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ZnO Nanoparticles and Pepper Fruit Extract Effect on *Streptococcus mutans* and *Staphylococcus aureus*

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Abstract

Objectives: To test the effect on *Streptococcus mutans* and *Staphylococcus aureus* isolates from dental cavities of different amounts of zinc oxide (ZnO) nanoparticles and Capsicum annuum L. extract. **Methods:** Zinc oxide nanoparticles (ZnO, purity: 99.8%, D50: 10–30 nm) were synthesised from Skyspring Nanomaterials. Pepper fruits were procured from local markets in Babylon. The fruits were cleansed using distilled water to eliminate soil, air-dried, and subsequently pulverised using an electric grinder. The extract was produced with Soxhlet equipment. **Results:** The inhibition zones for *S. mutans* at doses of 0.2, 0.4, 0.6, and 0.8 mg/ml were 2.12, 2.27, 2.58, and 2.98 mm, respectively. The inhibitory zones for *S. aureus* measured 2.02, 2.2, 2.45, and 2.68 mm, respectively. Significant antibacterial activities of the pepper fruit extract were demonstrated against both *S. mutans* and *S. aureus*. The inhibition zones for *S. mutans* at 100, 200, 3000, and 350 mg/ml doses were 1.24, 1.46, 1.54, and 1.7 mm, respectively. The inhibitory zones for *S. aureus* measured 1.35, 1.56, 1.78, and 1.89 mm, respectively. **Conclusion:** Both pepper fruit extract and ZnO nanoparticles possess antibacterial properties. ZnO nanoparticles at a dose of 1.0 mg/ml exhibited the most substantial inhibition zone against *S. mutans*. In contrast, pepper fruit extract at 300 mg/ml demonstrated the most significant inhibition zone against *S. aureus*.

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Introduction

For millennia, nature has served as a source of medicinal cures since the dawn of humanity. Despite this, synthetic drugs often have adverse effects, and it has become more and more apparent that microbes are resistant to these substances. Consequently, numerous developing countries have begun to emphasise herbal treatments and nanotechnology [1]. Zinc oxide nanoparticles (ZnO NPs) are important microelements that play important roles in many bodily functions. Consumption of water and food leads to the acquisition of zinc, which the small intestine primarily absorbs and transports to the blood plasma. Many businesses and drug companies use zinc oxide nanoparticles because they might be able to kill fungi, bacteria, diabetes, and inflammation, speed up wound healing, and protect cells from damage. Researchers have generated these nanoparticles using green synthesis techniques that use plants, fungi, bacteria, and algae [2,3].

The fruits of the pepper plant (Capsicum annuum L.) contain the alkaloid capsaicin, which imparts its spicy flavour. Capsaicin is present in elevated proportions in the fruits of spicy peppers. Alkaloids are essential molecules in the process of making medicines and pharmaceuticals. They also have big effects on the structure and function of cell membranes [4,5]. Paprika powder often comprises less than 0.5% essential oil. Pepper fruits possess a volatile oil level that is comparatively modest, varying from 0.1% to 2.6%, contingent upon the cultivar and the maturity stage at harvest. The primary chemical classes of compounds present in capsicum oil encompass terpenes and their derivatives, alcohols, aldehydes, ketones, carboxylic acids, esters of carboxylic acids, benzene derivatives, naphthalene derivatives, hydrocarbons, sulphur and nitrogen-containing compounds, phenolic compounds, and carotenoid derivatives [6].

Dental caries (cavitation) is notably severe and persistent among numerous dental issues. It is an irreversible microbial illness that impacts the calcified tissues of the teeth, marked by the demineralisation of the inorganic component and the degradation of the organic matrix. Dental caries recognises Streptococcus mutans and other

Vol 13, No 1 (2025) DOI 10.5195/d3000.2025.1039

streptococci as the principal causative agents [7]. Dental plaque, which develops on the tooth surface, particularly in the cervical region, comprises bacteria encased within an organic matrix. This plaque is associated with gingivitis, periodontal disease, and tooth caries [8]. Previous studies have demonstrated the management of dental plaque through physical removal and antimicrobial toothpaste and mouthwashes [9,10].
New types of bacteria, like Staphylococcus mutans and Staphylococcus aureus, are often linked to cavities and other periodontal diseases in the mouth [11,12]. These bacteria are becoming resistant to antibiotics, which is a major global health problem. This is especially applicable in the context of oral infections. These bacteria cause tooth decay and are also linked to the development of systemic illnesses. This shows the importance of effective antimicrobial treatments in dental care. Even though conventional antibiotics have been utilised in the fight against these diseases, the growing resistance to these medications has made it necessary to investigate alternative treatments [13].
Nanomaterials, including zinc oxide (ZnO) nanoparticles, are among the most promising options for antibacterial treatments. In oral health, zinc oxide (ZnO) has attracted significant attention due to its broad-spectrum antibacterial properties. These include the ability to combat both gram-positive and gram-negative bacteria, making it a potential therapeutic agent [14]. Recent studies have demonstrated that ZnO nanoparticles can effectively inhibit the growth of S. mutans and S. aureus. This could offer a new approach to treating oral microbial infections. However, the optimal concentration of ZnO nanoparticles needed for effective antibacterial activity while minimizing harm to human cells remains under investigation [15].
Researchers have long recognised plant-derived chemicals for their antibacterial characteristics and nanomaterials. Numerous researchers are investigating the possibility of natural extracts serving as adjuncts or replacements to traditional antibiotics [16,17].
Pepper fruit (Capsicum annuum), which is full of bioactive substances like capsaicin, flavonoids, and alkaloids, might be able to change the activity of microbes. This includes antibacterial actions against a wide range of pathogens. Using ZnO nanoparticles in conjunction with plant extracts, such as pepper fruit extract, could increase antibacterial action. This would reduce the requirement for higher doses of either agent on its own, potentially lowering the likelihood of resistance development.
This study aims to find out what happens to strains of *Staphylococcus aureus* and *Staphylococcus mutans* that have been taken from dental cavities when different amounts of ZnO nanoparticles and pepper fruit extract are mixed together. The study aims to help the ongoing search for long-lasting alternatives to traditional antibiotics in dental care by looking at how well these agents kill bacteria on their own and when mixed together. This research aims to provide insights into the potential of these agents as effective treatments for oral infections.

Materials and Methods

We synthesised zinc oxide nanoparticles/nanopowder (Specifications: ZnO, purity: 99.8%, D50: 10–30 nm, colour: white to light yellow) from Skyspring Nanomaterials (Fig. 1). The common contaminants in the nanoparticles consist of Cu (25 ppm), Cd (25 ppm), Mn (25 ppm), Pb (20 ppm), and As (20 ppm).

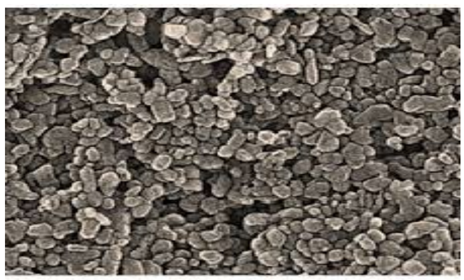

Figure 1. Zinc oxide nanoparticle.

We obtained the pepper fruits from local markets in Babylon. We rinsed the samples with distilled water to remove dirt and then dried them in the shade. After drying, we pulverised the samples using an electric mill [18].
The extraction was conducted via Soxhlet equipment. One hundred grams of the ground sample were placed in the thimble, and methanol at a 70% concentration was added to the round-bottom flask. The system was thereafter maintained at 60°C for 24 hours. Following the extraction process, the methanol was evaporated with a rotary evaporator to isolate the alcohol from the crude extract. The resultant extract was refrigerated until utilised [19].

Analysis of the Impact of Varied Doses on the Proliferation of Specific Pathogenic Microorganisms

We synthesised pepper fruit extract and ZnO nanoparticles using an MSA medium and incubated them anaerobically for 48 hours at 37°C before evaluating their antagonistic activity. Ten bacterial colonies were transferred to create the bacterial inoculum. To get a 0.5 McFarland Standard turbidity, which is equal to 1.5×10^8 cells/ml, the bacterial suspension was put into a test tube with 5 ml of Brain Heart Infusion (BHI) broth in a clean environment. The infected plates with MSA media were prepared by dispersing 0.1 ml of the suspension onto the plates. The plates were maintained at room temperature for 15 minutes to facilitate inoculum absorption.
A sterile cork borer with a diameter of 7 mm was employed to form wells in the inoculated agar plates. A micropipette was used to dispense 50 microliters of different concentrations of the test chemicals into the wells (0.2, 0.6, 0.8, and 1.0 mg/mL for ZnO nanoparticles; 100, 200, 300, and 350 mg/mL for the crude extract). Control plates were made by substituting the crude extract with 50 µl of sterilised distilled water in the wells.
The plates were kept at 37°C for 48 hours, and the results were judged by measuring the size of the inhibition zone, which is the area around the well where bacteria can't grow [20].

Statistical Analysis

The tests were executed as factorial experiments with three replications, employing a completely randomised design (CRD). The data were analysed with the least significant difference (L.S.D.) at a 1% probability threshold ($P \leq 0.01$)

Results

Table 1 demonstrates the strong impact of ZnO nanoparticles on *Staphylococcus mutans* and *Staphylococcus aureus*. At 0.3, 0.5, 0.7, and 0.9 mg/ml, the inhibition zones for *S. mutans* are 2.12, 2.27, 2.58, and 2.98 mm, and for *S. aureus* they are 2.02, 2.2, 2.45, and 2.68 mm.
Table 2 indicates a substantial impact of pepper fruit extract on S. mutans and S. aureus. At 100, 200, 300, and 350 mg/ml, the zones that stop S. mutans from growing are 1.24, 1.46, 1.54, and 1.7 mm, and the zones that stop S. aureus from growing are 1.35, 1.56, 1.78, and 1.89 mm.

Discussion

The study shows that ZnO nanoparticles can kill *Staphylococcus mutans* and *Staphylococcus aureus* bacteria, especially when 0.8 mg/ml of them are present. Nanoparticles may be responsible for this action. These nanoparticles may target subcellular compartments of the cell membrane, resulting in cellular damage and ultimately leading to cell death. Zinc oxide nanoparticles, also known as ZnO NPs, have demonstrated the ability to alter the construction of bacterial cell walls. ZnO NPs achieve this by influencing the linkage between N-acetylglucosamine and N-acetylmuramic acid in glycans. This, in turn, leads to the formation of pits and a weakening of the cell wall. Along with this, ZnO

Vol 13, No 1 (2025) DOI 10.5195/d3000.2025.1039

nanoparticles can stick to the surface of peptides, which breaks down the glycan structure of the bacterial cell wall [21,22]. The results of this observation are similar to those of earlier research [23], which showed how important the concentration of ZnO nanoparticles and the size of their particles are to antibacterial activity.

Numerous studies show that the antibacterial activity of ZnO nanoparticles is concentration-dependent. These nanoparticles are better at killing microbes because they have a larger surface area and are more reactive. The increased surface area results in an increase in the number of reactive sites that are available for interactions with bacterial cells. Some people also think that ZnO nanoparticles interact with the plasma membrane of bacteria, changing the surface chemistry and function of the membrane, lowering the amount of adenosine triphosphate (ATP), and messing up the cell's energy metabolism. In the end, these alterations have an effect on the stability of the bacterial cell membrane, which finally results in the membrane's disruption [24].

Some types of bacteria, like *Staphylococcus aureus*, Escherichia coli, and *Pseudomonas aeruginosa*, can be killed by zinc oxide nanoparticles (ZnO NPs). Researchers have found that 0.8%, 1%, and 1.5% of S. aureus bacteria survived at different concentrations of ZnO NPs; 1%, 1.5%, and 5% of P. aeruginosa bacteria survived; and 2%, 3.7%, and 6% of E. coli bacteria survived [25].

Researchers found that the presence of ZnO NPs was associated with these percentages. Compared to Gram-positive bacteria, which have thicker and more resistant cell walls, Gram-negative bacteria, such as E. coli, have thinner cell walls, which makes them more sensitive to metal nanoparticles. This is because Gram-negative bacteria are more susceptible to metal nanoparticles. Because of this structural difference, nanoparticles can more easily penetrate Gram-negative bacteria cells [26]. Nanoparticles can target various bacterial structures in addition to their action on the cell membrane [27]. These structures include respiratory chain dehydrogenases and bacterial chromosomes. It has also been shown that metallic nanoparticles like ZnO can make reactive oxygen species (ROS) that kill bacteria. These ROS are produced by creating oxidative stress and inhibiting the oxidation of metal ions that have been freed [28].

This research also indicates that pepper fruit extract possesses powerful antibacterial characteristics. Since phenolic chemicals allow the extract to penetrate bacteria cell walls, these properties may be linked to them. The findings of this study are consistent with those of other studies [29], which suggested that polyphenols are important bioactive chemicals that are responsible for antibacterial activity. There are hydroxyl groups or phenolic rings in these compounds that might interact with the proteins and membranes of bacteria. This could lead to the formation of complexes that stop the cell from working properly [30,31].

Researchers think that bioactive phytochemicals like flavonoids, alkaloids, and tannins, known to kill bacteria, make pepper extract bacteria-killing. These components are believed to be responsible for what makes pepper extract so effective. It has been observed in previous research [32, 33] that plant extracts abundant in phenolic compounds are particularly effective against bacteria because they disrupt their growth and metabolism. The active ingredients in pepper fruit extract, especially flavonoids and alkaloids, help stop the growth of bacteria. This is more proof that pepper fruits can be used as a natural antibacterial.

Conclusion

The conclusion is that ZnO nanoparticles and pepper fruit extract exhibit strong antibacterial effects. Microparticles of ZnO specifically target the cell walls and membranes of bacteria. On the other hand, bioactive chemicals like phenols, flavonoids, and alkaloids in pepper fruit extract make it work. These results show that ZnO nanoparticles and plant-based extracts may be useful in the fight against bacterial infections. They also offer a viable route for developing antimicrobial medicines based on natural materials and nanomaterials.

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Table 1. **Effect of different concentrations of ZnO nanoparticles on *S. mutans* and *S. aureus*.**

Concentrations mg/ml	*S. mutans*	*S. aureus*	Control
0.2	2.12	2.02	0
0.4	2.27	2.2	0
0.6	2.58	2.45	0
0.8	2.98	2.68	0
LSD =	1.50	1.25	

Table 2. **Effect of different concentrations of pepper fruit extract on *S. mutans* and *S. aureus*.**

Concentrations mg/ml	***S. mutans***	***S. auruas***	Control
100	1.24	1.35	0
200	1.46	1.56	0
300	1.54	1.78	0
350	1.7	1.89	0
LSD =	1.37	1.76	