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Antimicrobial Activity Of Ginger And Rosemary Mediated With Tio_2 Using Dental Varnish

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ABSTRACT

Aim: To investigate the antimicrobial activity of ginger and rosemary mediated with TiO_2 using dental varnish.
Introduction: Pharmaceutical corporations are substantially investing in the development of natural medicines derived from plants as a result of the rise in the usage of traditional medicinal herbs in recent years. There are numerous potential actions and fields for nanoparticles. Titanium nanoparticles (TiO_2) are produced in the current work using a green technique and floral extract.
Materials and method: Mueller Hinton Agar was utilized for this activity to determine the zone of inhibition. Mueller hinton agar was prepared and sterilized for 15 minutes at 121°C. Media poured into the sterilized plates and let it stable for solidification. The wells were cut using a 9mm sterile polystyrene tip and the test organisms were swabbed. The nanoparticles with different concentrations (25µL, 50 µL ,100 µL)were loaded and in the fourth well standard antibiotic amoxicillin was loaded
Result: At 25µl, 50µl and 100µl, the antimicrobial activity against lactobacillus was found to be significant when compared to the standard. At 50µl and 100µl, the antimicrobial activity against E. faecalis was found to be significant when compared to the standard. At 25µl and 100µl, the antimicrobial activity against S. aureus was found to be significant when compared to the standard.
Conclusion: The present study supports the antimicrobial activity of the ginger and rosemary with nanoparticle titanium oxide against S. mutans, E. faecalis and S. aureus, C. albicans, Lactobacillus along with dental varnish.

INTRODUCTION:

Man is turning towards nature as normal organically grown items are being progressively utilized in prophylaxis and treatment of various illnesses. As a result of its low occurrence of serious unfavorable impacts, minimal expense and their apparent viability, natural medication is acquiring significance and periodontal issues are the most widely recognized persistent infections around the world(1). The term "dental caries" refers to an infectious bacterial condition in which the calcified tissues of the teeth are destroyed. S. mutans appear to be one of the primary organisms associated with human dental caries. A caries counteraction technique is a complicated interaction including various perspectives(2). To arrive at this objective, restricting substrate, disturbing of plaque arrangement with brushing and flossing, modifying tooth surface with various types of fluoride, invigorating the spit stream, reestablishing cavitated tooth surface and changing cariogenic microflora to non-cariogenic ones with skin fluoride treatment, anti-toxin treatment or bactericidal mouth washes, for example, chlorhexidine can be applied . The brilliant norm for the mouthwashes is a diguanidohexane with articulated sterile properties, named chlorhexidine(3).
In The new past, there has been an expanded interest in the restorative properties of a few restorative plants and normal builds which have exhibited an enemy of cariogenic exercises in both in vitro and in vivo conditions(4).
Among these phytoconstituents, a few polyphenolic intensifies like tannins (catechins) and flavonoids appear to be the most encouraging biomolecules.
Green synthetic methods, for example, are gaining a lot of attention in materials science research and development right now(5). For the most part, green combination of nanoparticles, ready through guideline, tidy up, control, and remediation cycles will elevate their ecofriendliness. As a result, a few fundamental principles of biosynthesis can be described by a number of components, such as the prevention of waste, the utilization of non-toxic solvents, and renewable feedstock (6). An environmentally friendly and long-term strategy for avoiding the formation of harmful byproducts calls for biosynthesis. Numerous biological entities, including plant extracts, bacteria, and algae, can now be accommodated through the biosynthesis of metal and metal oxide nanoparticles(7). When compared to the algae-, fungi-, and bacteria-based prepared nanoparticles, the prepared green nanomaterials have a great application in the pharmaceutical industry, such as the preparation of novel pharmaceuticals, drug delivery personification procedures, and the synthesis of functional

nanodevices(5). Among the existing green approaches for the preparation of metal and metal oxide nanoparticles, using the plant is a rapid, easy, and simple process to synthesize nanoparticles at a large level.
The rise of anti-toxin safe microorganisms is the main source of nosocomial contaminations, which is considered as a serious general medical condition that prompted expanded horribleness and mortality overall (8). There is an urgent need to improve and develop methods and strategies for dealing with this problem because of the global escalation of bacterial resistance, which is now a major concern. The present study evaluates the antimicrobial activity of ginger and rosemary mediated with TiO_2.

MATERIALS AND METHOD:

Plant Material Collection and Preparation
The present study utilized two medicinal plants with well-documented therapeutic properties: Rosmarinus officinalis, commonly known as rosemary, and Zingiber officinale, commonly known as ginger. Rosemary is an aromatic herb belonging to the Lamiaceae family, native to the Mediterranean region but now cultivated worldwide for its culinary and medicinal applications. It contains numerous bioactive compounds including rosmarinic acid, carnosic acid, and essential oils with demonstrated antimicrobial, anti-inflammatory, and antioxidant properties. Ginger, a member of the Zingiberaceae family, is a perennial plant whose rhizome has been used for centuries in traditional medicine systems across Asia and other regions for treating various ailments, with its bioactive compounds including gingerols, shogaols, and zingerone contributing to its antimicrobial and anti-inflammatory activities.
Fresh leaves of Rosmarinus officinalis and fresh rhizomes of Zingiber officinale were collected from reliable sources and authenticated by a qualified botanist to ensure correct species identification. The plant materials were thoroughly washed under running tap water to remove any adhering soil particles, dust, or other contaminants, followed by a final rinse with distilled water. The cleaned plant materials were then spread in a single layer on clean paper and allowed to dry at room temperature in a shaded area away from direct sunlight to prevent degradation of heat-sensitive bioactive compounds. Drying continued until constant weight was achieved, indicating complete removal of moisture.
After complete drying, 100 grams of dried rosemary leaves and 100 grams of dried ginger rhizomes were weighed separately using a precision analytical balance. Each plant material was then ground into a fine powder using an electric grinder, with the grinding process continued until a uniform particle size was achieved to ensure efficient extraction of bioactive compounds. The powdered rosemary and ginger were then thoroughly mixed together to create a combined herbal powder for extract preparation.

Extract Preparation
For extract preparation, 100 milliliters of distilled water was added to the combined herbal powder in a clean glass container. The mixture was stirred thoroughly to ensure complete wetting of the powder and to facilitate extraction of water-soluble compounds. The mixture was then allowed to stand for approximately 30 minutes to permit initial extraction before filtration. Following this standing period, the mixture was filtered through Whatman No. 1 filter paper to separate the liquid extract from the solid plant residue. Filtration was performed carefully to obtain a clear filtrate free of particulate matter that could interfere with subsequent testing.The filtered extract was then subjected to gentle heating at a temperature of 50 degrees Celsius. This moderate temperature was selected to concentrate the extract by evaporating excess water while avoiding degradation of heat-sensitive bioactive compounds that could be damaged at higher temperatures. The heating was continued until the desired volume reduction was achieved, and the concentrated extract was allowed to cool to room temperature before being used for antimicrobial testing. The prepared extract was stored in sterile, airtight containers under refrigerated conditions until further use to maintain its antimicrobial activity.

Evaluation of Antimicrobial Activity
The antimicrobial activity of the prepared rosemary and ginger herbal extract was evaluated using the agar well diffusion method, a standard and widely accepted technique for assessing the antimicrobial potential of plant extracts and other test substances. This method is based on the principle that antimicrobial agents diffusing from wells cut into the agar medium will inhibit the growth of susceptible microorganisms, producing clear zones of inhibition around the wells.A panel of clinically significant oral pathogens was selected for antimicrobial testing, including Streptococcus mutans, the primary etiological agent of dental caries; Candida albicans, the most common fungal pathogen in the oral cavity; Enterococcus faecalis, a bacterium frequently associated with persistent endodontic infections and failed root canal treatments; Lactobacillus species, which contribute to caries progression through acid production; and Staphylococcus aureus, a Gram-positive coccus associated with various oral infections. This diverse panel encompasses both bacterial and fungal pathogens relevant to oral diseases and provides comprehensive information about the antimicrobial spectrum of the herbal extract.Culture Medium Preparation and InoculationMueller Hinton agar was prepared according to the manufacturer's specifications by suspending the dehydrated medium in distilled water and heating to ensure complete dissolution. The prepared medium was sterilized by autoclaving at 121°C for 15 minutes, then allowed to cool to approximately 45-50°C before being poured into sterile Petri plates to a uniform depth of approximately 4 mm. The poured plates were allowed to solidify at room temperature on a level surface to ensure uniform thickness.Fresh bacterial suspensions of each test

organism were prepared from overnight cultures grown on appropriate media. The turbidity of each suspension was adjusted to match the 0.5 McFarland standard, corresponding to approximately 1.5×10^8 colony-forming units per milliliter for bacteria, ensuring standardized inoculum for all tests. For Candida albicans, the inoculum was similarly standardized. The adjusted bacterial suspensions were then uniformly swabbed onto the surface of the Mueller Hinton agar plates using sterile cotton swabs, ensuring complete and even coverage of the agar surface to produce a confluent lawn of microbial growth after incubation.Well Preparation and Sample Loadingfter the inoculated plates were allowed to dry for a few minutes, wells of uniform size (approximately 6 mm in diameter) were cut into the agar using a sterile cork borer. The wells were carefully positioned to ensure adequate spacing between them to prevent overlapping of inhibition zones. Three different concentrations of the herbal extract, specifically 25 µL, 50 µL, and 100 µL, were incorporated into separate wells on each plate to evaluate concentration-dependent antimicrobial activity. This range of concentrations allows for assessment of whether increasing extract concentration correlates with enhanced antimicrobial effects.In addition to the test wells, a separate well on each plate was filled with the standard antibiotic amoxicillin to serve as a positive control. Amoxicillin is a broad-spectrum antibiotic commonly used in dental practice and provides a reference point for comparing the antimicrobial activity of the herbal extract. The plates were allowed to stand at room temperature for approximately 30 minutes after loading to permit pre-diffusion of the test substances into the agar before incubation.Incubation and MeasurementThe plates were then incubated at 37°C for 24 hours, conditions that support optimal growth of the test bacterial and fungal strains while allowing sufficient time for diffusion of antimicrobial agents and development of inhibition zones. After the incubation period, the plates were examined against a dark background with adequate illumination, and the zones of inhibition surrounding each well were measured. The diameter of each inhibition zone was recorded in millimeters using a calibrated ruler or digital calipers, with measurements taken across the widest diameter of the zone. The presence and size of inhibition zones provide both qualitative and quantitative information about the antimicrobial activity of the herbal extract, with larger zones indicating greater antimicrobial potency. All tests were performed in triplicate to ensure reproducibility of results, and the mean zone diameters with standard deviations were calculated for each test condition and each microorganism. The results were compared with the positive control to evaluate the relative efficacy of the herbal extract against the tested oral pathogens.

RESULT:

Overview of Antimicrobial Activity

The antimicrobial activity of the rosemary and ginger herbal extract was evaluated against a panel of five clinically significant oral pathogens using the agar well diffusion method. The results demonstrate that the herbal extract possesses broad-spectrum antimicrobial activity against all tested microorganisms, with the degree of inhibition varying depending on the specific organism and the concentration of extract applied. The zone of inhibition measured using different concentrations of herbal extract (25 µL, 50 µL, and 100 µL) shows clear concentration-dependent antimicrobial activity against Enterococcus faecalis (Figure 1), Candida albicans (Figure 2), Staphylococcus aureus (Figure 3), Lactobacillus species (Figure 4), and Streptococcus mutans (Figure 5). The positive control antibiotic, amoxicillin, produced varying zones of inhibition against the different test organisms, providing a reference for comparing the efficacy of the herbal extract.Antimicrobial Activity Against Enterococcus faecalisAgainst Enterococcus faecalis, a Gram-positive bacterium frequently associated with persistent endodontic infections and failed root canal treatments due to its ability to survive in nutrient-deprived environments and resist conventional antimicrobial agents, the herbal extract demonstrated substantial and concentration-dependent antimicrobial activity. At the lowest tested concentration of 25 µL, the extract produced a zone of inhibition measuring 28 mm in diameter, indicating significant antimicrobial activity even at this relatively low concentration. This substantial inhibition zone suggests that the extract contains bioactive compounds that are particularly effective against this resistant organism. At the medium concentration of 50 µL, the zone of inhibition increased to 30 mm, demonstrating enhanced antimicrobial activity with increasing extract concentration. The highest tested concentration of 100 µL produced the largest zone of inhibition, measuring 38 mm in diameter, representing the maximum antimicrobial effect observed against this organism. For comparison, the positive control antibiotic amoxicillin produced a zone of inhibition measuring 15 mm against Enterococcus faecalis, which was substantially smaller than the zones produced by all three concentrations of the herbal extract. This finding is particularly noteworthy, as it suggests that the rosemary and ginger extract may be more effective against this pathogen than the standard antibiotic, with the 100 µL concentration producing a zone more than twice the diameter of the antibiotic control.ntimicrobial Activity Against Candida albicansAgainst Candida albicans, the most common fungal pathogen in the oral cavity and a significant cause of opportunistic infections including oral thrush, denture stomatitis, and angular cheilitis, the herbal extract demonstrated concentration-dependent antifungal activity. At 25 µL concentration, the extract produced a zone of inhibition measuring 18 mm, indicating moderate antifungal activity. At 50 µL, the zone of inhibition increased to 22 mm, showing enhanced activity with increased concentration. The highest concentration of 100 µL produced a zone of 30 mm, representing substantial antifungal activity. The positive control antibiotic amoxicillin produced a zone of 20 mm against Candida albicans, which was comparable to the 50 µL concentration of the herbal extract but smaller than the 100 µL concentration. This finding indicates that the herbal extract at its highest concentration possesses antifungal activity superior to the standard antibiotic control against this important fungal pathogen.

Antimicrobial Activity Against Staphylococcus aureus

Against Staphylococcus aureus, a Gram-positive coccus associated with various oral infections including angular cheilitis, parotitis, and infections of the oral mucosa, and which can serve as a reservoir for systemic infections in immunocompromised individuals, the herbal extract produced uniform zones of inhibition across all three concentrations tested. At 25 µL, 50 µL, and 100 µL concentrations, the extract consistently produced zones of inhibition measuring 9 mm in diameter, indicating that even the lowest concentration tested was sufficient to achieve maximal antimicrobial activity against this organism. The lack of concentration-dependent increase suggests that the extract's active compounds are highly effective against S. aureus even at low concentrations, and that the minimum inhibitory concentration for this organism is at or below the 25 µL level. The positive control antibiotic amoxicillin produced a zone of 14 mm against S. aureus, which was larger than the zones produced by the herbal extract. This finding indicates that while the herbal extract possesses definite antimicrobial activity against S. aureus, the standard antibiotic remains more effective against this particular organism.

Antimicrobial Activity Against Lactobacillus Species

Against Lactobacillus species, which contribute to dental caries progression through their ability to produce acid from fermentable carbohydrates and survive in low pH environments, the herbal extract demonstrated the highest antimicrobial activity among all tested organisms. At 25 µL concentration, the extract produced a zone of inhibition measuring 34 mm, representing exceptionally strong antimicrobial activity. At 50 µL, the zone increased slightly to 35 mm, and at 100 µL, the zone reached 36 mm, showing a modest concentration-dependent increase. These large inhibition zones indicate that Lactobacillus species are particularly susceptible to the bioactive compounds present in the rosemary and ginger extract. The positive control antibiotic amoxicillin produced a zone of only 13 mm against Lactobacillus, which was substantially smaller than the zones produced by all three concentrations of the herbal extract. This finding is clinically significant, as Lactobacillus is an important contributor to caries progression, and the superior activity of the herbal extract against this organism suggests potential therapeutic applications.

Antimicrobial Activity Against Streptococcus mutans

Against Streptococcus mutans, the primary etiological agent of dental caries and a key target for caries-preventive strategies, the herbal extract demonstrated strong concentration-dependent antimicrobial activity. At 25 µL concentration, the extract produced a zone of inhibition measuring 22 mm, indicating significant activity against this important pathogen. At 50 µL, the zone increased to 24 mm, and at 100 µL, the zone reached 34 mm, showing a substantial increase in antimicrobial activity with increasing concentration. The positive control antibiotic amoxicillin produced a zone of only 9 mm against S. mutans, which was substantially smaller than the zones produced by all three concentrations of the herbal extract. This finding is particularly significant given the central role of S. mutans in caries pathogenesis, and suggests that the rosemary and ginger extract may be more effective against this organism than the standard antibiotic control.

Summary of Antimicrobial Activity

In summary, the rosemary and ginger herbal extract demonstrated broad-spectrum antimicrobial activity against all five tested oral pathogens, with the degree of activity varying by organism and concentration. The extract showed particularly strong activity against Lactobacillus species and Enterococcus faecalis, with inhibition zones exceeding 30 mm at the highest concentration. Against Streptococcus mutans and Candida albicans, the extract also showed substantial activity, with 100 µL concentrations producing zones of 34 mm and 30 mm respectively. Against Staphylococcus aureus, the extract showed consistent but more modest activity. In comparison with the positive control antibiotic amoxicillin, the herbal extract demonstrated superior activity against Enterococcus faecalis, Lactobacillus species, and Streptococcus mutans, comparable activity against Candida albicans, and slightly inferior activity against Staphylococcus aureus. These findings support the potential use of rosemary and ginger extract as a natural antimicrobial agent for preventing and managing oral infections, and provide a foundation for further research including isolation and characterization of active compounds, formulation development, and clinical evaluation.

Figure 1: Zone of inhibition of rosemary and ginger mediated with TiO2 nanoparticle extract by disk diffusion method showing antimicrobial activity against E. faecalis.

Figure 2: Zone of inhibition of rosemary and ginger mediated with TiO2 nanoparticle extract by disk diffusion method showing antimicrobial activity against C. albicans

Figure 3: Zone of inhibition of rosemary and ginger mediated with TiO2 nanoparticle extract by disk diffusion method showing antimicrobial activity against S. aureus.

Figure 4: Zone of inhibition of rosemary and ginger mediated with TiO2 nanoparticle extract by disk diffusion method showing antimicrobial activity against Lactobacillus.

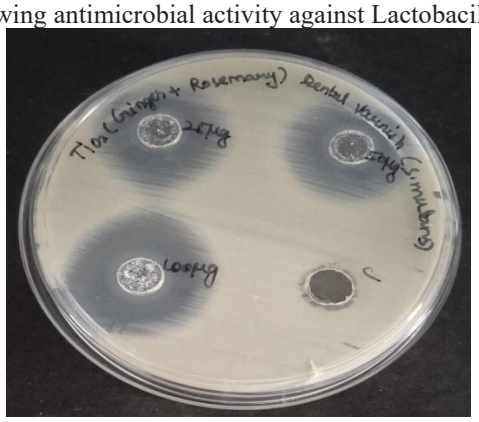

Figure 5: Zone of inhibition of rosemary and ginger mediated with TiO2 nanoparticle extract by disk diffusion method showing antimicrobial activity against S. mutans.

Figure 6: Represents MIC test of microbesE. faecalis, C. albicans, S. aureus, lactobacillus and S. mutans and in tabulation and graph where, X axis represents % of zone of inhibition and Y axis represents microbial pathogens.

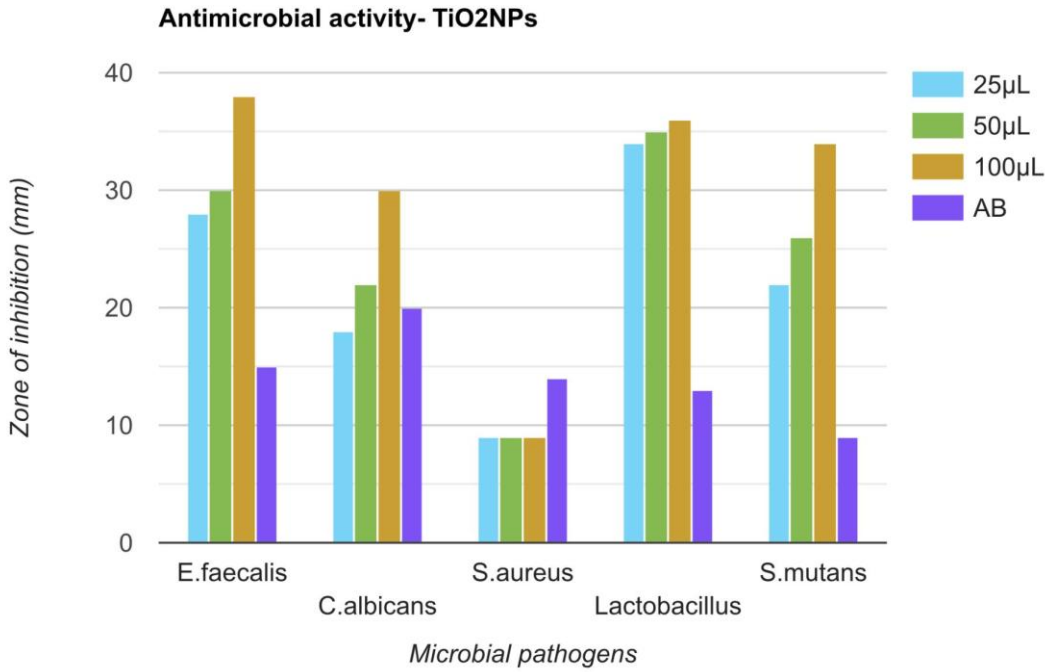

Table 1: Zone of inhibition using different concentrations of herbal extract against S. mutans, S. aureus, E. faecalis, lactobacillus and C. albicans.

organism	25µL	50µL	100µL	com
E faecalis	28	30	38	15
C albicans	18	22	30	20
S aureus	9	9	9	14
Lactobacillus	34	35	36	13
S mutans	22	26	34	9

DISCUSSION:

The present study was undertaken to assess the antimicrobial activity of a herbal dental varnish extract prepared from rosemary (Rosmarinus officinalis) and ginger (Zingiber officinale) and mediated with titanium dioxide nanoparticles. Dental varnishes represent an important vehicle for delivering therapeutic agents to the oral cavity, providing sustained release of active compounds and prolonged contact with oral tissues. The incorporation of herbal extracts with documented antimicrobial properties into dental varnish formulations offers a promising approach for preventing and managing oral infections while potentially avoiding some of the limitations associated with synthetic antimicrobial agents, including side effects and the development of antimicrobial resistance. The findings of this study demonstrate that the rosemary and ginger extract possesses broad-spectrum antimicrobial activity against a panel of clinically significant oral pathogens, supporting its potential use in dental varnish formulations.Comparison with Salvia officinalis StudiesRafael de Oliveira Jonatas and colleagues evaluated the antimicrobial activity of Salvia officinalis (sage) extract against oral bacterial and fungal species, providing a useful comparison for the present study. The antimicrobial activity of Salvia officinalis extract was tested against reference strains of Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus mutans, Candida albicans, Candida tropicalis, and Candida glabrata isolated from clinical oral cavity samples. The investigators

determined the minimum inhibitory concentrations, minimum bactericidal concentrations, and minimum fungicidal concentrations of the extract, as well as assessing its cytotoxic effects on mammalian cells. All isolates of Staphylococcus species, Streptococcus mutans, and Candida species were effectively treated with Salvia officinalis extract, and importantly, no cytotoxic effects were observed (9). This finding of broad-spectrum antimicrobial activity without cytotoxicity aligns with the results of the present study, where the rosemary and ginger extract demonstrated activity against all tested organisms including S. mutans, S. aureus, and Candida albicans. The absence of cytotoxic effects in the sage study supports the safety profile of plant-derived antimicrobials and suggests that similar biocompatibility may be expected for rosemary and ginger extracts, though specific cytotoxicity testing for the current formulation would be needed to confirm this.

Comparison with Solvent Extract Studies

Ghezelbash and colleagues evaluated the antimicrobial properties of Salvia officinalis extracts prepared using different solvents against the bacteria Bacillus anthracis, Bacillus cereus, Escherichia coli, and Staphylococcus aureus. The disc diffusion method was used to investigate three plant solvent extracts: ethanol, acetone, and deionized water. The outcomes showed that the inhibitory effects of acetone concentrate of Salvia officinalis were particularly notable, with minimum inhibitory concentrations of 10 mg/mL for Bacillus anthracis and 30 mg/mL for Staphylococcus aureus. Interestingly, the sensitivity of Gram-negative microorganisms to the extracts was greater than that of Gram-positive organisms in their study (10). This finding differs somewhat from the results of the present study, where strong activity was observed against both Gram-positive and Gram-negative organisms, though direct comparison is complicated by differences in extraction methods, test organisms, and assay conditions. The authors concluded that organic solvent extracts from this plant, particularly acetone leaf extracts, can be utilized as natural antimicrobial products, supporting the broader concept that plant-derived antimicrobials have potential for practical applications. The present study extends this concept by demonstrating that aqueous extracts of rosemary and ginger, prepared without organic solvents, also possess significant antimicrobial activity, offering a potentially more environmentally friendly and safer approach for therapeutic applications.

Comparison with Essential Oil Studies

Chenchen Cai and colleagues prepared and evaluated the antimicrobial activity of thyme essential oil, another plant-derived product with documented medicinal properties. Their results showed that thyme essential oil functions as a natural bacteriostatic agent and has the potential to be widely used in the food processing industry for preserving food products and preventing spoilage (11). This finding highlights the diverse applications of plant-derived antimicrobials beyond clinical medicine and supports the broader concept that natural products can serve as effective alternatives to synthetic antimicrobial agents in various contexts. The antimicrobial activity of thyme oil against foodborne pathogens and spoilage organisms parallels the activity observed in the present study against oral pathogens, suggesting that plant-derived antimicrobials may have utility across multiple applications.he authors Monika Sienkiewicz and colleagues examined the antimicrobial action of thyme essential oil against bacterial strains belonging to the Staphylococcus, Enterococcus, Escherichia, and Pseudomonas genera. The microbial growth inhibition of Thymus vulgaris oil at various concentrations was determined through agar diffusion testing, and susceptibility testing to conventional antimicrobials was performed using disc diffusion for comparison. Thyme essential oil emphatically repressed the development of the clinical isolates of microorganisms tested, demonstrating potent antimicrobial activity against a range of pathogens including some that are notoriously difficult to treat (12). The strong activity observed against Enterococcus species is particularly relevant to the present study, where the rosemary and ginger extract showed exceptional activity against Enterococcus faecalis, with inhibition zones reaching 38 mm at the highest concentration tested. This convergence of findings across different plant species suggests that plant-derived antimicrobials may share common mechanisms of action or target similar cellular structures in microorganisms.Linda and colleagues concentrated on the antimicrobial action of cinnamon oil against a comprehensive panel of microorganisms including Staphylococcus aureus, Escherichia coli, Enterobacter aerogenes, Proteus vulgaris, Pseudomonas aeruginosa, Vibrio cholerae, Vibrio parahaemolyticus, Salmonella typhimurium, Candida albicans, Candida tropicalis, Candida glabrata, and Candida krusei. The study found that cinnamon displayed antimicrobial activity against every one of the organisms that were tested, demonstrating remarkably broad-spectrum activity encompassing both Gram-positive and Gram-negative bacteria as well as multiple Candida species (13). This broad-spectrum activity is similar to that observed in the present study, where the rosemary and ginger extract was active against all five tested oral pathogens, including both bacterial and fungal species. The consistency of broad-spectrum activity across different plant species suggests that plants produce antimicrobial compounds as part of their defense mechanisms against a wide range of potential pathogens, and that these compounds may have utility in human medicine for similar purposes.Yasser Shabhazi and colleagues examined the chemical composition and antibacterial activity of essential oil extracted from the leaves of the Mentha spicata (spearmint) plant against Staphylococcus aureus and discovered that mint possessed antimicrobial activity against the organisms tested (14). This finding adds to the growing body of evidence that common culinary herbs possess significant antimicrobial properties that may be harnessed for therapeutic purposes. The activity against S. aureus observed in the mint study is consistent with the findings of the present

study, where the rosemary and ginger extract showed activity against this organism, though the zones of inhibition were relatively modest compared to those observed against other test organisms.

Comparison with Present Study Findings

The dental varnish extract of ginger and rosemary mediated with titanium dioxide nanoparticles tested in the present study showed significant antibacterial activity against Streptococcus mutans, Enterococcus faecalis, Candida albicans, Lactobacillus species, and Staphylococcus aureus at various concentrations, confirming the findings of the current study and extending them to demonstrate concentration-dependent effects. The particularly strong activity observed against Lactobacillus species and Enterococcus faecalis, with inhibition zones exceeding 30 mm at the highest concentration, suggests that these organisms may be especially susceptible to the bioactive compounds present in rosemary and ginger. The activity against Streptococcus mutans, the primary etiological agent of dental caries, is particularly significant and supports the potential use of this herbal extract in formulations aimed at caries prevention. The activity against Candida albicans, the most common fungal pathogen in the oral cavity, further broadens the potential applications of this extract to include management of oral fungal infections.The incorporation of titanium dioxide nanoparticles in the formulation may offer additional benefits beyond those provided by the herbal extract alone. Titanium dioxide nanoparticles have been shown to possess antimicrobial properties themselves and may enhance the stability and delivery of the herbal extract components. The combination of plant-derived antimicrobial compounds with nanoparticle technology represents a promising approach for developing effective oral care products that leverage the advantages of both natural and nanotechnological approaches.

Need for Clinical Trials

The authors note that clinical trials are necessary to verify the findings of this in vitro study and to establish the safety and efficacy of the rosemary and ginger dental varnish formulation for human use. While in vitro studies provide valuable preliminary data and allow for controlled evaluation of antimicrobial activity, they cannot fully replicate the complex environment of the oral cavity, where factors such as saliva flow, dietary influences, host immune responses, and the presence of complex microbial biofilms may affect the performance of antimicrobial agents. Clinical trials involving human participants are essential for determining optimal formulations, dosing regimens, and application protocols, as well as for assessing potential side effects and patient acceptability. The promising results obtained in this in vitro study provide a strong foundation for advancing to clinical investigation and support the potential development of this herbal dental varnish as a natural alternative or adjunct to conventional antimicrobial agents for preventing and managing oral diseases.

CONCLUSION

The present study successfully demonstrated the antimicrobial activity of a herbal dental varnish extract prepared from rosemary (Rosmarinus officinalis) and ginger (Zingiber officinale) and mediated with titanium dioxide nanoparticles against a panel of clinically significant oral pathogens. The extract exhibited concentration-dependent antimicrobial activity against Streptococcus mutans, Enterococcus faecalis, Candida albicans, Lactobacillus species, and Staphylococcus aureus, with particularly strong activity observed against Lactobacillus and E. faecalis, where inhibition zones exceeded 30 mm at the highest concentration tested. Notably, the herbal extract showed superior antimicrobial activity compared to the standard antibiotic control (amoxicillin) against E. faecalis, Lactobacillus, and S. mutans, comparable activity against C. albicans, and modest activity against S. aureus. These findings align with previous studies documenting the antimicrobial properties of various medicinal plants including sage, thyme, cinnamon, and mint, and support the growing body of evidence that plant-derived compounds can serve as effective natural alternatives to synthetic antimicrobial agents. The incorporation of titanium dioxide nanoparticles offers potential advantages in terms of formulation stability and enhanced delivery. The broad-spectrum antimicrobial activity demonstrated in this study, combined with the natural origin of the active ingredients and the established safety profiles of rosemary and ginger, supports the potential development of this herbal dental varnish for clinical applications in preventing and managing oral infections, including dental caries and candidiasis. However, clinical trials are necessary to verify these in vitro findings, establish appropriate formulations and dosing regimens, and confirm safety and efficacy in human subjects before this herbal formulation can be recommended for routine clinical use.

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