

Comparison of the Antibacterial Efficacy of Tea Tree Oil with 3% Sodium Hypochlorite and 2% Chlorhexidine against *E. faecalis*: An *in vitro* Study

Uday Kamath, Hina Sheth, Sai Ramesh, Keshav Singla

ABSTRACT

Background and objectives: Among the bacterial species which most often cause endodontic failure, *Enterococcus faecalis* is the most important. This study compared the antibacterial efficacy of tea tree oil with 3% sodium hypochlorite and 2% chlorhexidine as a root canal irrigant, against *E. faecalis*.

Materials and methods: Normal saline, tea tree oil, 3% sodium hypochlorite, 2% chlorhexidine and fresh stains of *E. faecalis* were used. Four round wells, 4 mm deep and 8 mm diameter, were punched using sterile cork borer in blood agar plates and divided into four groups: group A: normal saline (control), group B: 3% sodium hypochlorite, group C: 2% chlorhexidine and group D: tea tree oil. Agar well diffusion method was performed to compare the antibacterial efficacy of tea tree oil, 3% sodium hypochlorite and 2% chlorhexidine.

Results: The results were tabulated and statistically analyzed using analysis of variance (ANOVA). Tea tree oil showed comparable inhibition of bacterial growth with sodium hypochlorite and chlorhexidine. No zone of inhibition was shown by normal saline (control).

Interpretation and conclusion: Tea tree oil showed statistically significant antimicrobial activity against *Enterococcus faecalis*, which is very much similar to sodium hypochlorite and chlorhexidine.

Abbreviations: CHX: Chlorhexidine; *E. faecalis*: *Enterococcus faecalis*; NaOCl: Sodium hypochlorite.

Keywords: Chlorhexidine, *Enterococcus faecalis*, Inhibition zone, Sodium hypochlorite, Tea tree oil.

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INTRODUCTION

The major objective in root canal treatment is to disinfect the entire root canal system. Although cleaning, shaping and use of antimicrobial medicaments are effective in reducing the bacterial load, some bacteria do remain behind and multiply, causing reinfection of the canal.

Enterococcus faecalis is the most commonly isolated species from the canals of teeth presenting post-treatment diseases. *Enterococcus faecalis* account for up to 77% of therapeutic failures.¹ Residual pulpal tissue, bacteria and dentine debris may persist in the irregularities of root canal systems, even after meticulous mechanical preparation.²

Therefore, irrigant solutions should be used in combination with canal preparation.³

Root canal irrigants are used during chemomechanical procedures not only as antimicrobial agents but also to flush out loose debris, to lubricate the dentinal walls and to dissolve organic compounds in the canal. Sodium hypochlorite (NaOCl) has been the most widely used root canal irrigating solution. The main advantages of sodium hypochlorite are its ability to dissolve necrotic tissue and its antibacterial properties against microorganisms. However, it has several undesirable characteristics, such as tissue toxicity, risk of emphysema, allergic potential and disagreeable smell and taste.⁴

The constant increase in antibiotic resistant strains and side effects caused by synthetic drugs has prompted researchers to look for herbal alternatives. *Morinda citrifolia* juice,⁵ neem leaf extract (*Azadirachta indica*),⁶ ginger,⁷ triphala and green tea polyphenols⁸ had been evaluated previously as endodontic irrigants.

Tea tree or *Melaleuca alternifolia* (Fig. 1)⁴ is a native Australian plant, the oil of which has many properties that favor its use in dentistry. It is an antiseptic as well as an antifungal agent. It has mild solvent action and hence could hold potential applications in root canal treatment for dissolving the necrotic pulp tissue. Tea tree oil's major active component is terpinen-4-ol (typically 30-40%). This compound is responsible for its antibacterial and antifungal properties.⁴

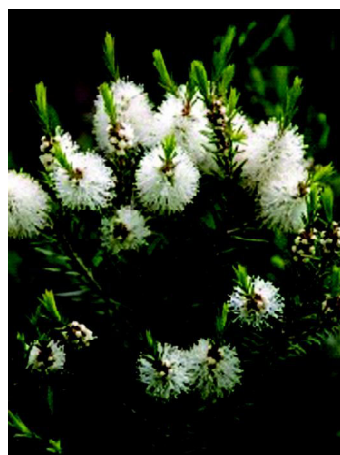


Fig. 1: Tea tree or *Melaleuca alternifolia*—native Australian plant (Source: Int J Drug Dev and Res 2011;3:68-77)

This literature had shown that tea tree oil is having antimicrobial and therapeutic effects suggesting its potential to be used as an endodontic irrigant, but there is lack of much documentation or data regarding tea tree oil research in endodontics.

AIMS AND OBJECTIVES

The purpose of this *in vitro* study was to compare the antimicrobial activity of tea tree oil with 3% sodium hypochlorite and 2% chlorhexidine (CHX) against *E. faecalis*, to assess the antimicrobial property of tea tree oil against endodontic pathogens, using the agar diffusion method.

MATERIALS AND METHODS

Tea tree oil was taken from Falcon Lab, Bengaluru (India), which was prepared by steam distillation of the foliage of *Melaleuca alternifolia*, then prepared to have miscibility in 85% (v/v) ethanol at 20° C, to get a concentration of 2% by volume. A total of 3% sodium hypochlorite (Venson India, Bengaluru) and 2% chlorhexidine (Azure Laboratories Pvt Ltd, Kochi, India) were taken. Normal saline was used as control. The bacterial stock culture of *Enterococcus faecalis* (ATCC 29212) was obtained from Curewell Diagnostic Center, Ludhiana, India.

Culture was grown overnight in nutrient broth, adjusted to 0.5 turbidity reading on McFarland scale (1.5×10^8 bacteria/ml). Blood agar plates were taken and 'Lawn cultures' were prepared on the agar plates by swabbing the sterile swab across the media. Four round wells, 4 mm deep and 8 mm diameter, were punched using sterile cork borer, named as groups A, B, C, D. 50 µl of the medicaments were added to the wells with the help of micropipette as follows:

- Group A: Normal saline (control)
- Group B: 3% sodium hypochlorite
- Group C: 2% chlorhexidine
- Group D: Tea tree oil

A total of three such inoculation agar plates with medicament were prepared. Agar plates were incubated at 37°C for 24 hours in an incubator. The diameter of bacterial inhibition zones around each well was recorded to the nearest size in mm with the help of vernier caliper.

RESULTS

The results were tabulated and statistically analyzed using analysis of variance (ANOVA). Mean zone of inhibitions were recorded (Table 1). All three medicaments used in this study showed well-defined zones of inhibition around their respective wells, but no zone of inhibition was shown by normal saline (control) as shown in (Fig. 2). ANOVA showed that there was no significant difference between the zone of inhibition diameters of tea tree oil, 3% sodium hypochlorite and 2% chlorhexidine against *E. faecalis*. Maximum antimicrobial activity was shown by 2% chlorhexidine (22.67 mm), followed by tea tree oil (22.33 mm) and 3% sodium hypochlorite (21.0 mm) (Graph 1). Tea tree oil showed comparable zone of inhibitions to sodium hypochlorite and chlorhexidine.

DISCUSSION

Owing to the potential side effects, safety concerns and ineffectiveness of conventional allopathic formulations, consumption of natural remedies like neem, tulsi and tea tree oil, have increased over the last few decades. This approach may be termed as phytotherapeutics or ethnopharmacology.⁴

Sodium hypochlorite and chlorhexidine have varying degree of antibacterial properties. But, they have several undesirable characteristics, such as tissue toxicity, allergic potential and inability to remove smear layer.

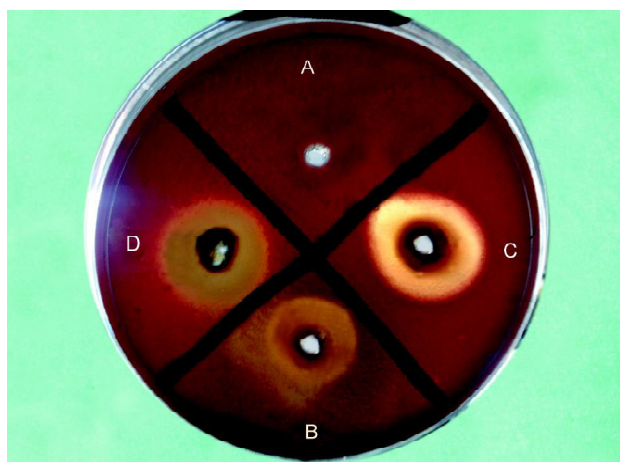


Fig. 2: Zone of inhibition: group A (normal saline)—0 mm, group B (sodium hypochlorite)—20.06 mm, group C (chlorhexidine)—21.5 mm, group D (tea tree oil)—22.46 mm

Table 1: Readings for mean zones of inhibition in all groups

Groups	Agent	N	Mean	Range	Mean ± SD	ANOVA
A	N. saline (control)	3	0	0	0.00	p < 0.001 (Highly significant)
B	Sodium hypochlorite	3	21.0	18-25	21.00 ± 3.61	
C	CHX	3	22.67	21-25	22.67 ± 2.08	
D	Tea tree oil	3	22.33	19-25	22.33 ± 3.06	

N: number of plates; SD: standard deviation; mean in mm

Tea tree oil can be extract from the leaves and branches of *Melaleuca alternifolia* through distillation. It has antiseptic activities like, antibacterial, antifungal, antiviral, antitumor and anti-inflammatory. Oil of *Melaleuca* has a wide spectrum of activity that is mainly attributed to terpeno-4-ol. It possesses effective antimicrobial activity against Gram-positive bacteria, such as *Staphylococcus aureus* and Gram-negative bacteria, such as *Escherichia coli* or *Salmonella typhimurium*.⁹

Mechanism of action is due to its hydrocarbon structure and attendant lipophilicity. Hydrocarbons do partition preferentially into biological membranes and disrupt their vital functions. This premise is further supported by data showing that tea tree oil causes lysis and the loss of membrane integrity and function by leakage of ions and the inhibition of respiration in microbes.¹⁰

The tea tree oil is believed to make an excellent wash for sore and weak eyes and also for the treatment of open sores and wounds due to its antiseptic, antifungal and mild solvent properties.¹¹ In an *in vitro* study, it was shown that tea tree oil which might disinfect the root canal system as effective as sodium hypochlorite and toxicity of tea tree oil is lesser than sodium hypochlorite.¹² Due to its properties, tea tree oil may have a role in the treatment of various infections.¹³ The recommended therapeutic concentration for tea tree oil is 2.5 to 5%, in which it can retain its antibacterial property without any toxic effect.¹⁴

The major advantages of using herbal alternatives are easy availability, cost-effectiveness, increased shelf life, low toxicity, lack of microbial resistance.

This study showed that tea tree oil have good inhibitory efficacy against *Enterococcus faecalis*. It is very important to treat extrapolations of *in vitro* to *in vivo* studies with care. Other properties beyond antimicrobial activity must also be investigated before the final choice of an irrigant solution for clinical use, such as minimum inhibitory concentration,

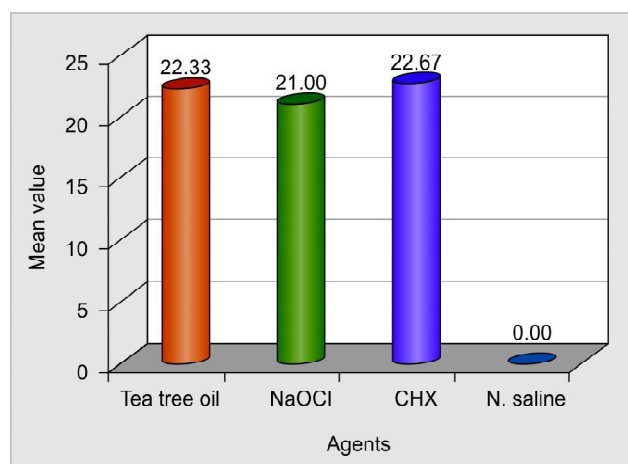
tissue dissolution capacity, detoxification of endotoxin and acceptable biologic compatibility.

CONCLUSION

Under the limitations of this study, it was concluded that tea tree oil has a significant antimicrobial effect against *E. faecalis*. Microbial inhibition potential of tea tree oil observed in this study opens perspectives for its use as a root canal irrigant. However, preclinical and clinical trials are needed to evaluate biocompatibility and safety before tea tree oil can conclusively be recommended as an intra-canal irrigating solution, but *in vitro* observation of tea tree oil efficacy appears promising.

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Graph 1: Mean inhibition zone in all groups

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