RESEARCH PAPER

Antibacterial Effects of ZnO Nanoparticles and *Capsicum Annuum* Extract on *Staphylococcus Mutans* and *Staphylococcus Aureus* Isolated from Dental Cavities

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ARTICLE INFO

Article History:
Received 28 April 2025
Accepted 26 June 2025
Published 01 July 2025

Keywords:
Antibacterial activity
Capsicum annuum
Staphylococcus aureus
Streptococcus mutans
ZnO nanoparticles

ABSTRACT

The purpose of this study is to determine the antibacterial effects of different concentrations of zinc oxide (ZnO) nanoparticles and extract from *Capsicum annuum L.* (pepper fruit) on *Staphylococcus mutans (S. mutans)* and *Staphylococcus aureus (S. aureus)* isolates that were obtained from dental caries. ZnO nanoparticles were achieved with a size range of 10–30 nm and a purity level of 99.8 %, and pepper fruit extracts were made using the Soxhlet extraction method. Several different concentrations were used in the agar well diffusion method to evaluate the effectiveness of antibacterial agents. According to the findings, ZnO nanoparticles exhibited inhibitory zones ranging from 2.11 to 2.97 mm for *S. mutans* and 2.01 to 2.66 mm for *S. aureus*, with 1.0 mg/mL for the maximum activity. The antibacterial activity of products increased with increasing concentration of the extract, reaching its maximum inhibition at 300 mg/mL. The combination of ZnO nanoparticles and extract from *Capsicum annuum* demonstrated dose-dependent antibacterial activity, indicating that these substances can be used in therapeutic applications against oral infections.

How to cite this article
Alsewidi W., Alfahdawi I. Antibacterial Effects of ZnO Nanoparticles and Capsicum Annuum Extract on Staphylococcus Mutans and Staphylococcus Aureus Isolated from Dental Cavities. J Nanostruct, 2025; 15(3):1109-1114. DOI: 10.22052/JNS.2025.03.028

INTRODUCTION

The formation of biofilms and the growth of microorganisms such as *Streptococcus mutans* and *Staphylococcus aureus* are the main reasons for dental caries, making it one of the most widespread infectious diseases in the world. Dental caries is one of the most common oral contagious illnesses. It is caused by the demineralization of tooth enamel and dentin due to acids created by bacterial fermentation of dietary carbohydrates [1]. It is necessary to develop alternative antibacterial agents, particularly those derived from natural sources and nanotechnology, because of the increasing prevalence of antibiotic resistance [2]. *Streptococcus mutans* is believed to be the principal etiological agent among the important microbial agents involved. This assumption is based on its powerful acidogenic and aciduric capabilities and the capacity to manufacture extracellular polysaccharides that facilitate biofilm formation [3]. Meanwhile, *Staphylococcus aureus*, which has historically been associated with systemic infections, has been found in the oral cavity at high rate and is now recognized as a

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contributor for secondary caries and periodontal disease [4]. Zinc oxide (ZnO) nanoparticles have gained a lot of interest due to large surface area and strong antibacterial properties against many types of germs. Studies have indicated that ZnO nanoparticles are effective against both gram-positive and gram-negative bacteria, which makes them potential candidates for applications in the field of oral health [5]. Similarly, plant extracts, like *Capsicum annuum,* have been known for their healing and bacteria-fighting properties. The capacity of *Capsicum annuum* in degradation of bacterial membranes and interfere with quorum sensing has been demonstrated in a previous study [6]. Because of the biofilm-protected nature of dental plaque and the increasing prevalence of bacterial resistance towards traditional antibiotics, newly developed antimicrobial medicines must be produced [7]. Metal oxide nanoparticles, have emerged as potentially useful options due to high surface area-to-volume ratio, simple to functionalize, and distinctive modes of microbial suppression [8]. The safety profile, affordability, and extensive antibacterial efficacy against oral and systemic infections for ZnO nanoparticles have attracted the attention of scholars [9]. The rupture of bacterial cell membranes, the formation of reactive oxygen species (ROS), and interference with the enzymatic activities of microorganisms are all examples of the antibacterial mechanisms of ZnO nanoparticles [10]. Jones et al. reported that ZnO nanoparticles were highly efficient against gram-positive and gram-negative bacteria. The intensity of the nanoparticles' activity increased in proportion to their size [5]. Botanical extracts are natural antibacterial agents. Pepper, known as *Capsicum annuum*, with medicinal and spice benefits has a high concentration of bioactive chemicals, including capsaicin, flavonoids, and phenolic acids, all contributing to its antibacterial capabilities [11]. It has been demonstrated that *Capsicum annuum*, the primary component responsible for the intense flavor of chili peppers, may disrupt the process of bacterial cell wall construction, the formation of biofilms, and the expression of virulence factors [12]. Previous research has proven that pepper extracts have modest antibacterial properties against oral and gastrointestinal infections. The effectiveness of pepper extracts is frequently impacted by the extract's concentration and the solvent used during the extraction process [13]. Few studies have examined the antibacterial ability of ZnO nanoparticles and extracts of *Capsicum annuum*, especially against *S. mutans* and *S. aureus* produced from dental caries. This study evaluates and compares the antibacterial activity of ZnO nanoparticles and *Capsicum annuum* extract at various concentrations against *S. mutans* and *S. aureus* isolated from dental cavities.

MATERIALS AND METHODS

This investigation was conducted from January to April 2025 at the Department of Microbiology and Tissue Culture, College of Pharmacy, Mustansiriyah University, Baghdad, Iraq.

Collection and identification of bacterial isolates

Dental plaque and carious specimens were active dental caries at local dental clinics in Baghdad. The specimens were obtained with sterile cotton swabs and covered in sterile tubes containing nutritional broth. Upon arriving at the laboratory, samples were cultivated on selected media:

- Mitis Salivarius Agar for *Streptococcus mutans*
- Mannitol Salt Agar for *Staphylococcus aureus*

Plates were incubated at 37°C for 24 to 48 h under suitable conditions (CO_2-enriched atmosphere for *S. mutans*). Colonies were characterized using morphological assessment, Gram staining, catalase and coagulase testing (for *S. aureus*), and biochemical profiling. Verification was further accomplished with commercial identification kits (API Strep and API Staph kits from bioMérieux, France).

Preparation of ZnO nanoparticle suspensions

ZnO nanoparticles, which are 99.8% pure and have a size between 10 and 30 nm, were obtained from Sky Spring Nanomaterials (USA). The nanoparticles were measured using an analytical balance and dispersed in sterile distilled water via sonication to achieve homogenous suspensions at concentrations of 0.2, 0.6, 0.8, and 1.0 mg/mL. Before each experiment, we made all suspensions and stored them in amber bottles at 4°C to prevent deterioration.

Extraction of Capsicum annuum

We obtained fresh pepper fruits (Capsicum annuum L.) from local marketplaces in Baghdad.

The fruits were meticulously cleaned with distilled water to eliminate surface impurities, air-dried for five days at ambient temperature, and processed into a fine powder using an electric grinder.

Soxhlet extraction procedure

100 g of fruit powders were placed in a thimble, and Soxhlet equipment was used to extract the ethanol for 6 h. We concentrated the extract at diminished pressure using a rotary evaporator at 45°C. The concentrated extract was diluted in ethanol to yield 100, 200, 250, and 300 mg/mL. Extracts were filtered using 0.22 µm syringe filters and preserved in sterile, amber glass vials at 4°C.

Antibacterial assay

The antibacterial efficacy was assessed utilizing the agar-well diffusion technique. Each isolate was produced in sterile saline using a standardized inoculum (0.5 McFarland standard, about 1.5 × 10^8 CFU/mL). The bacterial suspensions were inoculated onto Mueller-Hinton agar plates using a sterilized cotton swab.

- Wells (6 mm in diameter) were created in the agar with a sterile cork borer.
- 100 µL of each ZnO suspension and *Capsicum annuum* extract was allocated into distinct wells.
- Positive control: Chlorhexidine (0.2%)
- Negative control: Sterile distilled water or ethanol, contingent upon the test group
- Plates were incubated at 37°C for 24 h. The inhibitory zones were quantified with a calibrated digital caliper.

Statistical analysis

All tests were duplicated, and the average inhibition zone and standard deviation were computed for each treatment. Statistical significance was assessed by one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for group mean comparisons. A p-value less

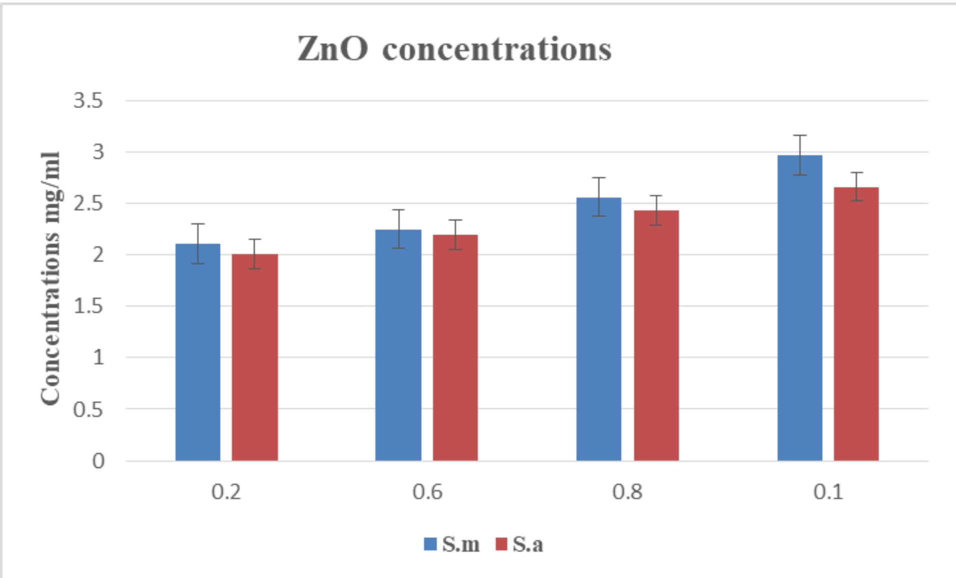

Fig. 1. Antibacterial activity of ZnO nanoparticles on *S. mutans* and *S. aureus*

Table 1. Inhibition zones (mm) of ZnO nanoparticles against *S. mutans* and *S. aureus*

Concentration (mg/mL)	*S. mutans* (mm)	*S. aureus* (mm)
0.2	2.11 ± 0.10	2.01 ± 0.11
0.6	2.25 ± 0.14	2.19 ± 0.10
0.8	2.56 ± 0.13	2.43 ± 0.12
1.0	2.97 ± 0.16	2.66 ± 0.14

than 0.05 was deemed statistically significant. Data were processed with IBM SPSS Statistics version 25.

RESULTS AND DISCUSSION

The antibacterial efficacy of ZnO nanoparticles and *Capsicum annuum* extract was assessed against *S. mutans and S. aureus* using the agar-well diffusion technique. The sizes of the inhibition zones were measured in millimeters, and the data are provided as the mean ± standard deviation from three repetitions.

Antibacterial activity of ZnO nanoparticles

ZnO nanoparticles exhibited a dose-dependent enhancement in antibacterial efficacy against both bacterial species. At the minimal dose (0.2 mg/mL), the inhibition zone for *S. mutans* measured 2.11 ± 0.10 mm, which incrementally reached 2.97 ± 0.16 mm at 1.0 mg/mL. The same pattern was seen against *S. aureus*, with inhibition zones ranging from 2.01 ± 0.11 mm to 2.66 ± 0.14 mm (Table 1, Fig. 1).

Antibacterial efficacy of Capsicum annuum extract

The minimum dose of 100 mg/mL for *S. mutans* yielded a mean inhibition zone of 1.22 ± 0.08 mm, which reached 1.69 ± 0.11 mm at 300 mg/mL. *S. aureus* exhibited marginally greater sensitivity, with inhibitory zones measuring between 1.34 ± 0.09 mm and 1.87 ± 0.12 mm (Table 2, Fig. 2).

The findings demonstrate that ZnO nanoparticles and *Capsicum annuum* extract display dose-dependent antibacterial properties against oral infections. ZnO nanoparticles demonstrated enhanced effectiveness, particularly at a concentration of 1.0 mg/mL, consistent with previous studies emphasizing the potent antibacterial properties of metal oxide nanoparticles attributed to oxidative stress and membrane disruption [8, 14]. Raghupathi et al. indicated that ZnO nanoparticles have greater efficacy against gram-positive bacteria owing to their robust peptidoglycan coating [15]. Sirelkhatim et al. also confirmed the significance of nanoparticle size in influencing antibacterial efficacy [16]. Furthermore, Rezaei et al. noted the

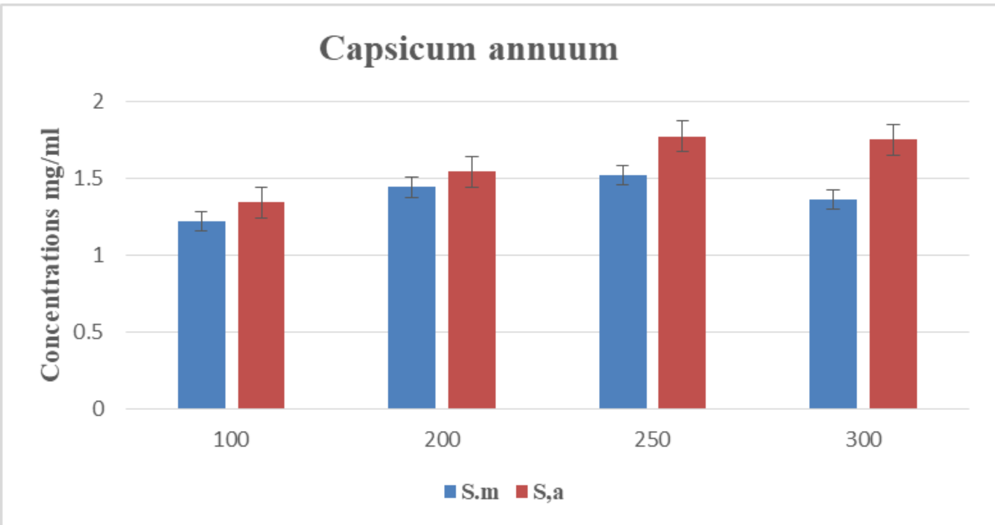

Fig. 2. Antibacterial activity of *Capsicum annuum* extract on *S. mutans* and *S. aureus*.

Table 2. Inhibition zones (mm) of *Capsicum annuum* extract against *S. mutans* and *S. aureus*

Concentration (mg/mL)	*S. mutans* (mm)	*S. aureus* (mm)
100	1.22 ± 0.08	1.34 ± 0.09
200	1.44 ± 0.10	1.54 ± 0.11
250	1.52 ± 0.12	1.77 ± 0.13
300	1.69 ± 0.11	1.87 ± 0.12

antimicrobial properties of *Capsicum extracts* on oral flora [17]. Nevertheless, the extract exhibited worse efficacy than ZnO nanoparticles, perhaps owing to restricted diffusion or a decreased concentration of active compounds. The enhanced efficacy of ZnO nanoparticles at a concentration of 1.0 mg/mL can be ascribed to many established processes. ZnO nanoparticles engage with bacterial cell walls, rupturing the membrane and leaking intracellular contents [18]. They also produce ROS agents like hydrogen peroxide, which provoke oxidative stress in bacterial cells, resulting in cell death [19]. Raghupathi et al. [15] showed that ZnO nanoparticles have superior antibacterial efficacy due to high surface reactivity and penetrating capacity. Sirelkhatim et al. [16] observed that ZnO nanoparticles have greater effectiveness against gram-positive bacteria like *S. aureus* due to variations in cell wall composition. The antibacterial efficacy of *Capsicum annuum* extract was dose-dependent but inferior to that of ZnO nanoparticles. The observed inhibitory zones exhibited the most pronounced inhibition against *S. aureus* at 300 mg/mL. It assumed that *Capsaicin*, the primary active component, destabilize bacterial membranes and interfere with intracellular signaling pathways, including quorum sensing [11]. Although both treatments exhibited activity against the bacterial strains, ZnO has shown superior efficacy against *S. mutans*. Conversely, *Capsicum annuum* had marginally greater efficiency against *S. aureus* at its maximum dosage. *S. mutans*, with greater acid tolerance and a propensity for biofilm formation, may be more vulnerable to oxidative stress generated by ZnO, whereas *S. aureus* may demonstrate high sensitivity towards the lipid-soluble characteristics of capsaicin and its membrane-targeting effects [16]. This study is the lack of combination therapies, which might provide synergistic effects. Prior research indicated that the amalgamation of nanoparticles with plant extracts can enhance antibacterial efficacy through synergistic mechanisms [15]. Investigations should examine this synergy, assess cytotoxicity in oral cellular models, and evaluate these agents in vivo for prospective applications in mouthwashes, toothpastes, or coatings for dental materials. Several bioactivities of *Capsicum annuum* extract presents molecular docking studies to substantiate its potential medicinal uses [20]. The potential of ZnO nanoparticles can formulate novel therapies for antibiotic-resistant bacterial infections [21].

CONCLUSION

This research revealed that ZnO nanoparticles and *Capsicum annuum* fruit extract possess notable antibacterial properties against *S. mutans* and *S. aureus* derived from dental caries. The antibacterial effectiveness of both agents depended on their concentration. ZnO nanoparticles at a 1.0 mg/mL concentration had the most potent antibacterial activity, particularly against *S. mutans*. This corroborates the growing evidence that nanoscale ZnO compromises bacterial cell integrity and metabolic functions *via* oxidative stress and direct membrane contact. *Capsicum annuum* extract exhibited quantifiable and incremental suppression of bacterial proliferation with rising doses, with the most pronounced impact observed at 300 mg/mL. The antibacterial properties are due to active phytochemicals, including capsaicin and flavonoids, which may disrupt bacterial viability and biofilm formation. The findings endorse the prospective application of both ZnO nanoparticles and *Capsicum annuum* extract as alternative agents in oral healthcare, namely in preventing and managing tooth caries and related infections.

ACKNOWLEDGMENTS

The author gratefully acknowledges the support of Al-Maarif University and the College of Dentistry.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

REFERENCES

1. Kidd E, Fejerskov O, Nyvad B. Infected dentine revisited. Dent Update. 2015;42(9):802-809.
2. Szul M. Causes Behind the Global Crisis of Increasing Antibiotic Resistance and Strategies to stop this. Polygence; 2024.
3. Loesche WJ. Role of Streptococcus mutans in human dental decay. Microbiol Rev. 1986;50(4):353-380.
4. Despotovic M, de Nies L, Busi SB, Wilmes P. Reservoirs of antimicrobial resistance in the context of One Health. Curr Opin Microbiol. 2023;73:102291.
5. Jones N, Ray B, Ranjit KT, Manna AC. Antibacterial activity of ZnO nanoparticle suspensions on a broad spectrum of microorganisms. FEMS Microbiol Lett. 2008;279(1):71-76.
6. Kaplan JB, Sukhishvili SA, Sailer M, Kridin K, Ramasubbu N. Aggregatibacter actinomycetemcomitans Dispersin B: The Quintessential Antibiofilm Enzyme. Pathogens.

2024;13(8):668.
7. Loyola-Rodriguez JP, Ponce-Diaz ME, Loyola-Leyva A, Garcia-Cortes JO, Medina-Solis CE, Contreras-Ramire AA, et al. Determination and identification of antibiotic-resistant oral streptococci isolated from active dental infections in adults. Acta Odontol Scand. 2017;76(4):229-235.
8. Rai M, Yadav A, Gade A. Silver nanoparticles as a new generation of antimicrobials. Biotechnol Adv. 2009;27(1):76-83.
9. Cheng L, Zhang K, Weir MD, Melo MAS, Zhou X, Xu HHK. Nanotechnology Strategies for Antibacterial and Remineralizing Composites and Adhesives to Tackle Dental Caries. Nanomedicine. 2015;10(4):627-641.
10. Premanathan M, Karthikeyan K, Jeyasubramanian K, Manivannan G. Selective toxicity of ZnO nanoparticles toward Gram-positive bacteria and cancer cells by apoptosis through lipid peroxidation. Nanomed Nanotechnol Biol Med. 2011;7(2):184-192.
11. TakÁCs-HÁJos M, Zsombik L. Total Polyphenol, Flavonoid and Other Bioactive Materials in Different Asparagus Cultivars. Notulae Botanicae Horti Agrobotanici Cluj-Napoca. 2015;43(1).
12. Zhou Y, Guan X, Zhu W, Liu Z, Wang X, Yu H, et al. Capsaicin inhibits Porphyromonas gingivalis growth, biofilm formation, gingivomucosal inflammatory cytokine secretion, and in vitro osteoclastogenesis. European Journal of Clinical Microbiology and Infectious Diseases. 2013;33(2):211-219.
13. Santos MMP, Vieira-da-Motta O, Vieira IJC, Braz-Filho R, Gonçalves PS, Maria EJ, et al. Antibacterial activity of Capsicum annuum extract and synthetic capsaicinoid derivatives against Streptococcus mutans. J Nat Med. 2011;66(2):354-356.
14. Zarrindokht E-K. Antibacterial activity of ZnO nanoparticle on Gram-positive and Gram-negative bacteria. Afr J Microbiol Res. 2012;5(18).
15. Raghupathi KR, Koodali RT, Manna AC. Size-Dependent Bacterial Growth Inhibition and Mechanism of Antibacterial Activity of Zinc Oxide Nanoparticles. Langmuir. 2011;27(7):4020-4028.
16. Sirelkhatim A, Mahmud S, Seeni A, Kaus NHM, Ann LC, Bakhori SKM, et al. Review on Zinc Oxide Nanoparticles: Antibacterial Activity and Toxicity Mechanism. Nano-Micro Letters. 2015;7(3):219-242.
17. Rezaei Z, Mohammadi S, Aghaei A, Pouragha H, Latifi A, Keshavarz-Mohammadi N. Assessment of risk factors for suicidal behavior: results from the Tehran University of Medical Sciences Employees' Cohort study. Frontiers in Public Health. 2023;11.
18. Lotha R, Shamprasad BR, Sundaramoorthy NS, Ganapathy R, Nagarajan S, Sivasubramanian A. Zero valent silver nanoparticles capped with capsaicinoids containing Capsicum annuum extract, exert potent anti-biofilm effect on food borne pathogen Staphylococcus aureus and curtail planktonic growth on a zebrafish infection model. Microb Pathog. 2018;124:291-300.
19. Khodaie L, Patel P, Deore S, Surana V, Byahatti V. Synergistic effects of plant extracts for antimicrobial therapy. Herbal Formulations, Phytochemistry and Pharmacognosy: Elsevier; 2024. p. 55-76.
20. Tunç T, Ozpinar H, Koçyiğit ÜM, Erkan S. Exploring the Antioxidant, Antimicrobial, Antidiabetic, and Anticancer Properties of Capsicum annuum L. (Samandağ Pepper): Biochemical and In Silico Insights. ChemistrySelect. 2024;9(48).
21. Makabenta JMV, Nabawy A, Li C-H, Schmidt-Malan S, Patel R, Rotello VM. Nanomaterial-based therapeutics for antibiotic-resistant bacterial infections. Nature Reviews Microbiology. 2020;19(1):23-36.