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RESEARCH ARTICLE
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Comparative Evaluation of Antimicrobial Efficacy of German Chamomile Extract, Tea Tree Oil and Chlorhexidine as Root Canal Irrigants against E-faecalis and Streptococcus Mutans - An In Vitro Study

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ABSTRACT

Background: In endodontic therapy, root canal irrigants are utilized to enhance mechanical debridement. However, conventional irrigants have certain drawbacks, such as being incapable of removing the smear layer, causing tissue irritation, toxicity, and unpleasant taste, especially in children. Therefore, there is a growing necessity to explore natural alternatives, such as herbal irrigants. This study sought to evaluate the antimicrobial effectiveness of German chamomile, tea tree oil (TTO), and chlorhexidine (CHX) as root canal irrigants against E. faecalis and Streptococcus mutans. Method: Fifteen test samples of German chamomile extract, tea tree oil, and chlorhexidine were taken. Indicator strains were grown in Brain Heart Infusion (BHI) agar at 37ºC for 24 hr. In preparation for the experiment, a 15 ml base layer of BHI agar was mixed with 300µl of each inoculum and placed in individual sterilized petri plates. Once the culture medium had solidified, four wells were created on each plate and subsequently filled with the designated testing materials. This methodology was employed to ensure methodological rigor and accuracy in the experimental process. In preparation for the experiment, a 15 ml base layer of BHI agar was mixed with 300µl of each inoculum and placed in individual sterilized petri plates. Once the culture medium had solidified, four wells were created on each plate and subsequently filled with the designated testing materials. This methodology was employed to ensure methodological rigor and accuracy in the experimental process. The plates were kept for 2 hours at room temperature for the diffusion of the material and then incubated at 37ºC for 48 hours. Antibacterial tests were repeated 5 times for homogeneity of the results. Results: The comparison of antimicrobial efficacy of chlorhexidine 2%, tea tree oil, and German chamomile showed a significant difference whereas the comparison between chlorhexidine 2% and tea tree oil was statistically insignificant. Conclusion: The effective reduction in the microbial count by all three test irrigants was observed to be significant. The antimicrobial efficacy of tea tree oil was found to be better than 2% chlorhexidine and German chamomile extract.

Keywords: Chlorhexidine, E-faecalis, German chamomile, Irrigants, Streptococcus mutans.

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INTRODUCTION

Successful endodontic treatment depends on three crucial elements: instrumentation, disinfection and obturation. In pediatric endodontics, the primary aim is to preserve deciduous teeth and aid in the emergence of permanent successors (Munaga et al., 2022). To achieve optimal results, irrigation is essential to access the intricate anatomy of root canals and remove tissues that can't be reached with mechanical cleaning (Neha et al., 2014). Sodium hypochlorite is the preferred and most commonly used irrigant in endodontics, but other options include Chlorhexidine (CHX), distilled water, Ethylene diamine tetra acetic acid (EDTA), Qmix, tetra clean, saline, Mixture of tetracycline acid and detergent (MTAD), and herbal alternatives such as green tea and Triphala (Bhavani et al., 2023).

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Endodontic infections are caused by a mixture of microorganisms, with facultative anaerobes like enterococci and streptococci being the major culprits behind treatment failure (Kairey et al., 2023). With the rise of antibiotic-resistant strains and unwanted side effects of chemical irrigants, there is a growing demand for natural remedies. Various plant extracts, such as neem and tulsi extracts, aloe vera, moringa centifolia, Curcuma longa, tea tree oil (TTO), chamomile and turmeric, possess therapeutic, antibacterial, and anti-inflammatory properties. TTO, an aromatic volatile oil from Melaleuca alternifolia, acts on bacterial cell walls by modifying cell membrane permeability, inhibiting bacterial growth, and disrupting oxygen usage. (Bagwe et al., 2023) It is utilized as an active component in topical preparations and as a mild solvent. German chamomile, a medicinal herb found in south and east Europe, interferes with bacterial ATP synthesis and pH balance, providing anti-inflammatory, antibacterial, sedative, healing, and spasmolytic effects (Karkare et al., 2015; Moukarab, 2020; Singhania et al., 2022; Susila et al., 2023).

Chlorhexidine (CHX) has emerged as a highly effective root canal irrigant owing to its potent antimicrobial activity against a broad spectrum of microorganisms and its ability to bind to dentin. Its mechanism of action involves the penetration of bacterial biofilm and disruption of cell membranes, leading to cell death (Mathur et al., 2018, Cherian et al., 2016). In addition, CHX has demonstrated substantivity for up to 12 weeks, indicating its long-lasting antimicrobial effects. Its molecular structure is conducive to biofilm penetration, thereby allowing it to infiltrate the matrix that envelops biofilm-ensconced microorganisms, following which it exerts its disruptive influence on the bacterial cell membrane by inducing structural alterations and impairing membrane integrity (Comparison of Antimicrobial Efficacy of Octenidine Dihydrochloride and Chlorhexidine as Endodontic Irrigant: A Systematic Review, n.d.). By binding to the lipid components of the membrane, CHX prompts intracellular leakage and disturbs essential cellular functions, ultimately leading to the demise of the bacterial cell. This process not only addresses the biofilm-protected bacteria but also those that are yet to become entrenched, thus impeding the establishment of biofilm formations and eradicating existing ones (Punathil et al., 2020). Notably, the sustained antimicrobial activity of CHX further augments its appeal as an irrigant, as it adheres to root canal surfaces, providing prolonged protection against bacterial recolonization (Afkhami et al., 2023). Its broad-spectrum efficacy extends to a wide range of microorganisms, encompassing both monoderm and diderm bacteria, as well as yeasts (Palipana et al., 2023).

Despite its evident virtues, caution must be exercised due to concerns about its potential cytotoxic effects on periapical tissues and interactions with dentin. The efficacy of German chamomile, tea tree oil and chlorhexidine against E. faecalis and Streptococcus mutans is not well established in the literature, prompting the design of this study.

MATERIALS & METHODS

A study was conducted at the Department of Microbiology, Rajeev Gandhi Institute of Biotechnology, Bharati Vidyapeeth, Pune, Maharashtra, with the aim of testing the antimicrobial activity of three indicator strains - German Chamomile (25%; Terra Firma botanicals), Tea Tree Oil (5%; Aroma magic –Aromatherapy Essential oil), and Chlorhexidine (2%; Consepsis-Chlorhexidine Antibacterial Solution). To conduct the research, 15 samples of each of these three materials were used. The indicator strains were grown on Brain Heart Infusion (BHI) agar at 37ºC for 24 hours, using sterilized petri plates (20 x 100mm) to prepare a base layer of 15ml BHI agar mixed with 300µl of each inoculum. Four wells of 7mm diameter and 4mm depth were made on each plate, and one of the testing materials was filled in each well. The plates were kept at room temperature for 2 hours to allow material diffusion, followed by incubation at 37ºC for 48 hours. Two organisms, Enterococcus faecalis (ATCC2912) and Streptococcus mutans (NCIM Accession no:5660), were tested in this study using the colony-forming unit (CFU) method to assess the antimicrobial activity of the organisms in vitro as it is considered the gold standard for such assessments. The study only included cultivable microorganisms that could start cell division and create colonies. The zones of bacterial inhibition and material diffusion were measured using a vernier calliper in millimeters and photographed to ensure homogeneity of results. (Fig. 1) The antibacterial tests were repeated five times to confirm the results' validity. The results were tabulated and subjected to statistical analysis, including ANOVA and Tukey's post hoc tests to compare the zone of inhibition among the groups and check for significant differences between the groups against both bacteria, respectively. A culture-dependent approach was used to identify bacteria in samples taken after antibiotic treatment, as it is one of the most reliable ways to do so.

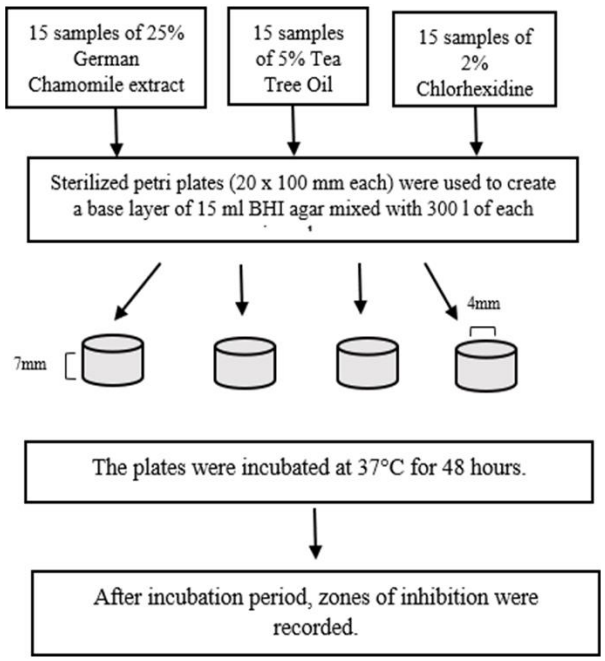

Fig. 1: Schematic representation of the grouping of samples.

RESULTS

Comparison of the zone of inhibition in terms of {Mean (SD)} for *E faecalis and Streptococcus mutans* among all the groups revealed that TTO with a mean of 15.27mm and 16.69mm had the highest zone of inhibition followed by CHX (15.09mm and 14.32mm) and German chamomile hydroalcoholic extract (11.58mm and 11.82mm), and also showed a statistically significant difference between all the three groups (p=0.001 and 0.005), respectively (Table 1 and 2).

Further using Tukey's post hoc analysis, a significant difference was observed between TTO and German Chamomile (p=0.001 and 0.003) for *E. faecalis and S. mutans,* respectively. On comparing the three groups, TTO showed higher antimicrobial efficacy when compared to

Table 1: Comparison of the zone of inhibition in terms of {Mean (SD)} among all the groups using ANOVA test for *E. faecalis*.

Group	*Mean*	*Std. Deviation*	*F value*	*P value*
Chlorhexidine	15.093	2.5594		
Tea tree oil	15.273	2.1255		
German Chamomile	11.580	1.7506		
Total	13.982	2.7301	13.806	<0.001**

(p < 0.05 - Significant*, p < 0.001 - Highly significant**)

Table 2: Comparison of the zone of inhibition in terms of {Mean (SD)} among all the groups using ANOVA test for *S. mutans*.

Group	*Mean*	*Std. Deviation*	*F value*	*P value*
Chlorhexidine	14.320	1.6463		
Tea tree oil	16.693	5.6939		
German Chamomile	11.820	2.9197		
Total	14.278	4.2355	6.122	0.005*

(p < 0.05 - Significant*, p < 0.001 - Highly significant**)

Table 3: Post-hoc analysis for *E. Faecalis.*

	Chlorhexidine	*Tea tree oil*	*German Chamomile*
Chlorhexidine	-	0.972	<0.001**
Tea tree oil	0.972	-	<0.001**
German Chamomile	<0.001**	<0.001**	-

Table 4: Post-hoc analysis for *S. mutans*.

	Chlorhexidine	*Tea tree oil*	*German Chamomile*
Chlorhexidine	-	0.216	0.184
Tea tree oil	0.216	-	0.003*
German Chamomile	0.184	0.003*	-

CHX 2% followed by German chamomile but the difference between TTO and CHX was statistically insignificant (Fig. 2, 3, and 4) (Table 3 and 4).

DISCUSSION

Tea tree oil, obtained from the Melaleuca alternifolia plant, has been found to offer a wide range of health benefits. These benefits include its ability to act as an antiseptic and anticancer properties. Its hydrocarbon structure and lipophilicity enable it to selectively partition into biological membranes, hindering their essential functions. Due to its mild solvent properties and antibacterial and antifungal qualities, TTO is considered an excellent treatment for open wounds, and sores. Recent research by Sheth et al. demonstrated that TTO is as effective as sodium hypochlorite in cleansing root canal systems but less harmful (Sheth et al., 2013). TTO can also be used to treat various ailments, but it should be administered in therapeutic doses of 2.5 to 5% to maintain its antibacterial properties. The study found that TTO was the most effective against E. faecalis and S. mutans.

In addition, several studies have evaluated the antibacterial efficacy of a 2% CHX solution as an endodontic irrigation (Khademi et al., 2006; Leonardo et al., 1999; White et al., 1997). In vivo and in vitro experiments have shown that CHX can reduce microbial activity in the root canal system for up to 48 hours and 72 hours, respectively. CHX can also be effective for up to 12 weeks following a 10-minute treatment, according to some studies (Khademi et al., 2006) (Rosenthal et al., 2004). Three concentrations of CHX solution (4%, 2%, and 0.2%) were recently tested for their antibacterial properties and the findings suggest that

Fig. 2: Zone of inhibition of chlorhexidine, tea tree oil and German chamomile extract on Streptococcus Mutans and E-Faecalis

Fig. 3: Zone of inhibition of chlorhexidine, tea tree oil and German chamomile extract on Streptococcus Mutans

Fig. 4: Zone of inhibition of chlorhexidine, tea tree oil And German chamomile extract on E-Faecalis

CHX can be a useful tool for endodontic irrigation and preventing microbial colonization of the root canal system (Mohammadi, 2008). The present study confirmed that CHX is a viable root canal irrigant after TTO.

According to the findings of the study, German Chamomile extract can be used as an endodontic irrigant,

but its antimicrobial efficacy value is comparatively lower than the other two. This may be due to the presence of nitric oxide, which can cause inflammation and central sensitization. Chamomile contains flavonoids and bisabolol, which can block peripheral nociceptors by inhibiting COX-2. Studies have shown that chamomile cream can reduce perineal discomfort following episiotomy (Husen et al., 2021). It also demonstrates inhibitory effects against common root canal pathogens like Enterococcus faecalis and Candida albicans (Pazelli et al., 2003; Mohan et al., 2023). Combining German Chamomile extract with other irritants like sodium hypochlorite may enhance its antimicrobial spectrum. Its biocompatibility with human tissues has also been observed (Mohammed and Selivany, 2021; Kebede et al., 2021). The study also found that chamomile was more effective than NaOCl alone in removing smear layers than NaOCl + EDTA (Murugesan et al., 2022). The research findings indicate that Tea Tree Oil (TTO) could serve as a viable root canal irrigant for young patients, exhibiting antimicrobial properties similar to Chlorhexidine (CHX). Nevertheless, it is important to note that the study has certain limitations. Firstly, the agar diffusion technique does not accurately reflect the dynamic changes in the root canal system or reproduce its complexity because it only reports antimicrobial activity in a static setting (Balouiri et al., 2016). Secondly, studies conducted in vitro do not take into consideration possible interactions between irrigants and other endodontic treatment materials such as sealers and obturation materials (Jose et al., 2021).

The study found that TTO, followed by CHX, and German Chamomile, demonstrated appreciable antibacterial effectiveness against both infections. More preclinical and clinical experiments are needed to assess biocompatibility and safety before TTO can be firmly suggested as an intra-canal irrigating solution.

Conclusion

According to the current study, the administration of three different test irrigants resulted in a significant reduction of bacterial count. Among the tested substances, tea tree oil demonstrated superior antibacterial activity relative to German chamomile hydroalcoholic extract and 2% chlorhexidine. Additionally, despite the fact that there was no statistically significant difference between German chamomile and 2% chlorhexidine, a comparison of the antibacterial efficacy of German chamomile, TTO, and 2% CHX revealed significant variances. The findings indicate that the implementation of Tea Tree Oil (TTO) as a root canal irrigant could be a promising approach, owing to its efficacy in impeding the proliferation of microorganisms. However, further research is required to ascertain its toxicity and biofilm formation inhibition properties before it can be applied in a clinical setting. Overall, these findings are significant and contribute to the ongoing effort to develop effective treatments for root canal infections.

Ethics Approval

Ethical Approval was taken from Bharati Vidyapeeth Dental College and Hospital, Pune.

Availability of the data

All the data will be made available on request.

Competing Interest

The authors declare that there is no conflict of interest.

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Authors' Contributions

All of the authors on the list made a significant, direct, and intellectual contribution to the work and gave their permission for publication.

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