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Resarch Article

Antibacterial Activity of Synthesized ZnO Nanoparticles in Combination with *Zataria multiflora* Essential Oil against Oral Cavity *Streptococcus mutans*

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ABSTRACT

The use of various nanoparticles and plant-based antibacterial substances can be a suitable alternative to conventional mouthwashes. The aim of this study was to investigate the antimicrobial effect of synthesized ZnO nanoparticles in combination with *Zataria multiflora* essential oil against *Streptococcus mutans.* ZnO-NPs were synthesized by zinc acetate and potassium hydroxide and their characteristics are evaluated by FE-SEM and XRD. *Z. multiflora* essential oil is extracted and its chemical compounds are analyzed by GC–MS. Antibacterial activity of essential oil and nanoparticles was studied by broth microdilution method against standard and clinical strains of *S. mutans* and was determined minimum inhibitory concentration (MIC). The antibacterial interactions of these materials are tested by checkerboard titration. The results showed that ZnO-NPs were spherical and were synthesized with an average diameter of 23.77nm. MIC of ZnO-NPs against *S. mutans* strains was reported in the range of 12.5-50 µg/ml, and the MIC of *Z. multiflora* essential oil was in the range of 0.031-0.25 µl/ml. It was observed that ZnO nanoparticles with essential oil of *Z. multiflora* have synergistic effects against *S. mutans* and can be a suitable alternative to mouthwashes such as chlorhexidine.

1. Introduction

Dental caries and periodontal problems are among the prevalent oral diseases throughout the world. *Streptococcus mutans* (*S. mutans*) is one of the main bacterial species responsible for tooth decay (Ismail et al., 2013). It is the most common bacterium found in cariogenic plaque, which produces short-chain organic acids that metabolize sucrose and lead to the synthesis of extracellular polysaccharides, promoting bacterial adhesion to dental surfaces and reinforcing the biofilm (Alejandra et al., 2020). The use of adjunctive methods such as mouthwashes has been shown to be effective for prevention of plaque accumulation (Motamedifar et al., 2016). Several methods have been used to prevent the growth of biofilms in resin composite samples using chlorhexidine which successfully inhibits microbial growth

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(Peedikayil et al., 2016); however, since chlorhexidine has a high solubility, it has a short-term effect on biofilms. Routine mouthwash like chlorhexidine, on the other hand, are associated with the disadvantages including enamel staining, taste disturbances and mucosal irritation (Thomas et al., 2016). Therefore, searching for an alternative antimicrobial agent with minimal side effects seems to be quite reasonable.

Nanotechnology has been introduced to the field of dental materials in recent years and nanoparticles have been inserted into the structure of the dental composites and disinfection (Davari et al., 2019). The antibacterial properties of metal ions depend on their surface contact area. Decreased size of nanoparticles (<100 nm in diameter) leads to increased surface area and thus increased interaction with organic and inorganic molecules (Amini Nia et ai., 2020). However, many of the properties of metal nanoparticles are still unknown solutions (Mirhosseini et al., 2019). Zinc oxide (ZnO) nanoparticles have long been used in medicine because of their bactericidal and bacteriostatic effects (Sadeghi, 2018). Zinc Oxide nanoparticles (ZnO-NPs) have a broad range of uses in the biomedical sciences, including antibacterial effects, treatment of burns, and targeted drug delivery. The size and concentration of ZnO-NPs play an important role in the antibacterial activity solutions (Almoudi et al., 2018).

Zataria multiflora Boiss has a place in the *Laminaceae* family, which grows wild in the central part of Iran and is one of Iran's endemic plants (Mahboubi & Bidgoli, 2010). This plant contains a large amount of phenolic compounds, flavonoids and antioxidant activity (Dini et al., 2022).The antibacterial activity of *Z. multiflora* has been shown against a number of Gram-positive and Gram-negative bacteria (Aghili et al., 2015; Agahi et al., 2022). This plant has positive effects in controlling some microbial diseases because of its antibacterial, antifungal, and anti-inflammatory properties, as well as its immunostimulation activity in humans and in some animal models (Shokri et al., 2006).

Investigating the antibacterial effects of medicinal plants in order to reduce the oral flora and as a result, making and using mouthwashes with minimal unwanted effects and using natural resources of the country can be of particular importance (Milho et al.,2021). On the other hand, the use of metal nanoparticles as a new mouthwash can act as an effective tool in plaque control and a suitable replacement for conventional mouthwashes. (Galvão et al., 2012). The Combining drugs with each other can lead to a greater inhibitory effect than the sum of the effects of each of these substances alone, and reduce the dosage of one or both substances (Biavatti, 2009). The aim of the present study was to determine the antimicrobial effect of synthesized ZnO nanoparticles and their combination with *Z. multiflora* essential oil against oral cavity *S. mutans* bacteria and comparing the results with the effect of chlorhexidine mouthwash.

2. Materials and Methods

2.1. ZnO Nanoparticles synthesis:

To prepare zinc oxide nanoparticles, 0.7 g of zinc acetate ($Zn(CH_3COO)_2(H_2O)_2$) was dissolved in 30 ml of methanol (CH3OH) in a two-portion balloon (solution 1). Potassium hydroxide solution was dissolved by magnetic stirring by adding 0.3 g of potassium hydroxide (KOH) in 10 ml of methanol and transferred to the decanter (solution 2). Solution 2 is slowly added to solution 1 for 30 minutes under stirring conditions. This reaction continued for 5 hours at 60° C under reflux conditions. The resulting white precipitates were centrifuged at 8000 rpm for 15 minutes and washed with methanol to remove impurities. Washing and centrifugation was repeated 3 times in the mentioned conditions. The resulting white precipitates were placed in an oven at 50°C for 24 hours to completely remove moisture and obtain a white powder of zinc oxide nanoparticles.

2.2. Techniques for the confirmation of ZnO-NPs synthesis:

To study the crystal structures, morphology, and size of ZnO-NPs. Field Emission Scanning Electron Microscopy (FE-SEM) (SIGMA VP-500, ZEISS) was used. Energy-Dispersive X-ray Spectroscopy (EDS) (detector made by Oxford UK) was used to detect the elemental composition of certain zones to map out the distribution of elements in the sample from the imaged area. X-Ray Diffraction (XRD) analysis (X`Pert Pro, Panalytical) recognizes the type of

crystallographic structure of nanoparticles, chemical composition and phase detection of ZnO-NPs. In this study, synthesized nanoparticles with CuKa coating and θ2 angle in the range of 0 to 100 degrees were evaluated.

2.3. Plant Material:

The branches of *Zataria multiflora* plant were collected from Shiraz, Iran, and the taxonomic identification of plant materials was confirmed by a botanist. A voucher specimen of the plant was deposited at the herbarium of the Islamic Azad University, Varamin-Pishva Branch under number HVP-310.

2.4. Essential Oil Preparation:

200 g of dried aerial parts were extracted for 3h by Clevenger's apparatus and dehydrated by anhydrous sodium sulfate. The sample stored in a dark closed container, away from light and at 4°C. The GC–MS analyses were performed using an Agilent technologies 7890A gas chromatograph equipped with an HP5MS capillary column (30 m, 0.25 mm, 0.25µm), and a mass detector MS 5975C VL MSD was operated in the EI mode. The identification of components was based on the comparison of their mass spectra with those of WILEY and NIST Libraries as well as on the comparison of their retention indices of the authentic standard with the literature (Adams, 2007).

2.5. Bacterial Strains:

In this study, 30 samples of dental caries of affected individuals were separated using sterile swabs for each individual and each was placed in 1 ml of transport medium containing PBS at pH = 7.2 and transferred to the laboratory. To isolate *Streptococcus mutans*, each of the samples was inoculated on a solid medium plate and heated for 72 hours at 37°C in 10% CO2. Identification based on standard tests such as colony shape, gram staining, catalase activity, and growth in 6.5% NaCl Broth, esculin hydrolysis, hemolytic activity and breakdown of lactose-mannitol-sorbitol-raffinose-inulin sugars, reactions in litmus milk, VP test and urease were performed. Standard strain of *Streptococcus mutans* (ATCC35668) were purchased from the institute of standard and industrial research of Iran and were grown overnight on Mueller-Hinton agar (Merck, Germany) plates at 37°C before use. After identification tests, 10 clinical strains and one standard strain of *S. mutans* ATCC 35668 were selected for further study.

2.6. Antibiotic Susceptibility Test:

Sensitivity of *Streptococcus mutans* isolates to antibiotics was determined using disk diffusion method (Bauer-Kirby) according to CLSI instructions. All culture media used were prepared by the German company Merck. The susceptibilities of 5 antibiotics Tetracycline (30µg), Erythromycin (15µg), Cefotaxime (30µg), Clindomycin (2µg), Chloramphenicol (30µg), (Padtan-Teb Co., Iran) were characterized by agar diffusion, according to CLSI (2019).

2.7. Determination of Minimum Inhibitory Concentrations:

The minimum inhibitory concentration (MIC) values of ZnO-NPs ad essential oil were determined by broth microdilution assay. The ZnO-NPs were serially diluted twofold with deionized water in concentrations ranging from 0.78-1600 µg/mL. The essential oil was serially diluted two-fold with 4% DMSO (Merck, Germany) containing 0.015-32 µl/mL of essential oil. After shaking, 100 mL of diluted ZnO-NPs/essential oil was added to each well of 96-well microtiter plates. Muller-Hinton broth (Merck, Germany) was used as the broth medium. Microbial suspensions were adjusted to 0.5 MacFarland and diluted to 1×10^6 cfu/ml, and then 100mL of the suspension was added to each well and incubated at 37°C for 24 hours. MIC values were determined as the lowest concentration of compound that inhibited bacteria after 24 hours. Negative and positive controls in this experiment were medium + ZnO-NPs/ essential oil well and medium + bacterial suspension well, respectively (Jahan peymay sabet et al., 2022; Agahi et al., 2022).

2.8. Determination of Minimum Bactericidal Concentrations:

After MIC determination, 50µL from all wells that showed no bacterial growth on

Mueller-Hinton agar plates were incubated at 37°C for 24 hours. The minimal bacterial concentration (MBC) endpoint was defined as the lowest concentration of antimicrobial agent that killed 100% of the initial bacterial population. MIC and MBC of chlorhexidine 0.2% (Shahr Daru Pharmaceutical Company) against *S. mutans* strains in dilutions of 0.25-512 µl/mL was carried out by the above method.

2.9. Checkerboard Microtiter Assay:

Eight serial, two-fold dilutions of ZnO-NPs and essential oil were prepared and used in the MIC tests. 50 µL of each dilution of oil essential was vertically added to the wells of the 96-well microtiter plates, and 50µL of ZnO-NPs dilution was added horizontally to the wells of the 96-well microtiter plates. 100µL of microbial suspension (1×10^6 cfu/ml) was added to each well and incubated at 37°C for 24 hours. Fractional inhibitory concentrations (FICs) were calculated using the MIC of the combination of ZnO-NPs and essential oil by the MIC of ZnO -NPs or essential oil alone. The interaction between the two antimicrobial agents was estimated by calculating the fractional inhibitory concentration (FIC) indices (FICI) using the following formula 1, 2:

$$(1)\ \text{FIC} = \frac{\text{MIC of the essential oil or nanoparticles in combination}}{\text{MIC of the essential oil or nanoparticles alone}}$$

(2) FICI = FIC of the essential oil + FIC of nanoparticles

The interpretation of the FIC results, according to the accepted criteria, is as follows: if the FIC index is $\leq$ 0.5, the combination is interpreted as being synergistic; if the FIC index > 0.5 and $\leq$ 1.0, the combination is interpreted as additive; if the FIC index is between 1 and 4, the combination is interpreted as indifferent; and if the FIC index is > 4, the combination is interpreted as antagonistic (Schwalbe et al., 2007).

2.10. Statistical Analysis:

All experiments were performed in triplicate. The data were analyzed with the descriptive statistics, Mann–Whitney *U* test using the IBM Statistical Package for Social Services (SPSS) version 21.0 (IBM, Armonk, NY, USA). A *p* value <0.05 was considered statistically significant.

3. Results

3.1. Chemical Composition of Zataria multiflora essential oil:

The wide variety of chemical compositions of the *Z. multiflora* essential oil, are presented in Tables 1, and the GC-MS chromatograms are shown in Figures 1. Thymol (33.336%) and Carvacrol (23.699) was the major component of followed respectively.

3.2. Results of zinc oxide nanoparticle synthesis:

Surface characteristics and size of ZnO nanoparticles were studied by FE-SEM field emission transmission electron microscope equipped with X-ray energy dispersive detector (EDX). ZnO nanoparticles are almost spherical and have relatively uniform size with an average diameter of 23.77 nm (Figures 2). Aggregation of nanoparticles is due to the adhesion of particles to each other by weak forces, which can lead to the formation of a cluster consisting of two or more nanoparticles. In fact, the density of nanoparticles occurs when the electron force on their surface loses its balance and the interaction between repulsive and attractive forces is not balanced. peaks in the EDS profile of ZnO-NPs indicated the presence of zinc, oxygen, and gold in its structure (Figure 3), which the discovered gold could be related to the preparation process of the sample for EDS and SEM analysis and the coating of zinc oxide powder on the gold adhesive tape for performing the relevant analyzes. The amount of zinc in the synthesized nanoparticles was 81.7% and

oxygen 18.3%, indicating that the synthesized nanoparticles are pure and there is no trace of impurities in other elements. The XRD pattern of ZnO-NPs is shown in Figure 4. As can be seen in this figure, the synthesized nanoparticles have a single-phase crystal structure and no peak of impurity is observed. The XRD plot of the pure-ZnO sample contained peaks at 2Θ of 31.7°, 34.4°, 36.2°, 39.5°, 56.5°, 62.5° and 67.8° attributed to the (100), (002), (101), (102), (110), (103), (112) and (201) crystal plates, respectively, demonstrating that ZnO-NPs have been prepared with high crystallinity and purity and have strong and clear peaks. All the peaks obtained from this spectrum confirm that the synthesized nanoparticles have a hexagonal wurtzite structure. The peaks were completely sharp, which indicate the crystal structure of the synthesized zinc oxide nanoparticles.

3.3. Antibiotic Susceptibility Pattern:

The pattern of antibiotic resistance of 30 strains of *S. mutans* against common antibiotics was investigated and the results are shown in Figure 2. 100% resistance to penicillin was observed in all *S. mutans* studied.

Resistance to Tetracycline (72.7%), clindamycin (72.7%), cefotaxime (72.7%), chloramphenicol (54.5%) and erythromycin (36.3%) were reported, respectively (Figure 5).

Synergistic and antibacterial activities of ZnO-NPs combined with Z. multiflora essential oil: The strains of S. mutans, were used to evaluate the possible antibacterial activity of ZnO-NPs and Z. multiflora essential oil. The MIC of ZnO-NPs against S. mutans strains was reported were in the range of 12.5-50 µg/ml, and the MIC of Z. multiflora essential oil was in the range of 0.031-0.25 µl/ml. as well as, MIC of chlorhexidine against bacteria was reported between 0.25-2 µl/ml. The MIC and MBC values of all agents against all strains are shown in Table 2. In all bacterial strains, the MIC value of ZnO-NPs in comparison with essential oil of Z. multiflora was found to be very high. The antimicrobial effects of ZnO-NPs in combination with essential oil of Z. multiflora are shown in Tables 2. The combination of ZnO-NPs with essential oil against S. mutans caused an additive effect, as defined by FICI values of 0.5078-0.625.

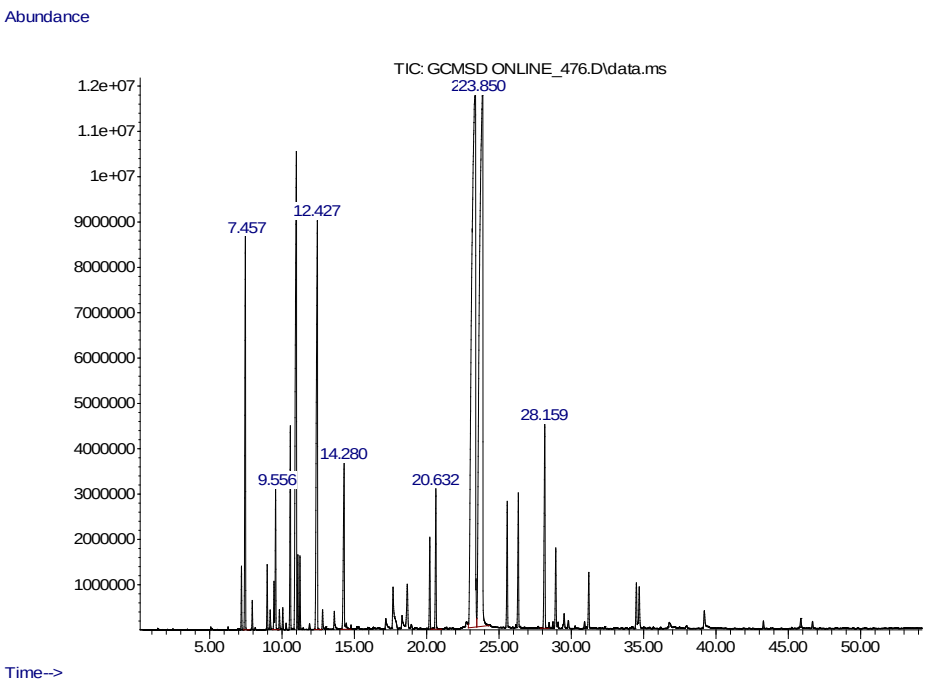

Figure 1. GC-MS chromatogram of the *Z. multiflora* essential oil,

Table 1. Chemical compositionof *Z. multiflora* essential oil

Ret Time	Compound	Mw	Molecular Formula	CAS Number	Area	%
7.457	Bicyclo[3.1.1]hept-2-ene, 2,6,6-trimethyl-	136.1	C10H16	000080-56-8	302018617	4.497
10.572	.Alpha. Terpinene	136.1	C10H16	000099-86-5	151525019	2.2562
10.975	Benzene	134.1	C10H14	000099-87-6	662738817	9.8681
12.427	1,4-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-	136.1	C10H16	000099-85-4	511452737	7.6154
14.28	Linalooll	154.1	C10H18O	000078-70-6	175863602	2.6186
20.216	2-isopropyl-5-methyl-1-methoxybenzene	164.1	C11H16O	000000-00-0	73781600	1.0986
20.632	Carvacrol Methyl ether	164.1	C11H16O	006379-73-3	116276401	1.7313
23.345	Thymol	150.1	C10H14O	000089-83-8	2238860808	33.336
23.85	Carvacrol	150.1	C10H14O	000499-75-2	1927435583	28.699
25.567	Acetylthymol	192.1	C12H16O2	000528-79-0	103347059	1.5388
26.332	Carvacryl acetate	192.1	C12H16O2	000000-00-0	115947982	1.7264
28.159	Trans-Caryophyllene	204.2	C15H24	000087-44-5	202249872	3.0115
31.204	Ledene	204.2	C15H24	021747-46-6	48261073	0.7186
34.497	8,9-Dehydro-neoisolongifolene	202.2	C15H22	000000-00-0	42250532	0.6291
34.695	Caryophyllene oxide	220.2	C15H24O	001139-30-6	43980845	0.6549
					6715990547	100

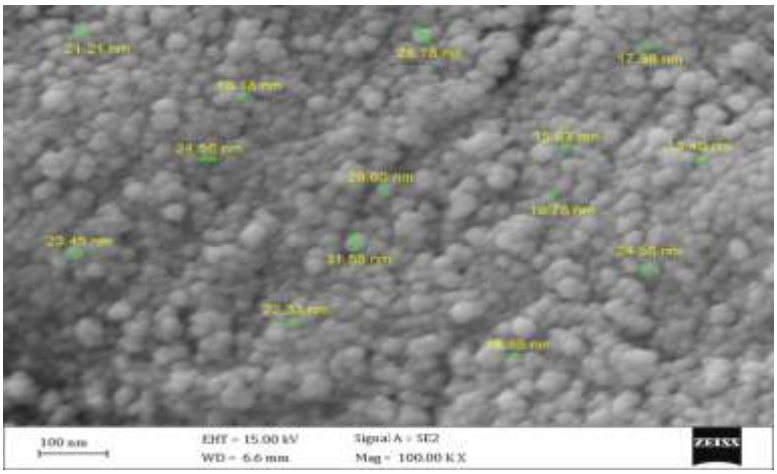

Figure 2. FE-SEM image of synthesized ZnO-NPs.

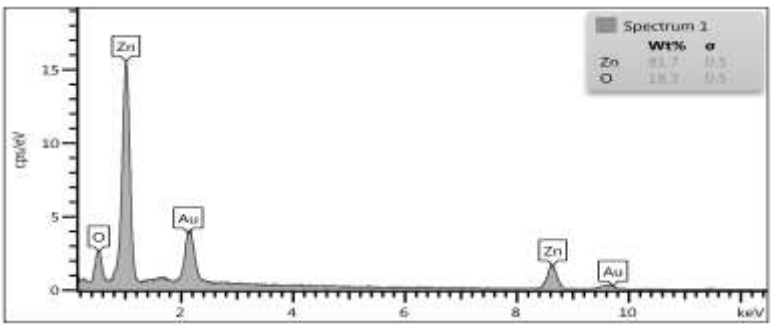

Figure 3. EDS profile of synthesized ZnO-NPs.

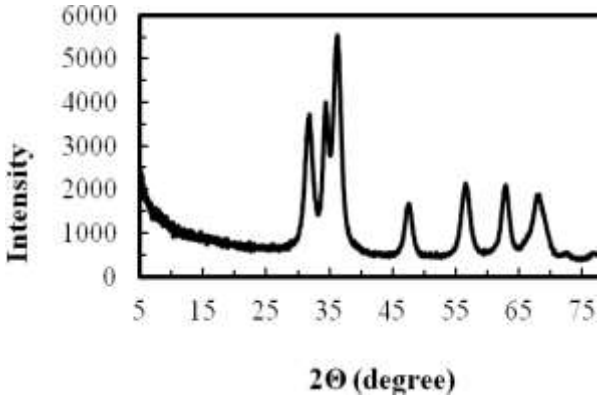

Figure 4. The XRD pattern of synthesized ZnO-NPs.

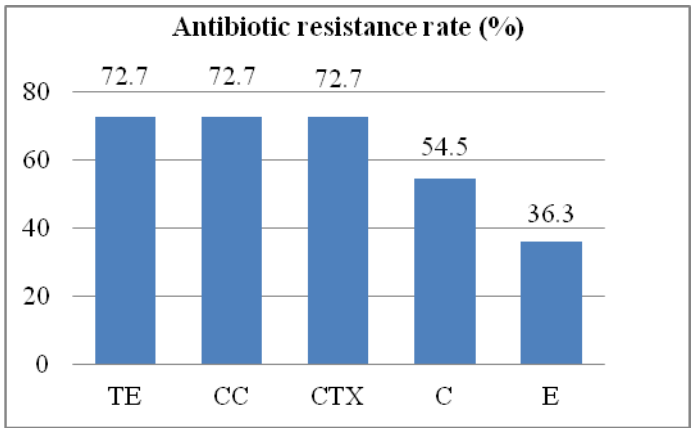

Figure 5. Comparison diagram of antibiotic resistance frequency distribution in Streptococcus mutans strains. Tetracycline (TE), Clindamycin (CC), Cefotaxime (CTX), Chloramphenicol (C) and Erythromycin (E).

Table 2. MIC and MBC activity of chlorhexidine, Z. multiflora Essential Oil, ZnO-NPs and antimicrobial activity of ZnO-NPs in combination with Z. multiflora essential oil

S. mutans	chlorhexidine (µl/ml)		*essential oil* (µl/ml)		ZnO-NPs (µg/ml)		FIC ZnO-NPs	FIC *essential oil*	FICI	Interaction
	MIC	MBC	MIC	MBC	MIC	MBC				
Strains 1	0.25	0.5	0.031	0.062	50	100	0.5	0.0078	0.5078	Additive
Strains 2	2	4	0.25	0.5	50	100	0.5	0.125	0.625	Additive
Strains 3	2	4	0.25	1	50	100	0.5	0.125	0.625	Additive
Strains 4	2	2	0.25	0.5	50	100	0.5	0.0078	0.5078	Additive
Strains 5	1	4	0.062	0.125	12.5	25	0.5	0.125	0.625	Additive
Strains 6	1	4	0.062	0.125	25	50	0.5	0.0624	0.5624	Additive
Strains 7	0.25	1	0.031	0.062	50	100	0.5	0.125	0.625	Additive
Strains 8	1	2	0.062	0.125	25	50	0.5	0.0624	0.5624	Additive
Strains 9	2	4	0.25	0.5	50	100	0.5	0.125	0.625	Additive
Strains 10	1	2	0.062	0.125	25	50	0.5	0.0624	0.5624	Additive
ATCC35668	0.25	1	0.062	0.125	12.5	25	0.5	0.0156	0.5156	Additive

4. Discussion

Tooth decay and periodontal problems are the most common oral and dental diseases in the world, and *Streptococcus mutans* is known as the main cause of tooth decay. Using mouthwash as a disinfectant solution can be effective in controlling oral and dental diseases. Despite this, the side effects caused by some mouthwashes, including various side effects such as changes in the sense of taste, burning and dryness of the mouth, the formation of dental pigments and negative systemic effects in case of swallowing, have limited their use (Shah et al., 2018).

Zataria multiflora essential oil of has strong antimicrobial effects due to the presence of Thymol and Caracrol. The mechanism of action of carvacrol and thymol, as the main components of *Z. multiflora* essential oil, has received much attention from researchers. Thymol and carvacrol are very similar to each other and their difference is in having a hydroxyl group in different positions in the phenolic ring, and both increase the permeability of the cell membrane. These two compounds are able to destroy the outer membrane of bacteria and cause the release of lipopolysaccharide (LPS) and increase cytoplasmic permeability to ATP (Sampaio et al., 2021).

Mirzaei et al. examined the effect of antimicrobial effects of *Z. multiflora*-based mouthwash on the microbial community of dental plaques and reported MIC levels for *S. mutans* 2mg/ml (Mirzaei et al., 2022). Yazdanian et al. reported MIC and MBC values of essential oil of *Z. multiflora* 187.5mg/ml and 375mg/mL, respectively for *S. mutans*. In our study, the MIC and MBC values of methanolic extracts against *S. aureus* were observed to be 1.56 mg/mL and 6.25 mg/mL, respectively (Yazdanian et al., 2022).

The bactericidal properties of mouthwashes containing metal nanoparticles have been shown in many studies. (Norouzzadeh et al., 2018). The antimicrobial activity of nanoparticles depends on various factors such as size, shape, concentration, surface charge, microbial cell sensitivity, etc. (Mirhosseini et al, 2019). ZnO-NPs are a non-toxic, biocompatible and stable compound compared to the processing conditions. These particles have selective toxicity on bacteria, but they have shown minimal side effects on human and animal cells. ZnO-NPs are used in dental restoration to produce filling compounds with high gloss and also in cosmetic products (Tiwari et al, 2022). In the present study, ZnO-NPs were chemically synthesized and structurally investigated. The synthesized nanoparticles have a crystalline structure and are spherical in shape and relatively uniform in size and around 23.77nm. The MIC of ZnO-NPs against *S. mutans* was reported between 12.5-50 µg/ml. The results of other studies indicated that ZnO-NPs effectively against *S. mutans*. The MIC using ZnO-NPs have ranged from 0.390 to 500 µg/ml. The MBC using ZnO-NPs ranged from 3.125 to 500 µg/ml (Almoudi et al., 2018). The antimicrobial effect of ZnO-NPs can be explained by several mechanisms, the induction of oxidative stress due to the production of reactive oxygen radicals, which react with DNA, proteins and lipids, leading to cell death. The destruction of the membrane arrangement due to the accumulation of nanoparticles in the bacterial membrane, as well as their accumulation inside the cell, as well as the release of zinc ions, which cause antimicrobial effects by attaching to the membrane of microorganisms (Gudkov et al., 2021).

In this study, the application of ZnO-NPs as an antimicrobial agent in combination with *Z. multiflora* essential oil was investigated by growing *S. mutans.* The combination of bioactive essential oil with ZnO-NPs is a novel applied method and could be beneficial (as a synergistic or additive interaction) or deleterious (as an antagonistic or toxic outcome). Our results confirm that these compounds exerted additive effects against *S. mutans* when ZnO nanoparticles were combined with essential oil. These results suggest that the additive effect of ZnO nanoparticles with *Z. multiflora* essential oil can be used as effective growth inhibitors in microorganisms, making them applicable to antimicrobial control systems. Although researchers have mentioned the antimicrobial property of ZnO nanoparticles, its safety for humans and the absence of environmental pollution due to its use as important features of these nanoparticles. But the findings of various researches show that the pathological changes induced by zinc oxide nanoparticles depend on

the method of exposure, size and quantity. On the other hand, excessive amounts of ZnO-NPs in body can affect tissues such as the liver (Sirelkhatim et al., 2015). The combined use of drugs in order to reduce their dosage and break bacterial resistance is important, and results of the present study confirmed the reduction of the dosage of ZnO-NPs and the increase of its antimicrobial activity when combined with essential oils.

Safari et al. investigated and confirmed the antimicrobial effect of hydroalcoholic extract of *Plantago major* leaves with and without zinc oxide nanoparticles on *Streptococcus mutans* (Safari et al., 2021). Farrokhpour et al. evaluated antibacterial activities of the essential oil and hydroalcoholic extract of *Eucalyptus microtheca* and silver nanoparticles (AgNPs) as compared to some different standard antibiotics, against *S. mutans*. The results showed that silver nanoparticles in combination with extract and essential oil of *E. microtheca* have antimicrobial activity against *S. mutans* (Farrokhpour et al., 2019). Sheikholeslami et al. assessed the antibacterial effects of silver nanoparticles solely and in combination with *Zataria multiflora* essential oil and methanolic extract on some photogenic bacteria. The results of the experiment showed that Silver nanoparticles have additive effects with essential oil against *S. epidermidis* and *S. aureus* (Sheikholeslami et al., 2016).

The use of chlorhexidine as a mouthwash has always been associated with side effects, including unpleasant taste, temporary changes in the sense of taste, and chlorhexidine causes the appearance of brown color on the teeth and tooth-colored fillings of the oral mucosa and tongue. A few cases of unilateral or bilateral swelling of the parotid gland due to chlorhexidine consumption have been reported (Davari et al., 2019). Based on the obtained results, it seems that the antimicrobial effect of ZnO nanoparticles in combination with of *Z. multiflora* essential oil against standard and clinical strains of *S. mutans* is significant and these compounds can prevent the growth of *S. mutans* and be a suitable alternative to chlorhexidine mouthwash. Further research on the antibacterial effects of combining nanoparticles with other plant extracts and essential oils is suggested.

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