Research Article

Antimicrobial activity of various natural oils on *Enterococcus faecalis*

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ABSTRACT

Aim: This study aims to study the antimicrobial activity of various natural oils on *Enterococcus faecalis*. **Background:** Antimicrobial resistance in bacteria raises serious concern for the continued efficacy of antimicrobial agents in medicine, agriculture, and industry. With the increase in the prevalence of microbial resistance to conventional antiseptics and antibiotics, attention is now turning to the use of natural antimicrobial compounds. The escalating demand for new antimicrobials has prompted several investigations into the antimicrobial effects of phytochemicals extracted from a range of botanic origins, most of which have been used traditionally for many years. **Materials and Methods:** The essential oils of neem and tea tree are diluted into aqueous solutions of varying concentrations of 1:5, 1:10, and 1:15 and subjected to minimum inhibitory concentration evaluation. The zones of inhibition were measured and tabulated. **Results:** It was observed that the antimicrobial efficacy of both the oils increased with their increase in concentration. However, the action of neem was more potent than tea tree oil. **Conclusion:** This study conducted suggests that the naturally occurring components of the essential oils can be a good alternative for the currently available root canal irrigants that might not guarantee the success of treatment due to their inability to combat *E. faecalis*.

KEYWORDS: Antimicrobial, *Enterococcus faecalis*, Essential oil, Neem, Tea tree

INTRODUCTION

In recent times, the use of naturally available substitutes in place of chemical materials used to treat various infections and dental infections are no exception. Medicines obtained from natural sources, also known as phytomedicines, have been increasingly used for their anti-inflammatory, antibiotic, analgesic, and sedative agents. They are a very good solution to tackle antibiotic overuse and misuse.

The dental pulp, which is the primary aspect to determine the vitality of the tooth structure, if invaded by micro organisms will lead to pathological changes like formation of dental caries. Most of these organisms are usually present in the oral cavity, due to the pathological changes the nutritional availability and the oxygen potential reductions in the root canals of the affected tooth are observed to have a limited number of microorganisms which cause the infection. The above-mentioned environment in the root canals facilitates the growth of facultative and anaerobic organisms, mainly *Enterococcus faecalis*. These organisms primarily survive as a single organism in the root canal and multiply causing infection and enhancing local bone resorption.[1] The failure of endodontic treatment due to *E. faecalis* is primarily due to their ability to invade dentinal tubules and adhere to collagen in the presence of human serum.[2] These organisms primarily grow due to biofilm transformation that enhances bacteria to stick and multiply on the surfaces.[3] Thus, the primary method to maintain the root canal patency and ensure the success of root canal treatment, proper debridement, and disinfection of the root canal system is necessary to prevent reinfection.[4,5]

In infected cases, along with proper chemomechanical preparation of the root canal to reduce the bacterial count, efficient intracanal medicament that possesses antibacterial action is required to maximize disinfection.[6] Several therapeutic agents are used such

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as sodium hypochlorite, 2% solution of chlorhexidine, and calcium hydroxide which have varies antibacterial efficacy.[7] The most commonly used root canal irrigant is sodium hypochlorite due to its tissue dissolving properties and antimicrobial action. However, they also possess many undesirable properties such as allergic potential, cause emphysema with overfilling, lack of potential to remove smear layer, and poor smell and taste. This lack to remove smear layer acts as an avenue for microleakage and a potential nidus for bacterial growth and ingress.[3,5,8] Chlorhexidine is used for canal irrigation due to its broad spectrum of antimicrobial activity and ability to disinfect the canals against *E. faecalis*. However, its primary disadvantage, includes discoloration of teeth and tongue, can cause loss of taste, burning sensation in the oral mucosa, and subjective dryness of the oral cavity. Furthermore, the efficacy of chlorhexidine is lesser when compared to that of sodium hypochlorite.[3] The use of calcium hydroxide as an irrigant is minimal as it fails to remove bacteria present in the dentinal tubules and recent studies show that *E. faecalis* present in the dentinal tubules were resultant to calcium hydroxide for over 10 days.[9,10]

Due to the insufficient efficacy of the available allopathic formulations with potential side effects and safety concerns, natural alternatives from medicinal plants are being considered to replace them. This study primarily focuses on the use of neem and tea tree oils as a potential alternative for the presently available irrigants.

MATERIALS AND METHODS

Essential Oils

Commercially available neem and tea tree oils were obtained to be used in this study. The oils were selected based on literature survey and their use in traditional medicine.

Test Organism

The test organism *E. faecalis* (ATCC 29212) was obtained from the Department of Microbiology, Meenakshi Dental College, Chennai, India. The cultures of bacteria were maintained in their appropriate agar slants at 4°C throughout the study.

Antibacterial Assay

The essential oils were tested to their antimicrobial efficacy with the help of disc diffusion method, which is usually used to select between essential oils for their efficiency. It was performed using 24 h culture at 37°C in 10 ml of Mueller-Hinton Broth. A sterile cotton swab was used to uniformly place the suspensions on the agar plates. The essential oils were dissolved in 10% aqueous dimethylsulfoxide and sterilized by filtration. Empty sterilized discs were impregnated with 50 µL of different concentrations (1:5, 1:10, and 1:20) of the respective essential oils and placed on the agar plates. The plates were left at room temperature for 30 min to allow the diffusion of the oil and later incubated. After the incubation period, the zones of inhibitions were measured and tabulated.

RESULTS

The zones of inhibition obtained for the action of neem and tea tree oils against *E. faecalis* obtained using the disc diffusion method have been tabulated [Table 1].

It is observed that both the essential oils tested showed significant antimicrobial action against the test organism and also that the degree of antimicrobial activity increased with the increase in concentration of the essential oil. Furthermore, tea tree oil was seen to have more antimicrobial action than that of neem with increase in concentrations.

On statistical analysis, independent *t*-test was applied between the three groups, namely Group I (sodium hypochlorite), Group II (neem), and Group III (tea tree). The data obtained are tabulated below [Tables 2-4]. It was observed that there was statistical significance between Groups I and II which was absent in case of the other comparisons.

DISCUSSION

Essential oils are volatile mixtures of hydrocarbons with a diversity of functional groups. Their use in natural medicine has been recognized for many years. The oils tested in this study, namely neem and tea tree oils, have had potential antimicrobial action in multiple studies conducted.

Table 1: The antimicrobial action of neem and tea tree oil against Enterococcus faecalis in varied concentrations

Essential Oil tested	Zone of Inhibition (diameter in mm)		
	5%	10%	20%
Neem oil	16	18	19
Tea tree oil	15	18	20
Sodium hypochlorite	17	18	18
Neem oil	13	15	16
Tea tree oil	17	18	22
Sodium hypochlorite	18	20	19
Neem oil	17	18	20
Tea tree oil	15	18	20
Sodium hypochlorite	19	20	23

Table 2: *t*-test between neem and sodium hypochlorite

Test group	*n*	Mean±SD	*t* value	*P* value
Neem	9	16.89±2.147	−2.399	0144
Sodium hypochlorite	9	19.11±1.763		

SD: Standard deviation

Table 3: *t*-test between tea tree and sodium hypochlorite

Test group	*n*	Mean±SD	*t* value	*P* value
Tea tree	9	18±2.121	−1.208	1222
Sodium hypochlorite	9	19.11±1.763		

SD: Standard deviation

Table 4: *t*-test between neem and sodium hypochlorite

Test group	*n*	Mean±SD	*t* value	*P value*
Tea tree	9	18±2.121	−1.104	1428
Neem	9	16.89±2.147		

SD: Standard deviation

Azadirachta indica (neem), also known as Indian lilac, is found to be one of the most versatile medicinal plants with a variety of biological activity. Its importance is recognized by the US National Academy and neem has been titled as "a tree for solving global problems." The primary active ingredients of neem include azadirachtin, meliacin, gedunin, salanin, nimbin, and valassin. It also possesses anti-adherence action by altering bacterial adhesion and reduces the capability of the organism to colonize. The oils obtained have a broad spectrum of action against Gram-positive as well as negative bacteria. Its action as an antifungal, antiviral, antibacterial, and anticariogenic agent makes a good candidate as a root canal irrigant. Its property as a biocompatible antioxidant strengthens its ability as a root canal irrigant as they will not cause potential damage as it occurs with sodium hypochlorite.[10] A study conducted by Naiyak Arathi showed that ethanolic extracts of neem had potential antimicrobial activity against *E. faecalis*.[11] Another study that compared the antimicrobial efficacy of neem, sodium hypochlorite, green tea, saline, and *Morinda citrifolia* suggested that neem had better antimicrobial efficacy than sodium hypochlorite.[12] Many other studies studied the effect of aqueous and alcoholic extracts of neem against *Streptococcus mutans* and *E. faecalis*.[13-16] These results are similar to the findings in the present study.

Tea tree oil (*Melaleuca alternifolia*) is a plant native to Australia which many properties such as a mild solvent, antiseptic, analgesic, anti-inflammatory, and antifungal agent.[17] They are used in cosmetics and in the health-care products. The primary antimicrobial oil terpinen-4-ol is responsible for most properties. It also has other compounds such as **α**-terpineol with similar properties.[17,18] A study conducted in the year 2006, SEM study was conducted using German chamomile extract and tea tree oil extract to test their disinfecting efficacy of the root canal systems, but the results obtained were contradictory to that obtained in the present study. Thus, it is necessary to conduct further studies to conclude their properties as an irrigant definitively.[19,20]

CONCLUSION

The major advantage of using herbal alternatives is easy accessibility, cost-effective, low toxicity, increased shelf life, and lack of microbial resistance so far. Even though the *in vitro* studies are successful, preclinical and clinical testing is necessary to assess the biocompatibility and safety factor before recommending them as intracanal irrigating solution and medicaments as an alternative to present available components. Thus, their potential risks, side effects, and drug interactions must be tested before using them in daily clinical practice.

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