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In Vitro Controlling of Oral Pathogens Using Commelina Benghalensis Assisted Zinc Oxide Nanoparticles Based Gel

Fathima shirin[1], S.Sangeetha[2]
[1,2]Department of Anatomy, Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical sciences (SIMATS), Saveetha University, Chennai – 600077, India.
*Corresponding author Email: sangeethas.sdc@saveetha.com

ABSTRACT
Introduction: *Many oral disorders, such as cold sores, oral thrush, periodontal disease, and dental caries, are brought on by mouth pathogens, which include viruses, bacteria, fungi, and parasites. The antibacterial action of plant phenolic compounds against human infections has been studied in great detail in an effort to find novel medications, therapeutic goods, and healthy food ingredients. Maintaining a healthy lifestyle, along with routine dental checkups, is crucial to preventing infections.* ***Aim:*** *In order to control oral infections, the purpose of this study is to evaluate the antibacterial activity of a gel based on Commelina benghalensis and zinc oxide nanoparticles (ZnONPs) in vitro. The study focuses on common oral microorganisms in particular.* ***Materials and methods****: Mueller Hinton agar was used in the study as the microorganism growing medium. The antibacterial capabilities of zinc oxide nanoparticles (ZnONPs) produced by green synthesis were investigated. Streptococcus mutans, Lactobacillus sp., Staphylococcus aureus, Candida albicans, and Enterococcus faecalis were among the bacterial and fungal strains. As controls, common antibiotics such as fluconazole for fungus and amoxyrite for bacteria were employed. Sterilised petri dishes, cotton swabs, polystyrene tips, an autoclave, and an incubator were necessary lab supplies.* ***Result:*** *For every pathogen examined, the study found discrete inhibitory zones at 25 µg, 50 µg, and 100 µg ZnONPs gel concentrations. The ZnONPs gel supported by Commelina benghalensis showed a noteworthy antibacterial activity, according to the data. Different pathogens had different inhibition zones, with most microorganisms exhibiting dose-dependent suppression. Candida albicans, on the other hand, displayed the maximum zone of inhibition at 100 µg/mL.* ***Conclusion:*** *Strong antibacterial qualities against a variety of oral infections were established by Commelina benghalensis-assisted ZnO NPs gel, indicating its possible use as a therapeutic agent in dental applications*
Keywords: *Zinc oxide nanoparticles, Commelina benghalensis, oral pathogens, antibacterial activity, in vitro study, Candida albicans, Streptococcus mutans, Staphylococcus aureus, Lactobacillus sp., Enterococcus faecalis.*

INTRODUCTION

Microorganisms in the mouth called oral pathogens are responsible for a number of illnesses and infections. They consist of viruses like herpes simplex virus, fungi like Candida albicans, bacteria like Streptococcus mutans and Porphyromonas gingivalis, and parasites. These microorganisms can cause cold sores, oral thrush, periodontal disease, and dental caries. In-depth research has been done on the antimicrobial activity of crude extracts of plant phenolic compounds against human infections to identify and create new, healthful dietary ingredients as well as pharmaceutical and medicinal goods[(1)]. Infections can be avoided by practising proper oral hygiene, seeing the dentist regularly, and leading a healthy lifestyle.

In vitro techniques for managing oral pathogens entail evaluating different antimicrobial substances or agents in lab environments to determine how well they suppress or eradicate dangerous germs. Prior to moving on with clinical trials, these techniques are essential for screening possible treatments. To assess the efficacy of various interventions, researchers use methods include time-kill kinetics investigations, minimum inhibitory concentration (MIC) assays, biofilm disruption assays, antimicrobial susceptibility testing, and molecular analysis.These in vitro methods are vital resources in the continuous fight against dental disorders and in favour of dental health.Essential oils with antibacterial properties, such thyme, tea tree, and peppermint, can also effectively combat oral diseases by acting as an intracranial antiseptic.[(2,3)]

Functional foods and medications derived from natural sources have gained popularity in recent years. Naturally abundant in bioactive substances are plants, and wild plants in particular have long been important in many societies.[(3)]For medical purposes, almost 3/4 of the world's population is dependent on plants and plant-based products[(4)]Commelina benghalensis is a perennial herb endemic to tropical Asia and Africa. It is also referred to as the Benghal dayflower, tropical spiderwort, or wandering Jew[(5)].It is frequently connected to disturbed soils.It is used as a medicinal herb in China, where it is said to have diuretic, febrifugal, and anti-inflammatory properties. In Pakistan, on the other hand, it is used as a laxative and to treat leprosy and skin swellings.

This medicinal plant has been utilised for many years for a variety of uses, including natural disease cures, alternative medicine, food preservation, and medicine. Natural products derived from plants are more appealing nowadays than those with artificial origins. Due to the real health benefits and lower risk of adverse effects, scientists, researchers, and doctors have been increasingly interested in natural therapies.It has been revealed that C. benghalensis severe extracts has antioxidant qualities in vitro. In vitro antioxidant capabilities are demonstrated by extracts of C. benghalensis root in ethanol, benzene, n-hexane, methanol, and chloroform.[(6)]

Zinc oxide, a chemical made of zinc and oxygen, can be found in nanoscale particles as zinc oxide nanoparticles. Because of their small size, high surface area-to-volume ratio, and quantum effects, these nanoparticles have special characteristics. Particle size has a significant impact on the dispersion and absorption of zinc oxide nanoparticles.((7))Due to their many uses, zinc oxide nanoparticles have drawn a lot of interest from a variety of sectors. They are useful in topical creams, ointments, and dressings because of their antibacterial, anti-inflammatory, and wound-healing qualities in medicine. Because of their semiconducting, optical, and photocatalytic qualities, they are also used in electronics, optics, catalysis, and environmental cleanup. Ongoing studies into the safety and environmental impact of zinc oxide nanoparticles have been spurred by worries about their possible toxicity. Zinc oxide nanoparticles have also been shown to infiltrate the bacterial cell membrane, cause damage to its wall, and subsequently concentrate there, as evidenced by SEM and TEM images.((8))

Zinc oxide's antibacterial and wound-healing qualities allow it to show a variety of therapeutic effects on oral infections. Zinc oxide has the ability to prevent the growth of a variety of oral infections, such as fungi like Candida albicans and bacteria like Porphyromonas gingivalis, when administered topically or integrated into dental materials. Its capacity to damage microbial cell membranes, obstruct vital cellular functions, and prevent the formation of biofilms is what is thought to be responsible for its antibacterial effect. It has been proposed that the antibacterial capabilities of bulk zinc oxide suspension can be described by external production of H_2O_2. (9)Furthermore, through promoting angiogenesis, collagen synthesis, and epithelial cell proliferation, zinc oxide has been demonstrated to aid in oral cavity wound healing. Because of these combined benefits, zinc oxide is a useful ingredient in mouthwash, toothpaste, and dental materials. It also helps with tissue healing and can be used to treat and prevent oral infections.

In comparison to currently available conventional medications, many plants-mediated (green synthesis) ZnO NPs exhibit excellent antimicrobial action against the pathogens and have prospective applications in the fields of antiviral, anticancer, and free radical scavenging. The production of ZnO NPs from plants could be a fantastic strategy for creating innovative and environmentally responsible biological applications(10,11).The possibility of using a zinc oxide nanoparticle-based gel aided by commelina benghalensis for the control of oral pathogens has been investigated in vitro. This novel method combines the inherent bioactive chemicals found in commelina benghalensis extract with the antibacterial capabilities of zinc oxide nanoparticles. The gel formulation has a number of benefits, such as improved penetration into oral pathogen biofilms and prolonged release of antimicrobial drugs. In vitro tests have shown how well this gel works to prevent the growth of common oral infections like Porphyromonas gingivalis and Streptococcus mutans, as well as to lessen the production of biofilms. Additionally, the combined actions of commelina benghalensis extract and zinc oxide nanoparticles may have anti-inflammatory and wound-healing properties.Another emerging trend is the use of nanoparticles in medicine. Studies on the potential effectiveness of combining copper nanoparticles with turmeric and tulsi in reducing oral infections have been conducted.This demonstrates the interaction between green plants and nanoparticles.(12)

AIM

This study aims to assess the antibacterial activity of a gel based on zinc oxide nanoparticles (ZnONPs) and Commelina benghalensis in vitro for the control of oral pathogens. By measuring the inhibition zones and bacterial count reductions of these common oral microbes—including Candida albicans, Streptococcus mutans, Staphylococcus aureus, Lactobacillus sp., and Enterococcus faecalis—at different ZnONPs gel concentrations, the study specifically aims to ascertain the efficacy of this gel against these microbes.

MATERIALS AND METHODS

The growing medium for the microorganisms in this investigation was Mueller Hinton agar, one of the materials employed in the experiment. Green-synthesised zinc oxide nanoparticles, or ZnONPs, were examined for their antibacterial capabilities. The bacterial and fungal strains that were examined were Streptococcus mutans, lactobacillus sp., staphylococcus aureus, candida albicans, and enterococcus faecalis. Standard antibiotics were employed as controls, amoxyrite for bacterial strains and fluconazole for fungal strains. Sterile petri dishes, sterile cotton swabs, sterile polystyrene tips, an autoclave, an incubator, and a ruler were all used in the investigation.

Mueller Hinton Agar Plate Preparation:

The medium was autoclaved to sterilise it before being transferred onto sterile Petri dishes to create Mueller Hinton Agar plates. This guaranteed that the growing media used to assess antimicrobial susceptibility was constant.

Microbial Suspensions Preparation and Standardization:

Overnight, in suitable broth media, pure cultures of bacterial (e.g., Streptococcus mutans, Staphylococcus aureus) and fungal strains (e.g., Candida albicans) were produced. Using spectrophotometry, the cultures were standardised to 10^6 CFU/mL to guarantee consistent initial concentrations.

Agar Plate Inoculation:

Standardised microbial suspensions were uniformly applied to the Mueller Hinton agar plate surface using sterile cotton brushes. The goal of this stage was to have the microbes grow uniformly on all the plates.

Dictionary of Agar Plates:

Using a sterile polystyrene tip, 9 mm diameter wells were made in the inoculated agar plates. This made it easier to apply ZnONPs solutions locally and regulate antibiotics to the agar surface.

ZnONPs Solutions Preparation:

Commelina benghalensis aided in the environmentally friendly synthesis of Zinc Oxide Nanoparticles (ZnONPs). The phytochemical characteristics of Commelina benghalensis aided in the green production of ZnONPs. Prior to application, the size and shape of the nanoparticles were assessed.

Filling the Wells with ZnONPs and Control Antibiotics:

On the inoculated agar plates, separate wells were filled with 100 µL of each ZnONPs concentration and control antibiotics (such as amoxyrite for bacteria and fluconazole for fungi). This made sure that drugs were applied and diffused under control into agar medium

Plate Incubation:

At 37°C, inoculated plates were incubated. In order to provide bacteria enough time to develop and establish inhibitory zones, bacterial plates were incubated for 24 hours and fungal plates for 48 hours.

Inhibition Zone Measurement:

Using a sterile ruler, the diameter of the inhibition zones surrounding each well was measured following the proper incubation period. To ensure precision, measurements were made in various directions. The average diameter of each inhibitory zone was then determined and reported in millimetres (mm).

Data Analysis and Calculation:

The inhibitory zone sizes at various ZnONP doses and control antibiotic concentrations were computed and compared.Analysis of variance (ANOVA) and other statistical techniques were used to determine the antibacterial efficacy of ZnONPs in comparison to conventional antibiotics and to identify any significant differences

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Figure2: C.benghalensis with distilled water.

Figure3: Filtration using. Whatman filter paper

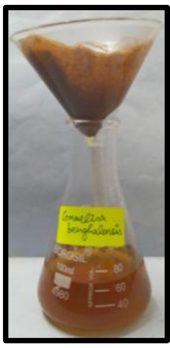


Figure1: Preparation of plant extract. mixed

Figure 4: Filtered plant extract solution.

Figure 5: Zinc sulphate solution

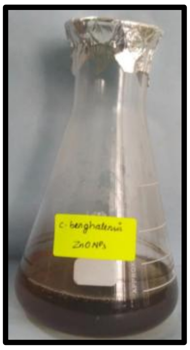


Figure 6: C.Benghalensis assisted ZnONPs

RESULTS

Five distinct bacterial and fungal strains are used in the study:Enterococcus faecalis (Plate 1), Lactobacillus sp. (Plate 2), Streptococcus mutans (Plate 3), Staphylococcus aureus (Plate 4), and Candida albicans (Plate 5). Zones of inhibition appear in every petri dish, demonstrating the ZnO NPs gel's antibacterial efficacy. There are distinct inhibitory zones for every pathogen at the gel concentrations that were tested, which are indicated as 25 µg, 50 µg, and 100 µg.

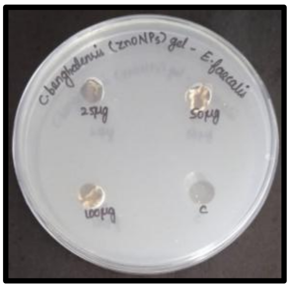


Plate1:E.faecalis.

Plate 2: Lactobacillus sp.

Plate 3:S.mutans

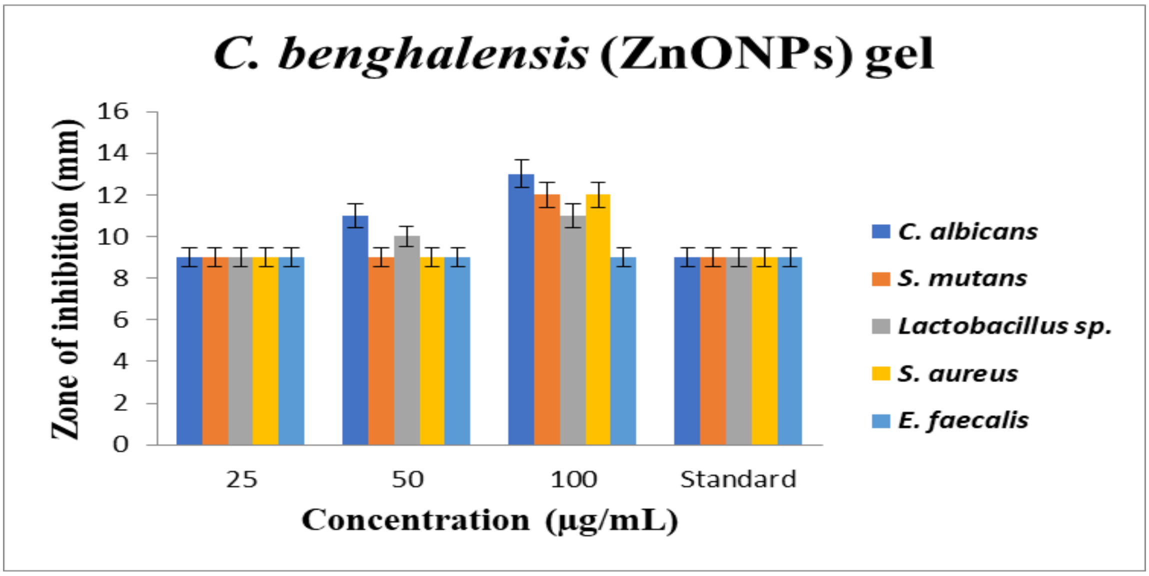
Plate 4: S.aureus

Plate 5: S.albicans

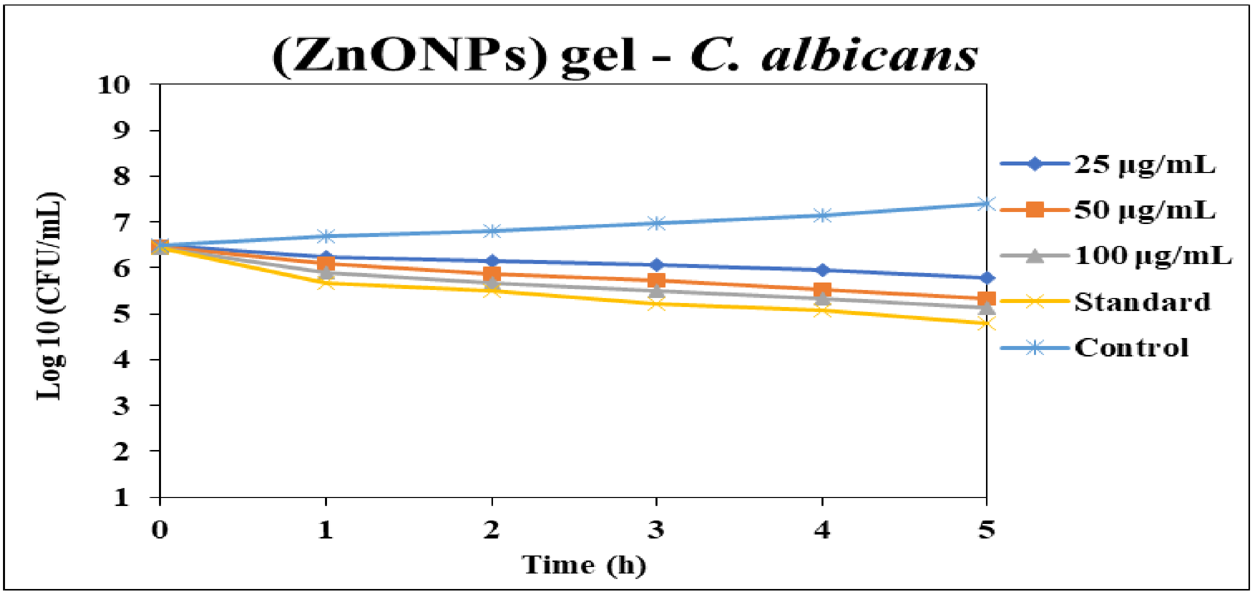
	25	50	100	Standard

C. albicans	9	11	13	9
S. mutans	9	9	12	9
Lactobacillus sp.	9	10	11	9
S. aureus	9	9	12	9
E. faecalis	9	9	9	9

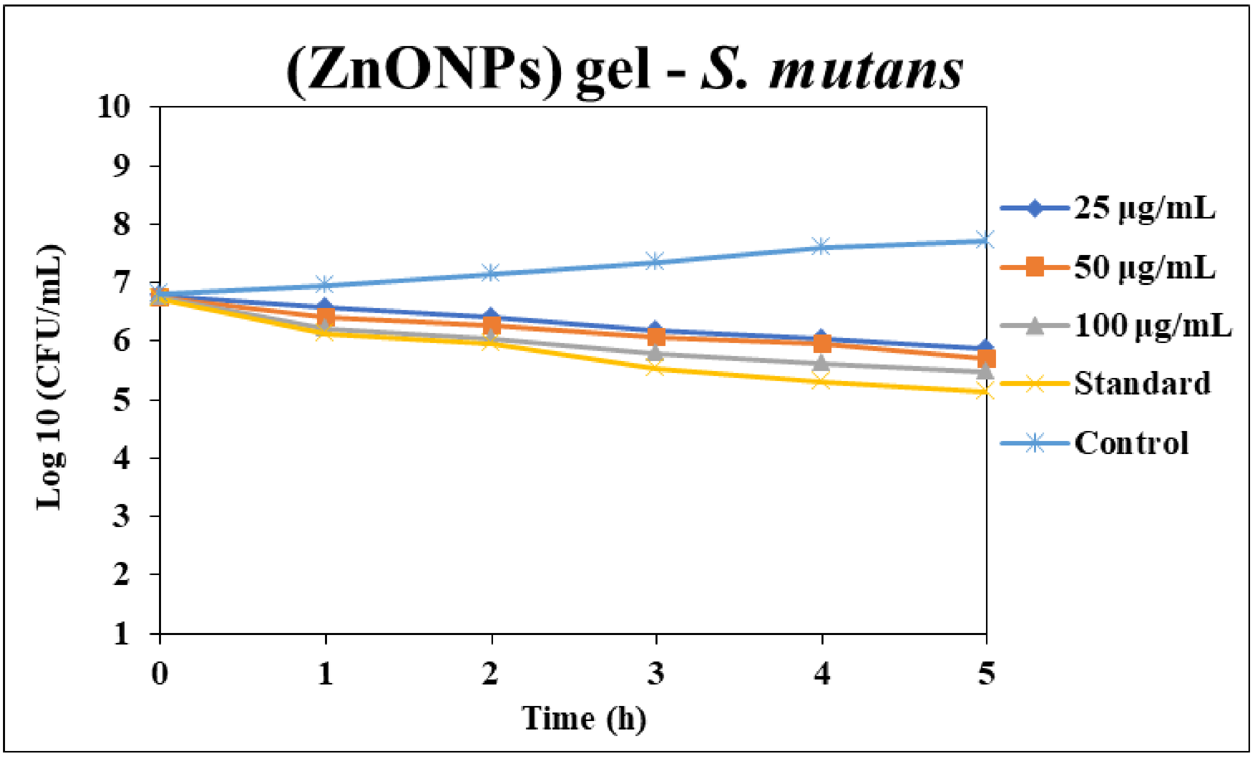
Table 1: The inhibition zones (measured in millimetres) against a variety of bacteria, such as Candida albicans, Streptococcus aureus, Lactobacillus sp., and Escherichia faecalis, at different concentrations (25, 50, and 100).



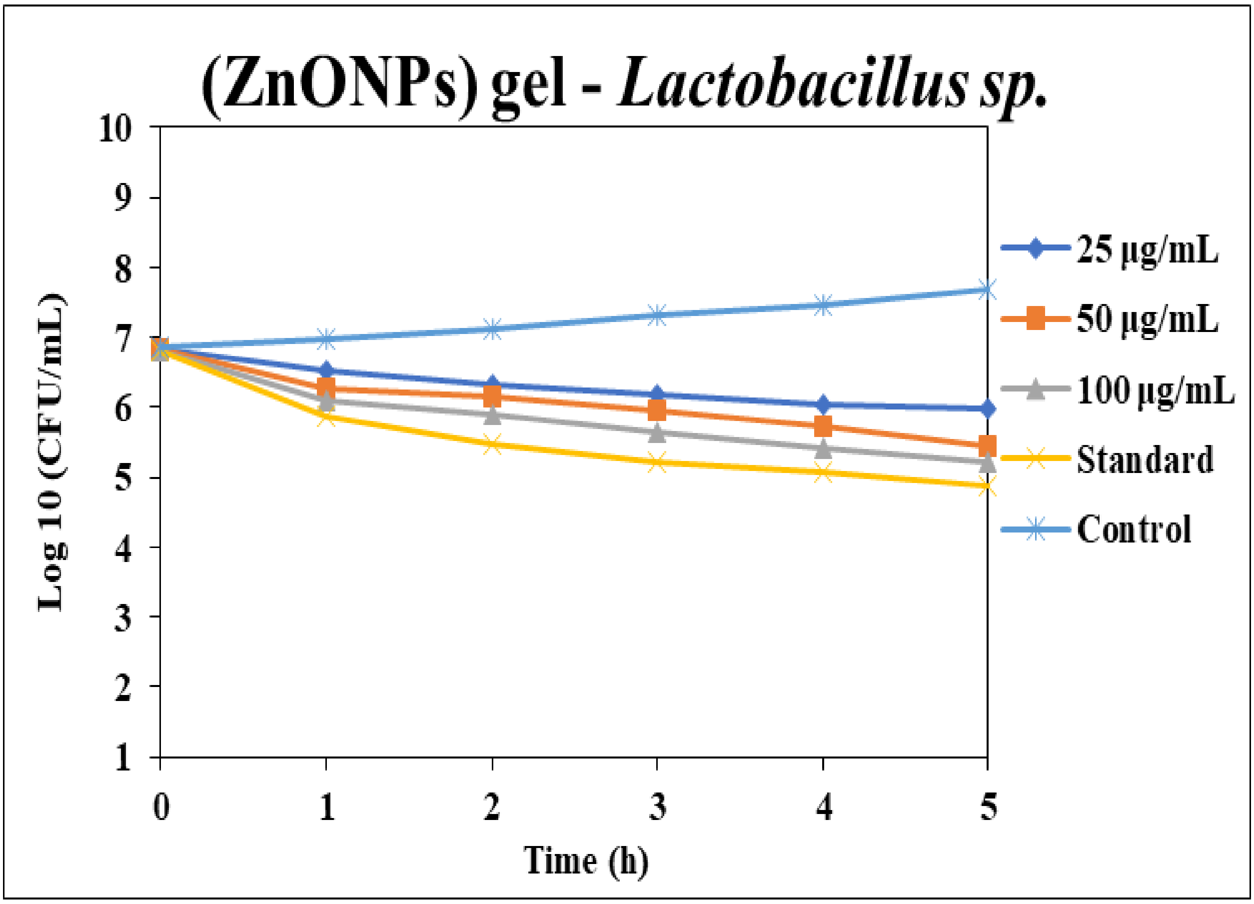
Bar graph 1: the antimicrobial activity of C. benghalensis (ZnONPs) gel against various microbes. On the X-axis, the concentration of the gel is represented in µg/mL, while the Y-axis depicts the zone of inhibition in millimetres (mm). The gel was tested at concentrations of 25 µg/mL, 50 µg/mL, and 100 µg/mL, alongside a standard and a control.



Time kill kinetic analysis graph 1: Impact of ZnONPs gel on C. albicans in 5 hour period. The Y-axis shows the bacterial count in logarithmic CFU (Colony Forming Units) per millilitre, while the X-axis shows the duration in hours (h).



Time kill kinetic analysis graph 2: Impact of ZnONPs gel on S. mutans in 5 hour period. The Y-axis shows the bacterial count in logarithmic CFU (Colony Forming Units) per millilitre, while the X-axis shows the duration in hours (h).



Time kill kinetic analysis graph 3: Impact of ZnONPs gel on S. mutans in 5 hour period. The Y-axis shows the bacterial count in logarithmic CFU (Colony Forming Units) per millilitre, while the X-axis shows the duration in hours (h).

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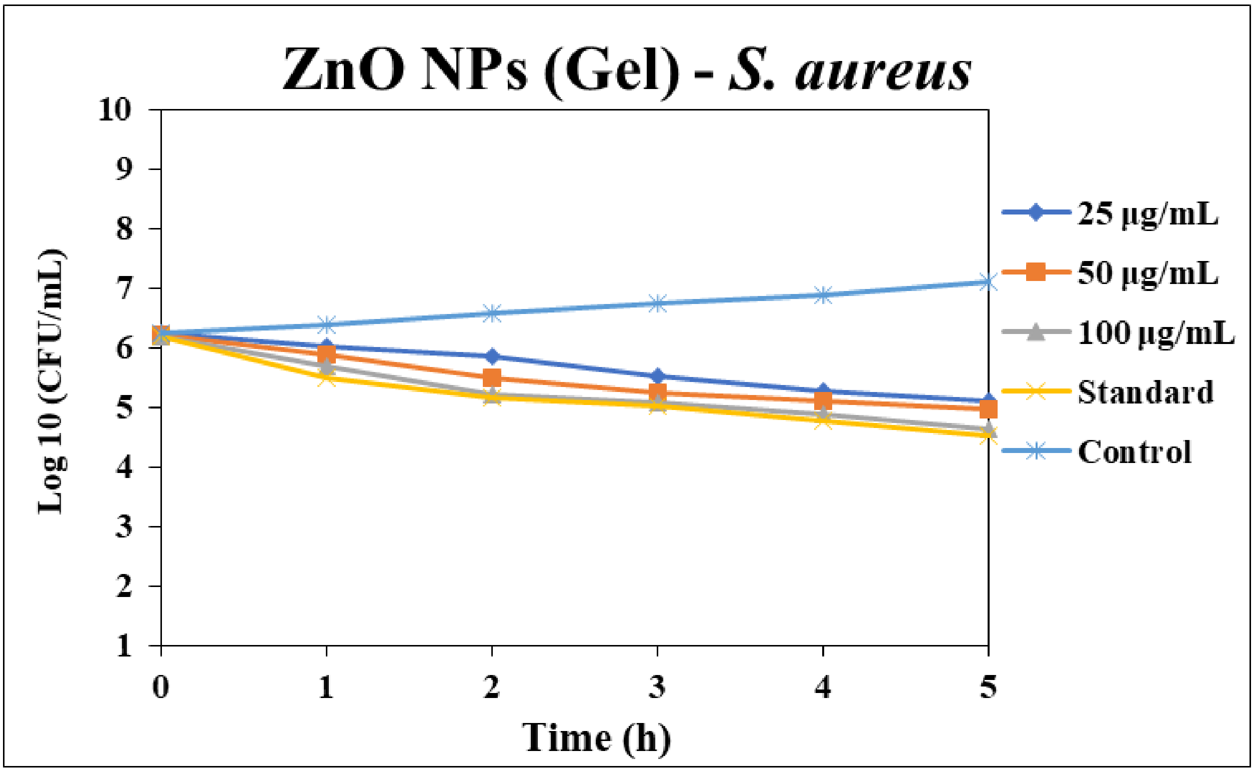
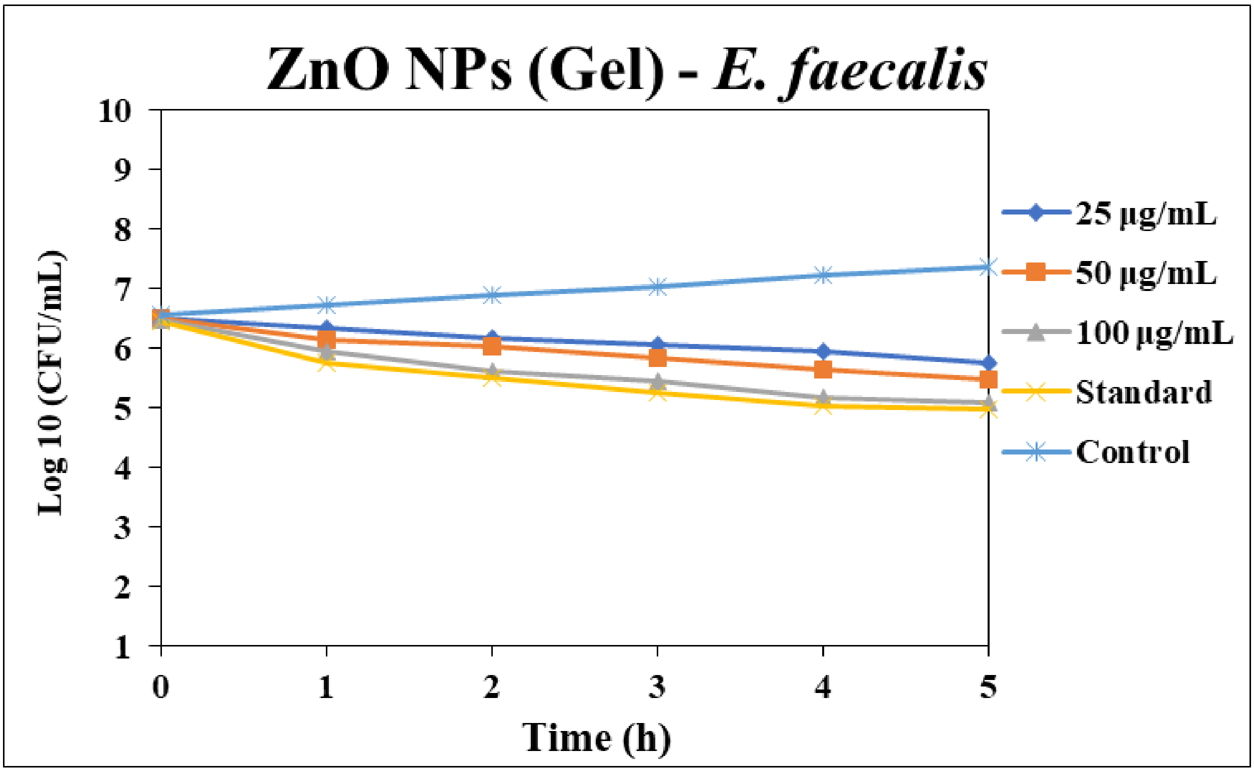


Time kill kinetic analysis graph 4: Impact of ZnONPs gel on S. aureus in 5 hour period. The Y-axis shows the bacterial count in logarithmic CFU (Colony Forming Units) per millilitre, while the X-axis shows the duration in hours (h).



Time kill kinetic analysis graph 5: Impact of ZnONPs gel on E.faecalis in 5 hour period. The Y-axis shows the bacterial count in logarithmic CFU (Colony Forming Units) per millilitre, while the X-axis shows the duration in hours (h).

DISCUSSION

The evaluation assessed the zinc oxide nanoparticles (ZnONPs) based gel assisted by Commelina benghalensis for its bactericidal and fungicidal efficacy against common oral pathogens, including Candida albicans, Streptococcus mutans, Staphylococcus aureus, Lactobacillus sp., and Enterococcus faecalis. To calculate CFU/mL, cultures treated with gel doses of 25, 50, and 100 μg/mL were sampled throughout a 5-hour period. The results showed that the gel is an efficient antimicrobial agent against the oral pathogens, which revealed significant, dose-dependent decreases in microbial counts. The largest decrease was achieved by the gel at the maximum concentration of 100 μg/mL.

In accordance with this study, common oral pathogens as Candida albicans, Streptococcus mutans, Staphylococcus aureus, Lactobacillus sp., and Enterococcus faecalis were significantly resistant to the ZnONPs-based gel when aided by Commelina benghalensis extract ($p<0.001$). E. coli and Lactobacillus species were one of the targets of the preceding study's discovery that selenium nanoparticles taken from Capparis decidua fruit had antibacterial action. The findings demonstrated that, in comparison to Lactobacillus, Selenium nanoparticles had more antibacterial efficacy against E. coli.(13)

For every oral pathogen examined, the ZnONPs-based gel showed significant inhibitory zones and decreased bacterial counts. Strong antibacterial activities are possessed by ZnONPs, as demonstrated by earlier research, especially when they are produced with plant extracts. Studies in the past have demonstrated that commercially available antibacterial treatments can be substituted with green generated copper nanoparticles based on Vernonia amygdalina extract. The position of the SPR band in the UV-Vis spectrum at 340 nm initially confirmed the green production of copper nanoparticles.(14) The antibacterial properties of various commercially available and laboratory-produced metal and metal oxide nanoparticles were evaluated against a variety of bacterial and fungal agents, including some freshwater cyanobacteria. Synthesised copper oxide (CuO), zinc oxide (ZnO), silver (Ag), and silver-doped titanium dioxide (Ag–TiO_2) were the only nanoparticles that exhibit broad spectrum antibacterial activity.(15)

Nevertheless, other research contends that the synthesis technique and kind of plant extract employed can have a substantial impact on the effectiveness of ZnONPs. Most studies backup the usage of ZnONPs for antibacterial purposes; several publications attest to their efficacy across a range of medical specialties. Because of their anti-C. albicans characteristics, ZnO and CuO nanoparticles may be employed to treat infections brought on by this fungus, which needs more in vivo research.(16) In spite of this, research is currently being done to maximise the effectiveness of synthesis techniques.Researchers gave out that natural treatments, such as O. sanctum L. gel, are better for treating periodontal disease because they have less adverse effects and, at 250 ug concentration, demonstrated effective antibacterial activity against anaerobic oral microbes—a greater level than CHX(17).(18)

One of the study's shortcomings is that the studies were conducted in vitro, which means that the intricate environment of the oral cavity might not be accurately replicated. Furthermore, differences in the production of ZnONPs may produce different outcomes. To guarantee consistent outcomes, future research should concentrate on standardising synthesis techniques and conducting in vivo testing.

Future studies should examine the clinical uses of the gel based on ZnONPs for treating oral infections, as well as its long-term effectiveness and safety. Examining ZnONPs in combination with other natural extracts may also improve their antibacterial qualities and provide them a wider range of activity.

CONCLUSION

This study set out to assess the antibacterial efficacy of a gel based on zinc oxide nanoparticles that was aided by an extract from Commelina benghalensis. The ZnONPs-based gel showed strong antibacterial action against common oral pathogens within the parameters of this investigation, indicating its potential as a substitute therapy for oral infections. It is necessary to conduct more research to validate these results and investigate their potential therapeutic uses.

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