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Evaluation of antibacterial efficacy of cinnamon and turmeric as endodontic irrigants against Enterococcus faecalis: An *In Vitro* study

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Abstract

Introduction: In order to achieve complete elimination of the debris and the micro-organisms from root canal system, instrumentation along with irrigants have been used. Sodium hypochlorite and chlorhexidine remain gold standard irrigants of choice in the field of endodontics. But due to its disadvantages such as toxicity, allergic potential, interest in herbal formulations has emerged. The aim of the present study was to evaluate herbal extracts of cinnamon and turmeric as endodontic irrigants against Enterococcus faecalis.

Materials and methods: Thirty extracted single rooted human mandibular premolar teeth were biomechanically prepared and autoclaved. Then the teeth were inoculated with suspension of E.faecalis and incubated for 14 days. The specimens were divided into three groups (n=10) and treated with the test irrigating solutions for 5 minutes. The dentinal shavings were collected and the colony forming units were determined.

Results: 2% sodium hypochlorite showed complete inhibition of E.faecalis in all the three dilutions whereas turmeric had a better antibacterial property than cinnamon.

Discussion: The antibacterial activity of turmeric can be attributed to curcumin and cinnamonaldehyde seen in cinnamon. Turmeric and cinnamon have been used in the traditional medicine due to their antimicrobial, anti-inflammatory properties

Conclusion: Due to the toxicity of the allopathic formulations, there is a need to develop herbal alternatives to root canal irrigants. The literature has shown various herbal ingredients to have potential use in the field of endodontics.

Keywords: Antibacterial activity, Enterococcus faecalis, cinnamon, turmeric, irrigants

Introduction

The goal of non-surgical root canal treatment is the elimination of microbes from the root canal system[1,2,3]. This is achieved through thorough instrumentation and the use of intracanal irrigants and medicaments[3,4]. Due to the complexity of the root canal system (due to the presence of isthmuses, fins, ramifications, etc) endodontic irrigants play a crucial role as mere instrumentation is not possible to eliminate the debris and micro-organisms[1] .Enterococcus faecalis is known for its typical characteristics and survives in root

canal without the support of other bacteria. Enterococcus faecalis is a facultative anaerobic gram positive rod which invades the dentinal tubules and sustains prolonged periods of starvation .It is known to possess virulence factors and lytic enzymes.[5]Various irrigants are used currently such as sodium hypochlorite, chlorhexidine, EDTA MTAD, tetraclean, ozonated water etc. Among these, sodium hypochlorite is the most commonly used in the field of Endodontics due to its ability to its tissue dissolving capabilities.[4,6]..But there is a downside to it such as tissue toxicity, risk of emphysema, allergic potential, foul odor and taste. Due to the potential side effects, safety concerns and ineffectiveness of the traditional allopathic formulations, the study about preparations from medicinal plants has increased. Therefore herbal products have gained sufficient momentum due to their high antimicrobial activity, biocompatibility, anti-inflammatory and anti-oxidant properties. Phytochemical extracts of Curcuma longa and Cinnamomum zeylanicum contain active ingredients such as curcumin and cinnamonaldehyde. It has a broad spectrum of activities such as anti-inflammatory, anti-carcinogenic[7,].Therefore the aim of this study was to evaluate and compare the antibacterial effects of Cinnamon and Turmeric against Enterococcus faecalis.

Materials and methods

The in vitro study was done at the Department of Conservative Dentistry and Endodontics and the Department of Microbiology, A J Institute of Medical Sciences, Mangalore.

Preparation of Enterococcus faecalis suspension:

ATCC strain 29212 of Enterococcus faecalis (Hi Media lab, Navi Mumbai) was obtained from our Department of Microbiology.

A subculture of E.faecalis was made onto Mac Conkey plate which was incubated for a period of 24 hours from a stock culture of ATCC 29212 .From this, the bacteria was inoculated into 50ml of BHI broth and incubated at 37 C for 24 hours and adjusted to a turbidity of 0.5 Mac Farland standard.

Preparation of the aqueous extracts:

Pure extracts of cinnamon and turmeric were purchased (Synthite Industries Ltd, Kolenchery, Kerala). 25g of each of these spices was mixed with 100ml of distilled water and shaken intermittently for a period of one week. After one week, they were centrifuged for 10 minutes and the supernatant was collected in a sterile container and stored at 4° C until use.

Selection of teeth and canal preparation:

30 extracted human mandibular premolar teeth were used in this study which were surfaced and disinfected as per the CDC protocol. The teeth selected had single root and a canal. They were then randomly divided into two experimental groups (cinnamon and turmeric were used test irrigants) and a control group (2% NaOCl) with sample size of 10 each.

- Group I- Cinnamon
- Group II- Turmeric
- Group III- 2% sodium hypochlorite.

Teeth were decoronated at the cemento-enamel junction using a rotary carborundum disc. Working length was determined at 1mm short of the apical foramen and canals were prepared upto F2 Protaper rotary instrument (Dentsply, Tulsa Dental, Johnson City, TN). The root apex was coated with cyanoacrylate to seal the apical foramen. The root canals were then irrigated with 10 ml of 3% of NaOCl and 10 ml of 17% ethylenediaminetetracetic acid (EDTA) and finally 10ml of NaOCl. Finally, the test specimens were sterilized at 121° C for 15 minutes at 26 psi.

Methodology

The specimens were handled using flamed tweezers in order to avoid contamination. Teeth were inoculated with Enterococcus faecalis under laminar air flow chamber and incubated at 37^0 C for 14 days .Teeth were then irrigated with the test irrigants. Dentinal shavings were collected with 40 H files and placed in test tubes with 10 ml of saline. Serial dilutions of 1:2, 1:4 and 1:8 were prepared and cultured into MacConkey agar plates and incubated at 37^0 C for 2 days. A semi-quantitative analysis was performed to determine the number of colonies of E.faecalis[8]. Data was collected, fed into SPSS version 16 and chi square test was used to analyse the results.

Results

Data was collected, fed into SPSS version 16 and chi square test was used to analyse the results. Chi square test was used to compare the different dilutions among the three test irrigants for their efficacy against E.faecalis. (Table-1) p value<0.05 was considered to be significant.

2% NaOCl showed complete inhibition in all the dilutions. Among the herbal extracts turmeric was more effective than Cinnamon (Table 1).

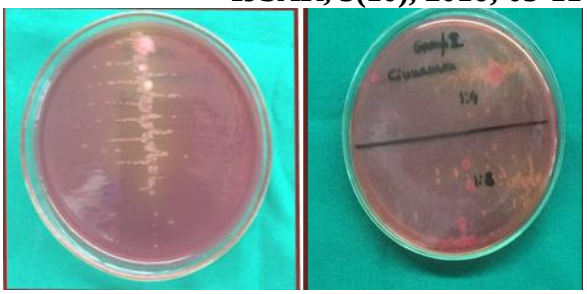


Figure 1: Mac Conkey agar plate (Group I).

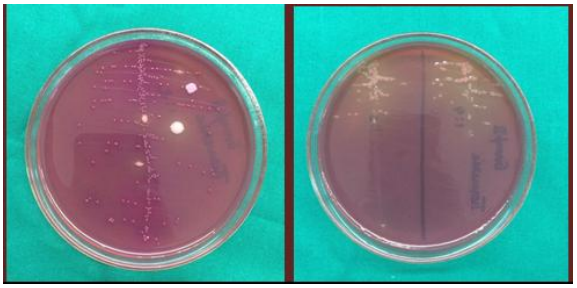


Figure 2: Mac Conkey agar plate (Group II).

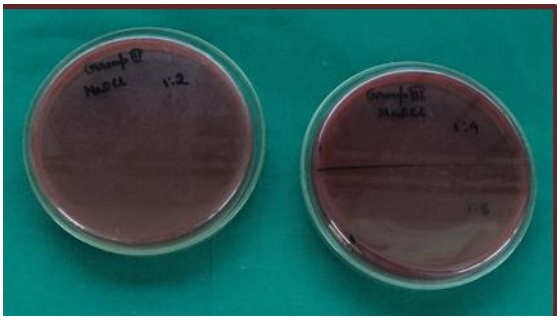
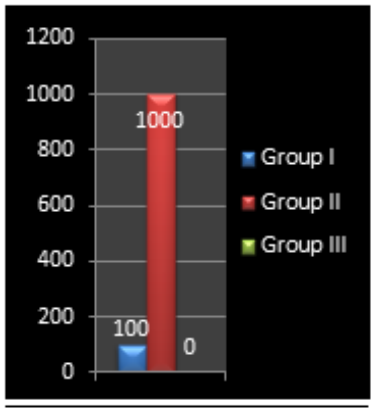


Figure 3: Mac Conkey agar plate (Group III).

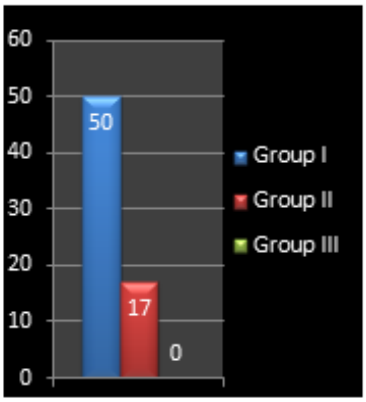
Table 1:

Dilution	Group I	Group II	Group III	p
1:2	100	1000	0	0.001<0.05
1:4	50	17	0	0.001<0.05
1:8	15	9	0	0.001<0.05

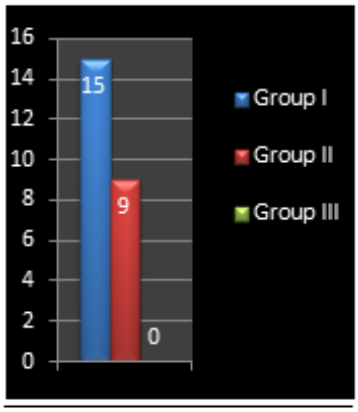
Graphical representation of number of colonies of *E.faecalis*



Graph 1-Representing the number of colonies of E.faecalis formed at 1:2 dilution



Graph 2-Representing the number of colonies of E.faecalis formed at 1:4 dilution



Graph 3-Representing the number of colonies of E.faecalis formed at 1:8 dilution

Discussion

There is a boom in the use of herbal extracts in dentistry for various applications such as eradicatingcaries, dental plaque and root canal irrigants[9].Primarily this can be attributed to their non-toxic nature and biocompatibility[10].Sodium hypochlorite and chlorhexidine are the gold standard root canal irrigants used in routinely in the field of Endodontics[8]. Enterococcus faecalis was selected as the test organism because it is a facultative gram positive anaerobic coccusthat is non -fastidious, easy to grow and efficiently colonises dentinal tubules[3] and the cause for post treatment failure cases at the rate of 63%[5].It is seen in 4- 40% of primary endodontic infections[9,11].Enterococcus faecalisis known to withstand extreme environmental conditions such as extreme alkalinity and salt concentrations. They resist heavy metals, ethanol, bile salts, azide and desiccation. They are capable of growing at temperature range from 10 to 45° C. Moreover, they can survive at temperature of 60° C for a period of upto 30 minutes[12]**.**

It can colonise dentinal tubules and survive without other bacteria. Therefore irrigating solutions like sodium hypochlorite etc have been used to eliminate it.In spite of its good efficacy, it comes with certain disadvantages of tissue toxicity, allergic potential and has foul taste and odour.Thus, there is a need to find herbal alternative to allopathic formulations to combat the adverse effects.

In the present study, 2% sodium hypochlorite had completely inhibited the growth of Enterococcus faecalis in all the three dilutions that is 1:2, 1:4 & 1:8 with similar results by Lakshmi (2013)[9] .In a study by Mohit et al , 5.25% of sodium hypochlorite had given 71.5% of disinfection[4]In a study by Mathew J et al(2015) 5.25% of NaOCl had antibacterial property against E.faecalis but to a lesser extent than 2% chlorhexidine[3].As stated earlier in this present study, turmeric had shown better antibacterial activity when compared to cinnamon at dilutions of 1:4 and 1:8.This can be attributed to the main ingredient in turmeric called curcumin and cinnamaldehyde in cinnamon.

Turmeric had better antibacterial efficacy than cinnamon as per this study (Table1) Turmeric (Curcuma longa) is commonly used as a spice, food preservative and colouring material in India, China and South East Asia. Curcumin (diferuloylmethane), the main yellow bioactive component seen in turmeric has been shown to antimicrobial, anti-inflammatory and antioxidant activities[11,13,14,15]. Curcumin is the major component of turmeric that is known for its biological activities. Its antibacterial activity occurs by virtue ofhydrogen bonding of the phenolic compounds to the membrane proteins, membrane damage, disruption of the electron transport chain and cell wall distraction[16].

In an in vitro study by Neelakantan (2013), it was shown that curcumin has significant antibacterial activity against E.faecalis and can be used as an alternative to NaOCl which is in support of present study[17]. Study by Rosina Khan et al. have found that cinnamon has antibacterial property Enterococcus faecalis. Moreover the herbal extracts were more effective against Gram-positive bacteria than Gram-negative bacteria towards the plant extracts[17]. In a study by Gupta et al. (2013) cinnamon had marked antibacterial activity against E.faecalis but not to the extent of NaOCl [19]Cinnamon (Cinnamomum zeylanicum) is primarily used for its aromatic bark as a spice.The antibacterial activity of cinnamon is due to the presence of cinnamaldehye compound which inhibits the amino acid decarboxylation activity in the cell which leads to energy deprivation and microbial cell death[20]. Therefore the results of the present study proved that turmeric and cinnamon have antibacterial property but not to the extent ofNaOCl.

Conclusion

Within the limitations of this study, it can be concluded that standard irrigant of 2% NaOCl showed complete inhibition and herbal extract of turmeric was better than that of cinnamon.

With increasing resistance to synthetic drugs and typical features of E.faecalis, herbal extracts of turmeric and cinnamon do have a promising role but further studies and clinical trials are to be conducted to promote its use in the field of Endodontics.

Limitation of the study

The present study was in vitro study having a limited sample size. Herbal extracts call for the need for a fresh preparation and modifications in its taste for clinical use.

Further research

Herbal irrigants when compared to the other allopathic formulations are easy to procure, non-toxic, less expensive, and therapeutic action and antibacterial property against oral bacteria. Therefore this warrants for further studies to determine their role root canal microbes.

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Conflict of interest: None

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