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Antibacterial activity dental varnish prepared using titanium oxide nanoparticles using ginger and rosemary against Staphylococcus aureus and Enterococcus faecalis

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Abstract: Nanomedicine, which combines medicine and nanotechnology, has a number of benefits over more conventional forms of therapy. Due to their distinctive characteristics compared to their bulk equivalent, metal nanoparticles have increased tremendous attention in the field of nanomedicine. To study the mechanism of action of rosemary and ginger-mediated titanium oxide nanoparticles (TiO2 NPs)-based dental varnish against Streptococcus mutans (S. mutans) and E.faecalis. Materials and methods:MHA broth was prepared. Bacterial suspension (*Staphylococcus aureus and MRSA*) was added. The incubation is done under suitable conditions for varied time intervals. Then the percentage of dead cells is calculated at a wavelength of 540 nm at regular time intervals. Result: The results have demonstrated that when concentration rises, optical density values fall, demonstrating a bactericidal action. The results show a great difference between the values of optical density of the test samples at various concentrations in the order of 25, 50, and 100 µL at various time intervals from 1hr, 2hr, 3hr, 4hr, 5hr being the highest when compared with control and antibiotic groups against Streptococcus mutans and E.faecalis. The results have proved that the greenly generated dental varnish has demonstrated good antibacterial and antibiofilm properties. Conclusion: From our study we concluded titanium oxide dental varnish prepared using plant extract of rosemary and ginger showed considerable antibacterial activity against s. Aureus and e.faecalis

Keywords: nanoparticles, titanium oxide, health, dental varnish,child.

1.Introduction: Technology that works with nanometer-sized items is called nanotechnology. Nanotechnology is anticipated to advance on a number of levels, including those of materials, electronics, and systems. The level of nanoparticles is currently the most developed in terms of scientific understanding and practical applications. Nanoparticles were investigated ten years ago due to their size-dependent physical and chemical characteristics. They are now in the commercial exploring phase [1]**.**Almost all scientific disciplines have been impacted by nanotechnology, which has allowed for the creation of innovative solutions to a number of research bottlenecks. The use of nanomedicine, which combines medicine and nanotechnology, has a number of benefits over more conventional forms of therapy. Due to their distinctive characteristics compared to their bulk equivalent, metal nanoparticles have increased tremendous attention in the field of nanomedicine [3]. Noble metal nanoparticles have shown potential for use in biomedical applications among the metal nanoparticles. Nanoparticles' small size makes it simple for them to interact with biomolecules both outside and inside of cells, producing stronger signals and greater target specificity for treatments and diagnostics. Nanoparticles have the potential to replace antibiotics and treat bacterial infections brought on by Salmonella typhi, Klebsiella pneumoniae, Staphylococcus aureus, and Staphylococcus epidermidis [5,6]. TiO2 nanoparticles stand out among metal oxide nanoparticles because of their photocatalytic abilities, chemical stability, and lack of toxicity. Numerous applications exist for it in the cosmetics business, solar energy, electrochemical devices, pollution prevention, and antibacterial coatings [7]**.** These particles have been utilized as food coloring, colors, medications, cosmetics, and toothpaste because they shield the skin from UV rays. TiO2 nanoparticles are particularly antibacterial and antioxidant compared to other metal nanoparticles because of their photocatalytic ability [7,8]. TiO2 based drug delivery devices have been demonstrated to enhance cancer treatment and lower tumorigenesis risk. TiO2 has a high refractive index, photocatalytic activity when exposed to light, is biocompatible, and is chemically and physiologically inert. Consequently, photodynamic and sonodynamic therapy for cancer use TiO2 nanoparticles[9,10 11]

Plant extracts have been created recently and used in foods as antimicrobials. These extracts may have anti-microbial characteristics in addition to their antioxidant activity since they include a variety of phenolic compounds, including abietane diterpenes , carnosol, and ursolic acid [12,13]. Rosemary (Rosmarinus officinalis L.), a member of the Lamiaceae family of plants, is a very significant medicinal and aromatic shrub. There is evidence that ancient Egyptians employed rosemary herbs for their therapeutic, culinary, and cosmetic benefits. China, Mesopotamia, and India. Antibacterial, anticancer, antidiabetic, anti-inflammatory, antinociceptive, antioxidant, antithrombotic, antiulcerogenic, reducing cognitive impairments, antidiuretic, and hepatoprotective properties are only a few of the medical uses of rosemary that have been pharmacologically established.[9 14]. The Zingiberaceae family includes ginger (Zingiber officinale).It is a blooming plant whose rhizome or root is frequently employed as a spice and in traditional medicine. According to reports, ginger isIt is said that ginger oil has great therapeutic value. Major active elements in ginger oil are reportedly sourced from the tropical rainforests of the Indian subcontinent to Southern Asia, where ginger plants exhibit some genetic variety [15]. A type of diarrhea that is the main cause of newborn death in impoverished nations is resistant to ginger chemicals. Numerous studies have shown that ginger is useful for treating nausea brought on by chemotherapy, morning sickness, and nausea brought on by being seasick; however, post-operative nausea was found to be where ginger outperformed a placebo[16]. Dental varnishes are simple to use and a safe way to supply active substances to the teeth, including fluoride or chlorhexidine. Its local action at the tooth/plaque interface, where it promotes remineralization while reducing demineralization, is the fundamental advantage of varnish's anti-caries function. Streptococcus mutans are also prevented from producing acid [1718]. The aim of our study is to find the antibacterial activity of dental varnish prepared by titanium oxide using plant extract of ginger and rosemary against Staphylococcus aureus and Enterococcus faecalis.

2.Materials and methods:

2.1 Study setting: The study was conducted in Chennai, Tamil Nadu, India's Saveetha Institute of Medical and Technical Sciences (considered to be a university, Department of Pharmacology, Nanomedicine Laboratory, Saveetha Dental College and Hospitals. In the current investigation,Staphylococcus aureus and Enterococcus faecalis was the oral pathogen employed to investigate the antifungal activity. The microbe was obtained from the Dental College and Hospitals nanomedicine laboratory culture lab in Chennai, Tamil Nadu, India's Saveetha Institute of Medical and Technical Sciences.

2.2 Antibacterial activity : The antibacterial activity of the green synthesized titanium oxide nanoparticles was evaluated using the agar well diffusion technique. Mueller Hinton agar plates were prepared and sterilized using an autoclave at 121°C for 15- 20 minutes. After sterilization, the medium was poured on to the surface of sterile Petri plates and allowed to cool to room temperature. The bacterial suspension (*Lactobacillus sp, Staphylococcus aureus)* was spread evenly onto the agar plates using sterile cotton swabs. Wells of 9mm diameter were created in the agar plates using a sterile polystyrene tip. The wells were then filled with different concentrations (25 µg, 50 µg, 100 µg) of titanium oxide NPs. An antibiotic (e.gBacteria-Amoxyrite, Fungi- Fluconazole) was used as a standard. The plates were incubated at 37°C for 24 hours and 48 hours for fungal cultures.The antimicrobial activity was evaluated by measuring the diameter of the inhibition zone surrounding the wells. The diameter of the zone of inhibition was measured using a ruler and recorded in millimeters (mm) and the zone of inhibition was calculated.

2.3 Time kill curve assay:

1. MHA broth was prepared, sterilized and 6 mL was added to all the five test tubes.
2. Bacterial suspension (*Staphylococcus aureus and MRSA*) was added to all five test tubes in the range of 5×10^5 CFU/ml.
3. The first three tubes contain the rosemary and ginger extract with three different concentrations and the fourth tube is considered as the growth control and fifth tube as standard (Amoxyrite).
4. The incubation is done under suitable conditions for varied time intervals (1h, 2h, 3h, 4h, 5h).
5. Then the percentage of dead cells is calculated at a wavelength of 540 nm at regular time intervals.

2.5 Figure 1 : Plant extract of rosemary and ginger-mediated TiO2, NPs-based

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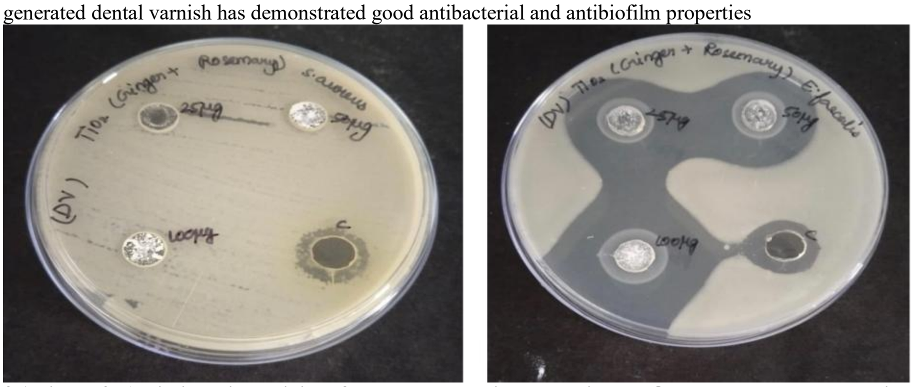
3. Result:

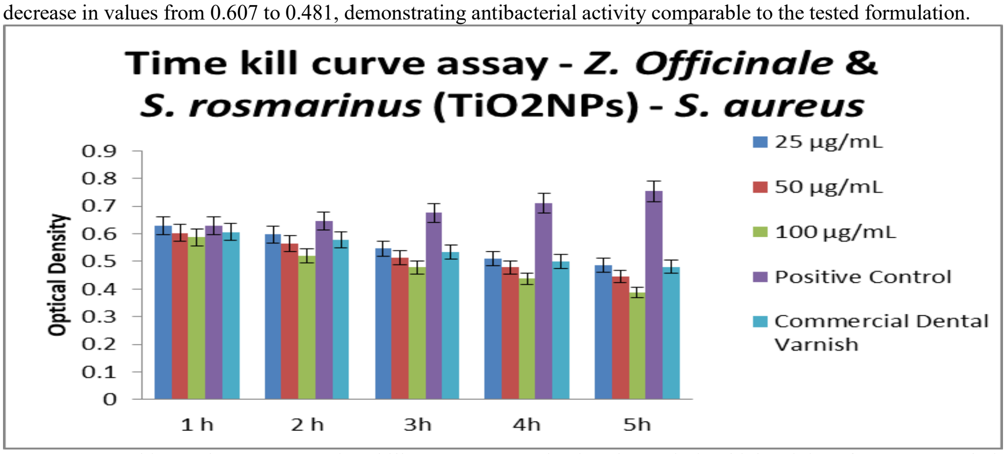
The results have demonstrated that when concentration rises, optical density values fall, demonstrating a bactericidal action. The results show a great difference between the values of optical density of the test samples at various concentrations in the order of 25, 50, and 100 µL at various time intervals from 1hr, 2hr, 3hr, 4hr, 5hr being the highest when compared with control and antibiotic groups against Streptococcus mutans and E.faecalis. The results have proved that the greenly generated dental varnish has demonstrated good antibacterial and antibiofilm properties



3.1 Figure 2: Antimicrobial activity of rosemary and ginger-mediated TO, NPs-based dental varnish

	1h	2h	3h	4h	5h
25µL	0.629	0.597	0.546	0.511	0.486
50µL	0.603	0.565	0.512	0.479	0.446
100µL	0.587	0.521	0.437	0.437	0.388
Positive control	0.629	0.647	0.676	0.712	0.754
Commercial dental varnish	0.607	0.577	0.534	0.501	0.481

3.2 Table 1: Shows the time-kill curve assay results against Staphylococcus aureus measured at different time intervals (1–5 hours) for varying concentrations of the test sample (25 µL, 50 µL, and 100 µL), along with a positive control and commercial dental varnish. The results show a progressive decrease in absorbance values over time for all tested concentrations of the sample and for the commercial dental varnish, indicating increased antibacterial activity and reduction in bacterial growth with longer exposure time. At 25 µL, the value decreased from 0.629 at 1 hour to 0.486 at 5 hours, suggesting gradual bacterial inhibition. Similarly, 50 µL showed a reduction from 0.603 to 0.446, while the 100 µL concentration demonstrated the greatest reduction, decreasing from 0.587 at 1 hour to 0.388 at 5 hours, indicating stronger antibacterial activity at higher concentration. In contrast, the positive control exhibited an increase in absorbance values from 0.629 to 0.754 over the 5-hour period, indicating continued bacterial growth in the absence of the test agent. The commercial dental varnish also showed a gradual decrease in values from 0.607 to 0.481, demonstrating antibacterial activity comparable to the tested formulation.



3.3 Graph 1: This graph represents a time-kill curve assay evaluating the Antimicrobial activity of rosemary and ginger-mediated TO, NPs-based dental varnish against s.aureus species tested at various concentrations from 25, 50, 100ML, at various time intervals from 1hr, 2hr, 3hr, 4hr, 5hr and the percentage of inhibition. The X-axis shows the time interval of the NPs and the Y-axis shows the optical density

	1h	2h	3h	4h	5h
25µL	0.583	0.552	0.513	0.487	0.445
50µL	0.561	0.528	0.476	0.436	0.401
100µL	0.532	0.488	0.445	0.402	0.365
Positive control	0.612	0.645	0.679	0.712	0.749
Commercial dental varnish	0.554	0.511	0.489	0.443	0.409

3.4 Table 2: Shows the time-kill curve assay results against Enterococcus faecalis recorded at different time intervals (1–5 hours) for various concentrations of the test sample (25 µL, 50 µL, and 100 µL), along with a positive control and commercial dental varnish. The results demonstrate a gradual decrease in absorbance values over time for all tested concentrations, indicating progressive inhibition of bacterial growth with increasing exposure time. At 25 µL, the absorbance value decreased from 0.583 at 1 hour to 0.445 at 5 hours, showing moderate antibacterial activity. Similarly, 50 µL showed a reduction from 0.561 to 0.401, while the 100 µL concentration exhibited the greatest reduction, decreasing from 0.532 at 1 hour to 0.365 at 5 hours, suggesting stronger antibacterial activity at higher concentration. In contrast, the positive control showed an increase in absorbance values from 0.612 to 0.749 over the 5-hour period, indicating continuous bacterial growth in the absence of the test agent. The commercial dental varnish also demonstrated antibacterial activity, with values decreasing from 0.554 at 1 hour to 0.409 at 5 hours, showing a comparable inhibitory effect.

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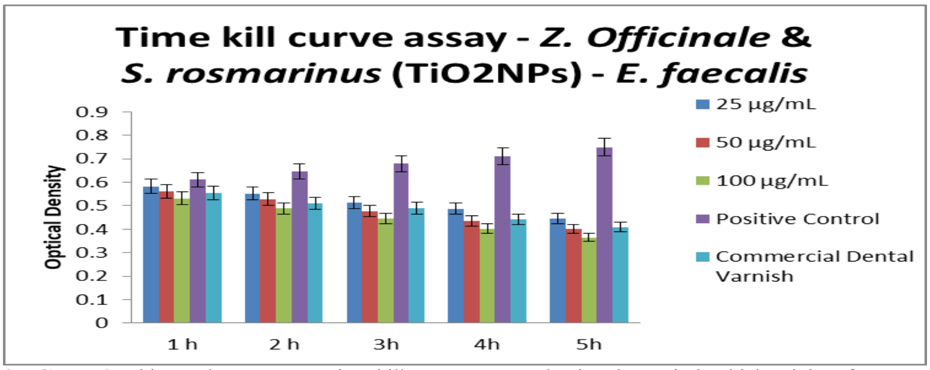

3.5 Graph 2: This graph represents a time-kill curve assay evaluating the Antimicrobial activity of rosemary and ginger-mediated TO, NPs-based dental varnish against E.faecalis species tested at various concentrations from 25, 50, 100ML at various time intervals from 1hr, 2hr, 3hr, 4hr, 5hr and the percentage of inhibition. The X-axis shows the time interval of the NPs and the Y-axis shows the optical density

4.Discussion: One of the most prevalent oral health issues, dental caries has a high incidence and prevalence and, if left untreated, can cause irreparable harm. Simply expressed, different intra- and extraoral stimuli may cause a biofilm over a tooth to start caries production on the tooth surface. For the preservation of oral hygiene, a range of preventive measures and products have been created in an effort to incorporate antimicrobial substances into the biofilm[19]. In previous research done by Canto et al [20] calcium silica nanoparticles used for treatment can prevent the progression of enamel erosion. In a different study JJ Chokkattu et al 2023 The results demonstrated that a dental varnish formulation based on TiO, NPs mediated by clove and ginger has proved to have an effective antibacterial action. In another study done by JJ Chokkattu et al dental varnish prepared by clove and ginger mediated Tio NP showed anti caries activity against dental caries causing microbes[17]. In previous research done by B. harini et al dental varnish prepared using *P. longum*- and chitosan-assisted nano zinc oxide showed *Staphylococcus aureus* to be very sensitive with a maximum zone of inhibition followed by *Streptococcus mutans*, *Enterococcus faecalis*, and *Candida albicans. P. longum- and chitosan-assisted nano zinc oxide–based dental varnishes will be a better choice for infections caused by S. aureus and S. mutans* [17,21]. In another research AuNP dental varnish showed considerable re mineralizing property but it was not superior to dental varnishes like SnF2 dental varnish [22]. In another research AgNPs added to varnish which showed dental remineralization and strengthened the enamel and protects against dental caries [23]. In another research dental varnish containing nanosilver showed antimicrobial effect against S. mutans and S. salivarius and can be used in amalgam restoration to prevent microbial population [23,24]. In another research *Citrullus lanatus* based AgNp dental varnish showed antibacterial activity against common oral microbes such as *S. mutans, C. albicans, E. faecalis, and Lactobacillus sp* [25].

5. Limitations: The investigation was carried out in an in vitro setting with few test samples. Therefore, it's possible that the antibacterial efficacy data by themselves are insufficient to demonstrate its therapeutic efficacy.Scope of future research-The precise mechanism of action of these NPs against various pathogens needs to be determined through additional clinical trials and ex vivo studies.

6. Conclusion: From our study, we conclude that the dental varnish formulation containing TO and nanoparticles mediated by rosemary and ginger effectively inhibits bacterial growth. The results demonstrated maximum antibacterial activity at higher concentrations and longer exposure times. The formulation showed significant antibacterial efficacy comparable to commercial dental varnish. However, further in vivo studies are required to confirm its effectiveness and potential clinical applications.

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8. Conflict of Interest: The authors reported no conflict of interest while performing this study.
9. Funding Agency: The present project is supported/funded/sponsored by
- Saveetha Institute of Medical and Technical Sciences
- SaveethaDental College and Hospitals.
- Saveetha University

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