters, and the zone of inhibition value was computed (Rajeshkumar *et al.* 2019a).

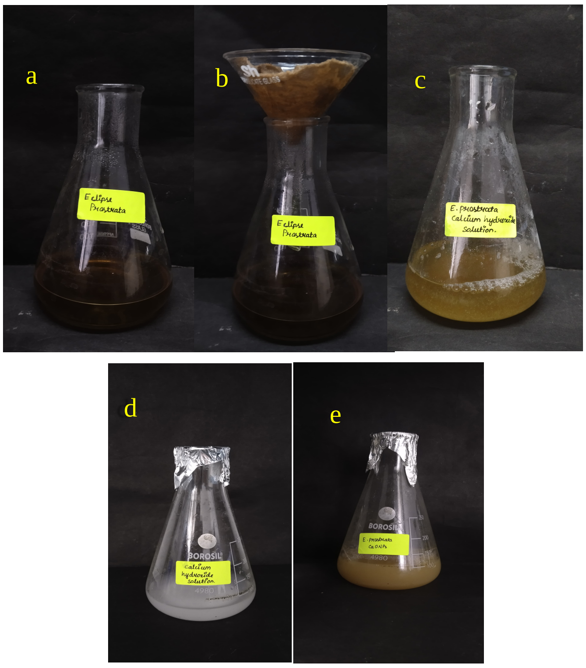

Fig. 1: Preparation of plant extract (a) *Eclipta prostrata* in distilled water, (b) boiled and filtered form of *Eclipta prostrata* extract, (c) calcium hydroxide solution, (d) mixture of *Eclipta prostrata* and calcium hydroxide solution, and (e) *Eclipta prostrata*-mediated CaO NPs with dark yellow color

2.3 Cytotoxic Effect

2.3.1 Brine Shrimp Lethality Assay

Saltwater preparation200 ml of distilled water was used to dissolve 2 g of iodine-free salt to create a saline solution. The next step was to use a six-well ELISA, where each well held 10–12 ml of saline solution. Ten nauplii (5, 10, 20, 40, and 80 µg/ml) were then progressively added to each well. After that, the nanoparticles were added to the solution until the appropriate concentration was reached. The plates were incubated for 24 hours. The ELISA plates were inspected, and the number of dead nauplii was calculated using the formula below:

Percentage of death =

[Number of dead nauplii / (number of dead nauplii + number of live nauplii)] × 100

3. RESULTS AND DISCUSSION

3.1 Antimicrobial Activity

The mouth rinse prepared using *Eclipta prostrata* and CaO NPs was evaluated for its antimicrobial activity against oral pathogens. Compared to the commercial mouthwash, which had a 9 mm zone of inhibition, the S. mutans group showed a 9 mm zone of inhibition at 25 µg/ml concentration, 50 µg/ml concentration, and 10 mm zone of inhibition at 100 µg/ml concentration. This indicates that the antimicrobial activity was lower in the case of S. mutans. When compared to the commercial mouthwash, E. faecalis showed a greater zone of inhibition, indicating good antimicrobial activity. At 25 µg/ml concentration, a 9 mm zone of inhibition was seen, at 50 µg/ml concentration, a 10 mm zone of inhibition was seen, and at 100 µg/ml concentration, a 13 mm zone of inhibition was seen followed by Streptococcus mutans. Candida albicans and Lactobacillus species with a maximum zone of inhibition of 9mm at 25, 50 and 100 25 µg/ml concentrations (Fig. 2).

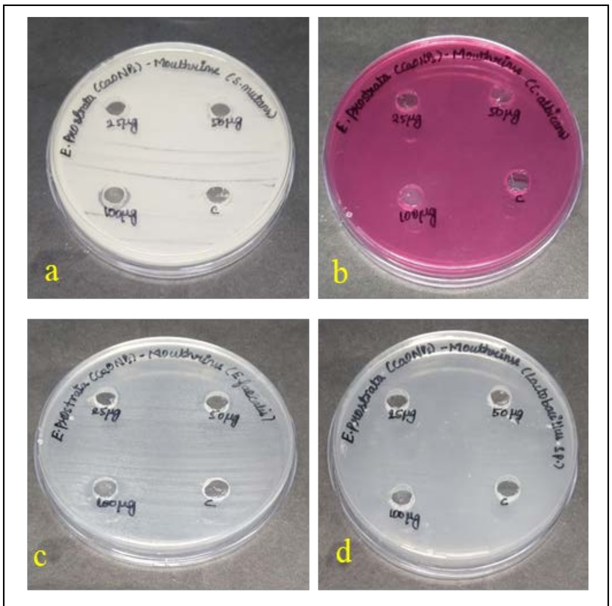

Fig. 2: Antimicrobial activity of Cao NP-mediated moutrinse

Fig. 3 shows the graph of the antimicrobial activity of *Eclipta prostrata* containing CaO NPs against four organisms: *S. mutans*, *C. albicans*, *E. faecalis*, and *Lactobacillus sp*. The antimicrobial activity was measured by the zone of inhibition (in mm) at three different concentrations of CaO NPs: 25 µg/ml, 50 µg/ml, and 100 µg/ml, and compared to a standard commercial mouthwash.

E. faecalis is more susceptible to higher concentrations of the CaO NP mouth rinse. The antimicrobial activity of CaO NP mouth rinse at 100 µg/ml was comparable to the standard for most microorganisms, but more effective against E. faecalis with a maximum inhibition zone.

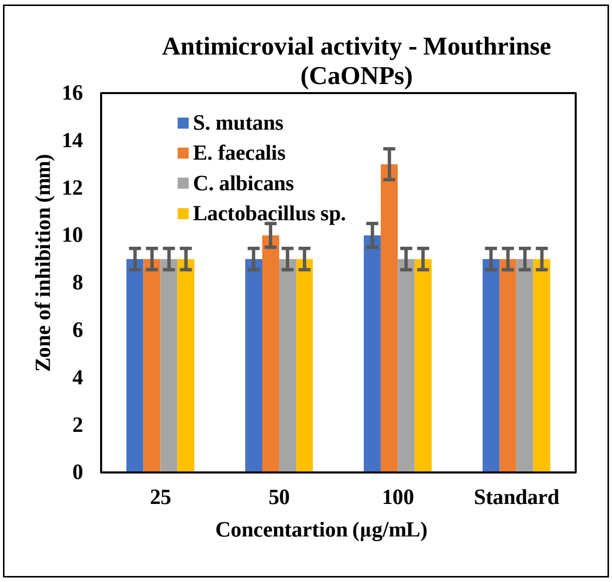

Fig. 3: Antimicrobial activity of *Eclipta prostrata*-mediated Calcium nanoparticle mouth rinse with a maximum zone of inhibition of E. faecalis at 100 µg/ml

The antimicrobial activity is represented by the reduction in colony-forming units (CFU) per ml, plotted on a logarithmic scale ($\log_{10}$ CFU/ml). The activity was measured for three different concentrations (25 µg/ml, 50 µg/ml, and 100 µg/ml) and compared with a standard treatment and a control as shown in Fig. 4.

3.2 Cytotoxic Activity

The cytotoxicity of the mouth rinse was evaluated using the brine shrimp lethality assay, which measured the percentage of live nauplii at varying concentrations over two days. On Day 1, the results showed no significant cytotoxic effects across all tested concentrations. The survival rate of nauplii in both the treatment and control groups remained at 100%, indicating that neither the commercial mouthwash nor the *Eclipta prostrata*-mediated CaONPs mouth rinse induced acute lethality within the first 24 hours of exposure. However, on Day 2, a concentration-dependent cytotoxic effect was observed with the *Eclipta prostrata*-mediated CaONPs. At concentrations of 20 µg, 40 µg, and 80 µg, the percentage of live nauplii decreased compared to the control group, demonstrating increased cytotoxicity. This effect was most pronounced at the highest concentration (80 µg), where the survival rate dropped to 70%, in contrast to the commercial mouthwash, which exhibited a lesser effect. These findings highlight a delayed cytotoxic response associated with higher concentrations of the *Eclipta prostrata*-mediated CaONPs mouth rinse (Fig 5)

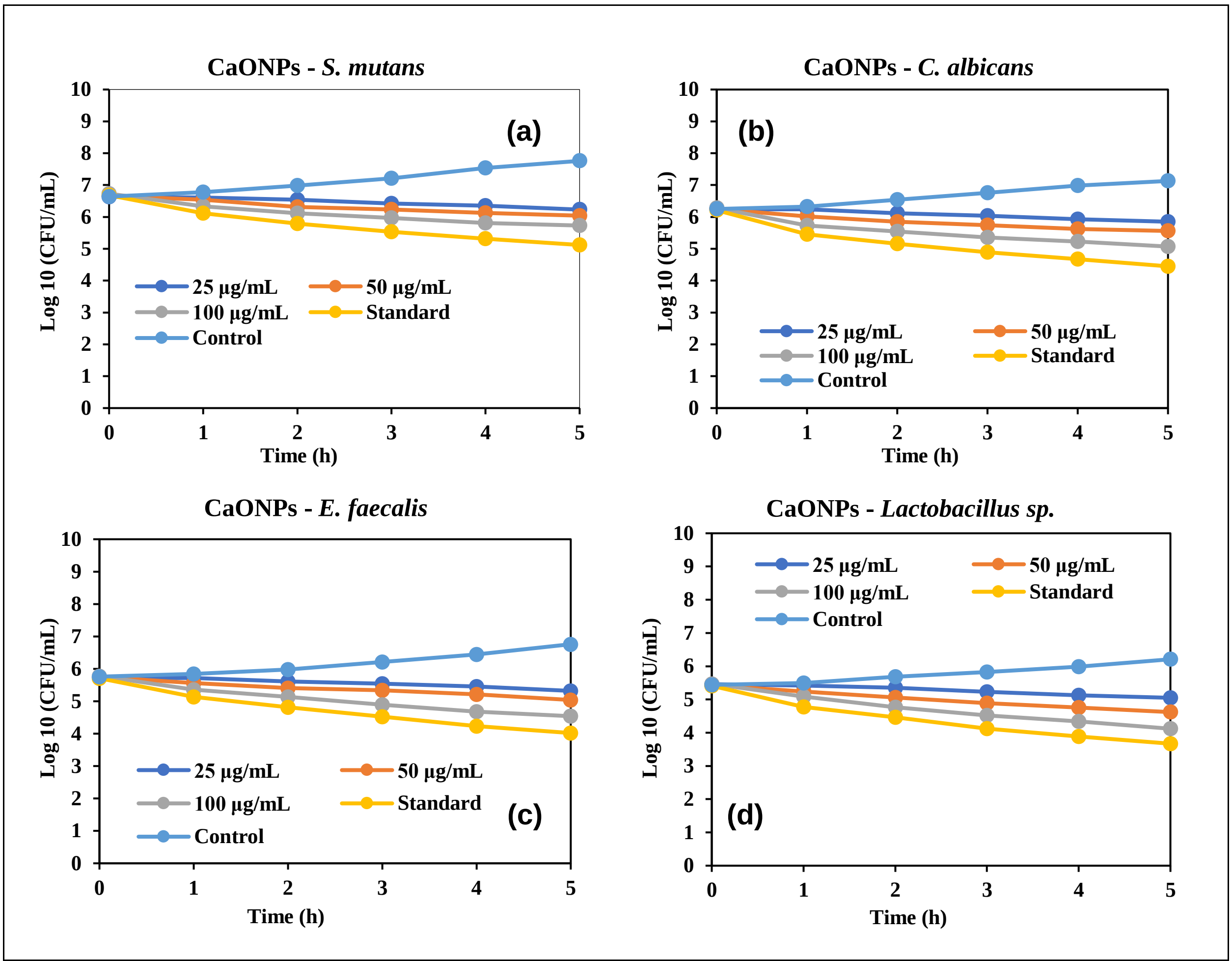

Fig. 4: Antimicrobial activity of *Eclipta prostrata* mediated CaO NPs against (a) S. mutans(b) C. albicans (b) E. faecalis (d) Lactobacillus sp. for 5 hours

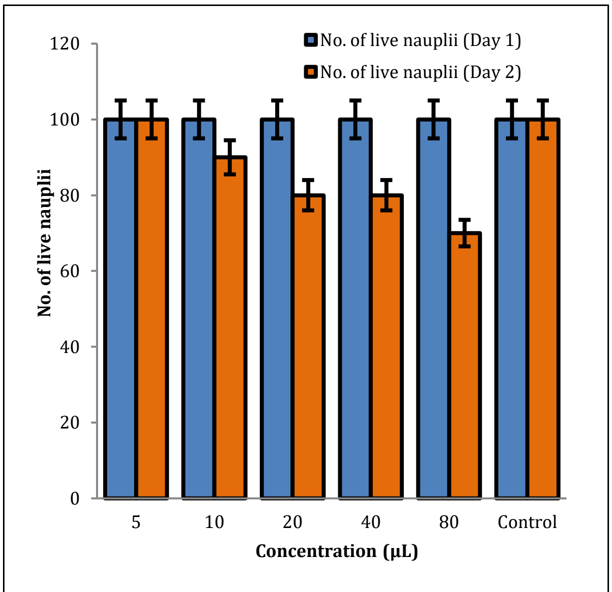

Fig. 5: Cytotoxic effect of *Eclipta prostrata* CaO NP-mediated mouth rinse showed a negative correlation with a decrease in several live nauplii with the rise in concentration

The evaluation of the antimicrobial activity of *Eclipta prostrata*-mediated calcium oxide nanoparticles incorporated into a mouth rinse revealed notable findings in this study. The results demonstrate a dose-dependent antimicrobial effect against various oral pathogens, including E. faecalis, S. mutans, C. albicans, and Lactobacillus species. The antimicrobial activity observed in this study was consistent with earlier research, which has demonstrated the efficacy of CaO NPs synthesized using plant extracts. Studies reported significant antibacterial properties of CaO NPs against oral pathogens (Loka *et al.* 2024). However, the current study extends these findings by providing a detailed comparison of antimicrobial activity at different concentrations and against multiple oral pathogens. In this study, E. faecalis exhibited the highest susceptibility to the CaO NP mouth rinse, with a zone of inhibition reaching 13 mm at a 100 µg/ml concentration. This was in line with the previous reports that have highlighted the potent antibacterial activity of CaO NPs against E. faecalis (Louwakul *et al.* 2017; Jose *et al.* 2022). The superior performance of the CaO NP mouth rinse, compared to a commercial mouthwash that had a 9 mm zone of inhibition, highlights the potential of this formulation to target stubborn pathogens in the oral cavity.

S.mutans showed moderate sensitivity to the CaO NP mouth rinse, with a zone of inhibition increasing from 9 mm to 10 mm at 100 µg/ml. This result was somewhat lower than the antimicrobial activity reported in the previous studies. Another study suggested that mouthwash from herbal extract-mediated nanoparticles synthesized using different methods exhibited higher efficacy (Tatekalva *et al.* 2020; Barma *et al.* 2021). The slight variation could be due to differences in nanoparticle synthesis protocols, the stability of the mouth rinse formulation, or the specific strains of S. mutans tested. C. albicans and Lactobacillus species demonstrated relatively low susceptibility to the CaO NP-based mouth rinse, with a consistent 9 mm zone of inhibition across all tested concentrations. This outcome indicates a less pronounced antimicrobial effect compared to the commercial mouthwash, which suggests that while CaO NPs have broad-spectrum activity, their effectiveness against fungal pathogens like C. albicans might be limited. This aligns with the previous studies which noted varying degrees of antimicrobial efficacy depending on the microbial species targeted.

The dose-dependent antimicrobial activity observed in this study was a crucial finding. As the concentration of CaO NPs increased, the zones of inhibition for E. faecalis and S. mutans also increased, demonstrating a clear relationship between nanoparticle concentration and antimicrobial effectiveness. This trend is indicative of the potential for optimizing nanoparticle concentrations to achieve desired antimicrobial effects in oral care products. Most studies to date have been conducted in vitro, with a limited number of in vivo studies, and remain untested in an oral environment - the reason why there is a lack of proof for the effectiveness and safety of these nanoparticles. This gap raises concerns about the translation of in vitro findings to practical applications.

4. CONCLUSION

Eclipta prostrata-mediated calcium oxide nanoparticles represent a novel and promising approach in the formulation of mouth rinses. Their combined antimicrobial, biocompatible, and re-mineralizing properties make them a superior alternative to conventional mouth rinses. To fully establish their safety and efficacy in human subjects, more clinical trials and in vivo investigations are necessary. Embracing such innovative solutions could significantly enhance oral healthcare, reducing the prevalence of dental diseases and improving the quality of life.

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CONFLICT OF INTEREST

The authors declared no conflict of interest in this manuscript regarding publication.

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