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ORIGINAL RESEARCH

Combination of zinc oxide nanoparticles with *Syzygium aromaticum* and *Acacia nilotica* extracts as effective agents against common pathogens in dental applications

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சுருக்கம்:
ஸிங்க் ஆக்சைடு நானோ துகள்களின் (ZnO NPs) நுண்ணுயிர் எதிர்ப்பு திறன், மருத்துவ தாவர சாறுகளான *Syzygium aromaticum* (கிராம்பு) மற்றும் *Acacia nilotica* (கருவேல மரம்) ஆகியவற்றுடன் இணைந்து, வாயில் காணப்படும் பொதுவான நோய்க்கிருமிகளுக்கு எதிராக ஆய்வு செய்யப்பட்டது. ZnO NPs, வீழ்படிவு முறை மூலம் தயாரிக்கப்பட்டு, UV-Vis நிறமாலைமானி, FTIR மற்றும் டைனமிக் லைட் ஸ்கேட்டரிங் (DLS) ஆகியவற்றைக் கொண்டு இயல்பு கண்டறியப்பட்டது. ZnO NPs, *Syzygium aromaticum* மற்றும் *Acacia nilotica* சாறுகள் மற்றும் அவற்றின் கலவைகளின் நுண்ணுயிர் எதிர்ப்பு செயல்திறன், *Escherichia coli* (E. coli) மற்றும் *Staphylococcus aureus* (S. aureus) ஆகியவற்றுக்கு எதிராக காலனி எண்ணிக்கை முறை மூலம் மதிப்பீடு செய்யப்பட்டது. ZnO NPs உடன் *Syzygium aromaticum* மற்றும் *Acacia nilotica* சாறுகளின் கலவை சிறந்த கிருமி அழிப்பு விளைவை வெளிப்படுத்தியது; குறிப்பாக 1 mg/ml வரை *E. coli* மற்றும் *S. aureus* வளர்ச்சியை முழுமையாகத் தடுத்தது கண்டறியப்பட்டது. இந்த ஆய்வு, தாவர சாறுகள் மற்றும் ZnO NPs ஆகியவற்றின் தனித்தனி மற்றும் கலந்த சிகிச்சைகளின் முக்கியத்துவத்தை எடுத்துக்காட்டுகிறது. ZnO NPs மற்றும் தாவர சாறுகளின் கலவை, வாயில் காணப்படும் பொதுவான பாக்டீரியாக்களுக்கு எதிரான திறனுள்ள காரணிகளாக செயல்படுகின்றன என்று இந்த ஆய்வு பரிந்துரைக்கிறது.

Abstract:
Antibacterial efficacy of zinc oxide nanoparticles (ZnO NPs) combined with medicinal plant extracts *Syzygium aromaticum* and *Acacia nilotica* was studied against common oral pathogens. ZnO NPs were prepared by the precipitation method and characterized using a UV-Vis spectrophotometer, FT-IR, and dynamic light scattering (DLS). The antibacterial activity of ZnO NPs, *S. aromaticum*, and *A. nilotica* extracts and their combinations was assessed by the colony counting method against *Escherichia coli (E. coli)* and *Staphylococcus aureus (S. aureus)*. The combination of ZnO NPs with *S. aromaticum* and *A. nilotica* extracts shows a better bactericidal effect, with complete inhibition of *E. coli* and *S. aureus* growth observed up to 1 mg/ml. This study has shown the importance of combinations and individual treatments of plant extracts and ZnO NPs. It suggests a combination of ZnO NPs and plant extracts acts as effective agents against common oral bacteria.

Keywords: zinc oxide nanoparticles, *Syzygium aromaticum*, *Acacia nilotica*, characterization, antibacterial activity

Introduction

In recent days, one of the major global health concerns is oral infection, and it affects billions of people across all age groups. The World Health Organization (WHO) reports that oral infection is ranked as the most prevalent health problem worldwide. The overuse of antimicrobial agents develops a multidrug-resistant organism (MDR), and its treatment is highly challenging due to biofilm-mediated pathogen resistance. This determinant indicates the need for development of novel and targeted therapeutic strategies (1). Different common pathogens can cause disrupted, major

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alterations in the oral environment, such as changes in pH, dietary habits, poor oral hygiene, and the use of orthodontic appliances, which can disrupt this equilibrium and lead to the overgrowth of pathogenic species, infection, and oral health complications (2). Common oral microbes include *Enterococcus faecalis (E. faecalis), Staphylococcus aureus (S. aureus), Escherichia coli (E. coli), and Candida albicans (C. albicans)* (3, 4). Recent progress has explored the integration of antimicrobial agents into dental materials against the challenges posed by such resilient pathogens. Many studies have focused on incorporating nanoparticles into resin-modified glass ionomer cements, wires, bands, and orthodontic adhesives and elastomerics, as well as coating orthodontic brackets (5).

Many studies have demonstrated that nanoparticles can specifically inhibit the formation and proliferation of microbial biofilms (5). Metal-based nanoparticles have developed as potent antimicrobial agents due to their unique physicochemical properties and higher reactivity (6). Zinc oxide nanoparticles (ZnO NPs) are widely investigated for their antimicrobial properties, which represent a key reason for their growing application. It has various properties like thermal stability, robustness, chemical properties, long shelf life, and antimicrobial activity (7). It can be synthesized through many approaches, including physical, chemical, and biological (green chemistry) methods (8, 9). ZnO NPs have found applications across multiple branches due to their versatile properties like dentistry, including endodontics, regenerative endodontics, oral medicine, restorative dentistry, periodontics, prosthodontics, orthodontics, cancer diagnostics, and dental implantology (10, 11). In addition to nanoparticles, natural products such as medicinal plants have also gained attention for their antimicrobial potential (12). Many plant-derived substances have been used to maintain oral hygiene and treat oral diseases (13). Medicinal plant extracts are rich in bioactive compounds, many of which possess potent antibacterial, antifungal, and antiinflammatory properties. These natural agents offer a sustainable, biocompatible alternative to conventional antimicrobial therapies (14). *Syzygium aromaticum* (clove) is a conventional medicinal spice, and it has antiseptic and analgesic properties to treat various infections, including oral infections. It exhibits broad-spectrum antimicrobial and antiinflammatory effects due to the presence of the bioactive compound eugenol. *S. aromaticum* extract is highly effective against pathogens in endodontic infections, periodontal diseases, and dental caries. It disrupts the bacterial membranes and inhibits enzymes to kill the bacteria (15). Babul tree (*Acacia nilotica*) has different therapeutic properties, such as antimicrobial, antiplasmodial, and antioxidant activities. Many traditional plant extracts have been evaluated to find their therapeutic application. The last few studies have reported the antibacterial efficacy of *A. nilotica and S. aromaticum* plant extracts against oral cavities (16). Therefore, the current study was designed to evaluate and compare the antibacterial efficacy of ZnO NPs in combination with plant extracts, specifically *A. nilotica* and *S. aromaticum*, against common oral pathogens. This study is based on the hypothesis of combining ZnO NPs with phytochemicals present in the medicinal plants and providing a synergistic antimicrobial effect to enhance overall efficacy while minimizing the risk of antimicrobial resistance.

Methodology

Zinc oxide nanoparticles synthesis

0.5 M of zinc acetate was weighed and dissolved in 100 ml of distilled H_2O kept under magnetic stirrer. 100 ml (2 M) of NaOH was added drop wise under stirring. The formation of precipitation indicated the presence of ZnO NPs and the solution was kept under stirring for 2 h. It was transferred to a crucible and kept in a muffle furnace for calcination (300°C for 1 h). The collected powder was stored for the further use (17).

Plant extracts preparation

Syzgium aromaticum (S. aromaticum)

S. aromaticum was collected from the local market, Chengalpattu, India, and grinned to get a coarse powder. 0.5 g of *S. aromaticum* powder was mixed in 20 ml of distilled H_2O and kept in a boiling water bath for 20 min. After cooling, it was filtered using Whatman filter paper and stored in an air-tight container in the refrigerator (18).

Acacia nilotica (A. nilotica)

A. nilotica stem was collected from a local area near Chengalpattu, India, and dried for 1 week at ambient temperature. It was then grated roughly to get a coarse powder. 0.5 g of *A. nilotica* powder was mixed in 20 ml of distilled H_2O and kept in a boiling water bath for 20 min. After cooling, extract was filtered using Whatman filter paper and stored in an air-tight container in the refrigerator (19).

Antibacterial activity

Colony counting using the spread plate method was used to evaluate antibacterial activity. Serial dilution (up to 10^{-5}) of *S. aureus*-ATCC 25923 *and E. coli*-ATCC 25922 in nutrient broth was used. ZnO NPs and plant extracts (*A. nilotica* and *S. aromaticum*) at different concentrations of 100, 50, 25, 5, 2.5, 1, 0.5, and 0.1 mg/ml were prepared. 50 µl of each concentration was mixed with broth containing bacterial cultures. To evaluate antibacterial activity, a combination of ZnO NPs with plant extracts in the ratio of 1:1 was prepared and inoculated similarly. Samples were incubated overnight at 37°C, subjected to dilution

up to 10^{-3}, the spread plate technique was performed, and the number of colonies was counted after overnight incubation (20).

Results and discussion

Characterization of ZnO NPs

Confirmation of the synthesized ZnO NPs was exhibited by the blue-shifted absorption maximum wavelength at 378 nm (21), shown in **Figure 1a**. **Figure 1b** shows the Fourier Transform Infrared Spectroscopy (FT-IR) spectrum of synthesized ZnO NPs, and the peaks at 1635 and 1420 cm^{-1} (22) were symmetric and asymmetric. Hydroxyl group stretching can be seen at the adsorption peak of 3377 cm^{-1} (23). The functional group of the ZnO NPs was also mentioned in **Table 1**. The size of the ZnO NPs was measured using the dynamic light scattering (DLS) analysis, and the hydrodynamic diameter of 220 nm, on average, is shown in **Figure 1c**. The zeta potential of chemically synthesized ZnO NPs with the adverse value of about −11 mV indicates a moderate colloidal stability (**Figure 1d**) (24).

Antibacterial activity by colony counting method

Antibacterial activity of ZnO NPs, A. nilotica extract, and S. aromaticum extract

Antibacterial efficacy of ZnO NPs, *A. nilotica* extract, and *S. aromaticum* extract was assessed against two common oral pathogens, *E. coli* and *S. aureus*. The results demonstrated a concentration-dependent inhibitory effect for all tested agents and were mentioned in **Table 2**. ZnO NPs exhibited significant antibacterial activity against both bacterial strains, with complete inhibition of colony formation observed from 100 mg/ml down to 5 mg/ml for *E. coli*. Few colonies appearing at 1 mg/ml (13 ± 0.05 colonies), and a gradual increase was noted at lower concentrations (91 ± 0.02 and 130 ± 0.08 colonies at 0.5 and 0.1 mg/ml, respectively). In contrast, *S. aureus* showed higher susceptibility to ZnO NPs, with no visible colony growth up to 1 mg/ml. At 0.5 and 0.1 mg/ml,

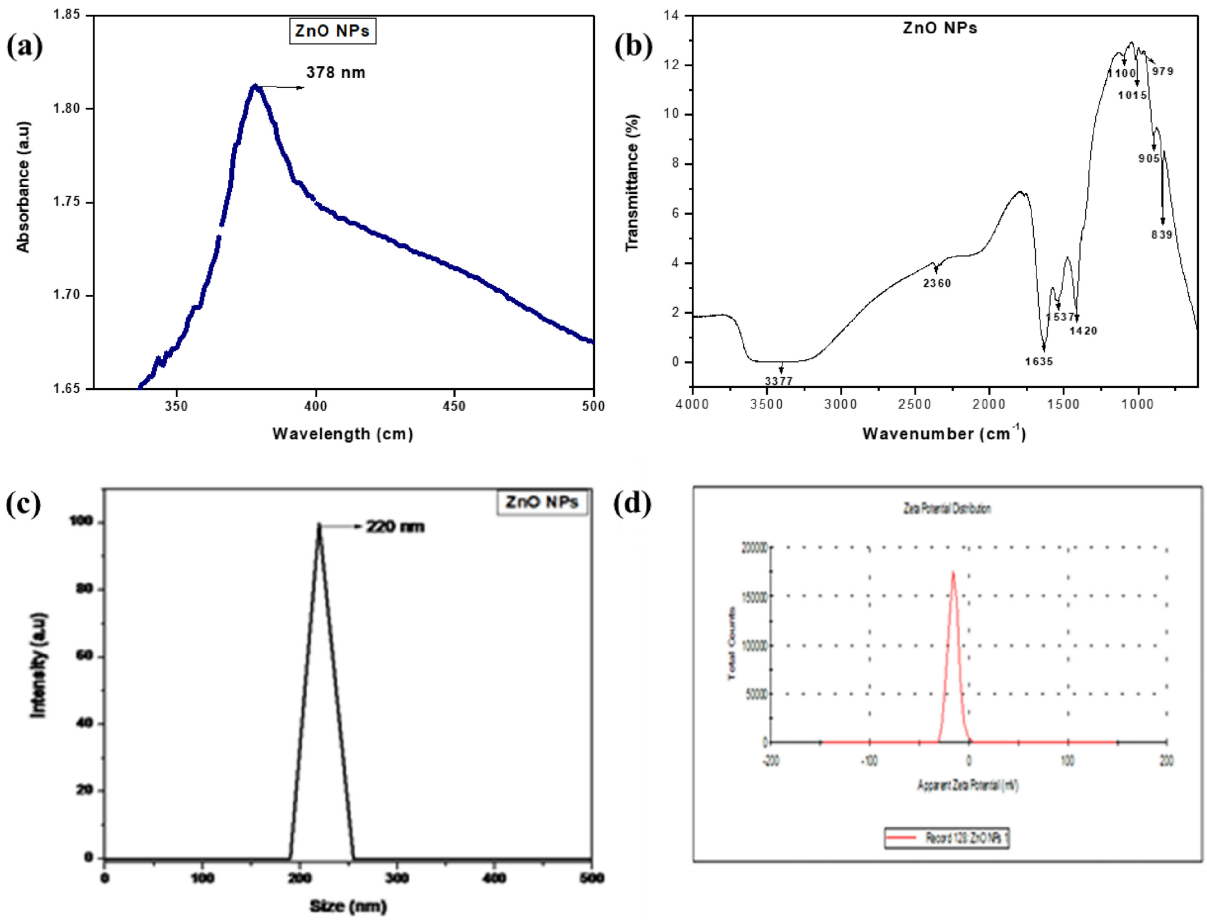

FIGURE 1 | (a) UV-visible spectrum, (b) Fourier Transform Infrared Spectroscopy (FT-IR) spectrum, (c) size, and (d) charge of zinc oxide nanoparticles (ZnO NPs).

TABLE 1 | Wavelength and functional group of ZnO NPs.

S. no	Wavelength	Bond	Functional group	References
1	3377	N-H stretching	Aliphatic primary amine	(25)
2	2360	C=C conjugated and C≡C	Amides	(26)
3	1635	C=C stretching	Alkene	(27)
4	1537	N-O stretching	Nitro compound	(28)
5	1420	C=C stretching	Aromatic	(29)
6	1100	C-O stretching	Alcohol	(30)
7	1015	C-O stretching	Alcohol	(30)
8	975	C=C bending	Alkene	(28)
9	839	C=C bending	Alkene	(28)

TABLE 2 | Antibacterial activity of ZnO NPs, *A. nilotica* extract, and *S. aromaticum* against *E. coli and S. aureus*.

S. no	Concentration (mg/ml)	ZnO NPs		*A. nilotica*		*S. aromaticum*	
		E. coli	*S. aureus*	*E. coli*	*S. aureus*	*E. coli*	*S. aureus*
1	100	0	0	0	0	0	0
2	50	0	0	0	0	0	0
3	25	0	0	0	0	0	0
4	5	0	0	72 ± 0.1	30 ± 0.09	32 ± 0.02	0
5	1	13 ± 0.05	0	102 ± 0.03	76 ± 0.02	85 ± 0.03	13 ± 0.01
6	0.5	91 ± 0.02	10 ± 0.03	183 ± 0.01	143 ± 0.04	160 ± 0.03	107 ± 0.6
7	0.1	130 ± 0.08	35 ± 0.06	277 ± 0.08	220 ± 0.04	197 ± 0.03	152 ± 0.01
8	Negative control	Numerous	Numerous	Numerous	Numerous	Numerous	Numerous
9	Positive control	0 ± 0.1	0 ± 0.02	0 ± 0.07	0 ± 0.02	0 ± 0.05	0 ± 0.01

only 10 ± 0.03 and 35 ± 0.06 colonies were observed, respectively, indicating a more pronounced bactericidal effect on *S. aureus*.

A. *nilotica* extracts are also showing better inhibitory effects against *E. coli and S. aureus*. Number of colonies was observed at 5 mg/ml (72 ± 0.1 colonies) against *E. coli*; bacterial colony growth was increased at lower concentrations. A similar flow was observed in *S. aureus*, but with some colonies at each concentration, offering better antibacterial efficacy against *S. aureus*. In the case of *S. aromaticum* extract, it shows better activity up to 25 mg/ml, with no bacterial growth observed for *E. coli*. The bacterial growth was observed at 5 mg/ml (32 ± 0.02 colonies) and increased with further dilution. For *S. aureus*, *S. aromaticum* extract inhibited growth up to 5 mg/ml and showed a more generous antibacterial effect than *E. coli*, especially at 1 and 0.5 mg/ml concentrations.

When compared with all the groups, untreated control samples have shown high bacterial colony growth against both the bacteria. The study resulted in ZnO NPs having the most potent antibacterial activity, followed by *S. aromaticum* extract and then *A. nilotica*, with all three agents showing better effectiveness against *S. aureus* than *E. coli*.

Synergistic antibacterial Activity of ZnO NPs in combination with A. nilotica extract and S. aromaticum extract

The antibacterial effects of ZnO NPs combined with *A. nilotica* and *S. aromaticum* extracts were assessed against *E. coli* and *S. aureus*, and the results are illustrated in **Table 3**. The combination of ZnO NPs and *A. nilotica* demonstrated significantly enhanced antibacterial activity, with complete inhibition of *E. coli* growth observed up to 5 mg/ml.

Minimal bacterial growth was observed, with no colony formation up to 1 mg/ml, with a slight increase at lower concentrations (20 ± 0.01 colonies at 0.5 mg/ml and 53 ± 0.02 colonies at 0.1 mg/ml). A similar inhibitory effect was studied against *S. aureus*; in the concentration of 1 mg/ml, zero colonies were observed, and for 0.5 and 0.1 mg/ml, 6 ± 0.03 and 23 ± 0.01 colonies were observed, respectively. This indicates highly conclusive antibacterial activity in combinations against *S. aureus* and is consistent with trends observed in individual agent tests.

The combination of ZnO NPs with *S. aromaticum* extract showed a high bactericidal effect. The concentration of 1 mg/ml mentioned complete inhibition of *E. coli*. At the concentration of 0.5 mg/ml, 5 ± 0.01 colonies were observed,

TABLE 3 | Antibacterial activity of *A. nilotica, S. aromaticum*, and ZnO combination against *E. coli and S. aureus*.

S. no	Concentration (mg/ml)	ZnO NPs + *A. nilotica*		ZnO NPs + *S. aromaticum*	
		E. coli	*S. aureus*	*E. coli*	*S. aureus*
1	100	0	0	0	0
2	50	0	0	0	0
3	25	0	0	0	0
4	5	0	0	0	0
5	1	0	0	0	0
6	0.5	20 ± 0.01	6 ± 0.03	5 ± 0.01	3 ± 0.01
7	0.1	53 ± 0.02	23 ± 0.01	85 ± 0.03	40 ± 0.02
8	Negative control	Numerous	Numerous	Numerous	Numerous
9	Positive control	0 ± 0.06	0 ± 0.04	0 ± 0.1	0 ± 0.05

and for 0.1 mg/ml, 85 ± 0.03 colonies were observed. Similar results were noted against *S. aureus*, where total inhibition occurred at 1 mg/ml. At 0.5 mg/ml, colony formation of 3 ± 0.01 colonies was observed, and moderate growth of 40 ± 0.02 colonies was recorded at 0.1 mg/ml. These results indicate that both combinations generated nearly double the bactericidal effect compared to the individual agents used alone.

The improved antibacterial action may be explained by a synergistic relationship between ZnO NPs and the active phytochemicals present in *A. nilotica* and *S. aromaticum*. The nanoparticles may enhance the delivery and stability of plant-derived compounds, thereby promoting greater damage to bacterial cell membranes and interfering with essential cellular activities. The stronger effect observed against *S. aureus* could be related to its Gram-positive cell wall structure, which is generally more accessible to antimicrobial agents. The outer membrane of Gram-negative *E. coli* may limit the penetration of active compounds, resulting in reduced susceptibility at lower concentrations.

The untreated control groups showed the highest bacterial growth, confirming the significant antimicrobial efficacy of the combined treatments. Overall, these findings suggest that the integration of ZnO nanoparticles with medicinal plant extracts produces a strong synergistic antibacterial effect and may offer promising applications in the development of advanced antimicrobial and oral care formulations.

Conclusion

This study finds the bactericidal effects of ZnO NPs, plant extracts, and their combination against common oral pathogens. Different concentrations of ZnO NPs, *S. aromaticum* extract, *A. nilotica* extracts, and their combinations were studied against *E. coli* and *S. aureus*. At the concentration of 5 mg/ml complete inhibition of bacterial growth in ZnO NPs was observed. Plant extracts also attained complete reduction of *E. coli* and *S. aureus* up to 25 mg/ml concentration, representing a lesser antibacterial effect compared to ZnO NPs.

In the case of the combination of ZnO NPs with *A. nilotica*, the antibacterial activity was showed to be noticeably higher. The complete inhibition was observed at a low concentration of 1 mg/ml, and the related effect was observed for ZnO NPs with *S. aromaticum* combination, which also fully inhibited both pathogens at 1 mg/ml. These results highlight the valuable interaction of ZnO NPs and plant extracts, showing the combined formulation has a strong bactericidal effect against oral pathogens.

Such synergistic combinations have possible applications in the dental field, reducing plaque; controlling periodontal infections, including preventing dental caries; inhibiting biofilms; and aiding post-surgical healing. When the treatment of ZnO NPs is less effective alone, the combination with plant extracts helps to overcome bacterial resistance. Overall, the combination of ZnO NPs with plant extracts presents a promising and versatile approach for improving oral health and managing dental diseases.

Ethics committee/institute for this study

Not applicable

Ethics statement

Not applicable

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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