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Evaluation of Antimicrobial Efficiency of Herbal Root Canal Irrigants (Neem, Garlic Extract, Tridax Procumbens) and Sodium Hypochlorite against Enterococcus Faecalis

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ABSTRACT

Objectives: The present research was conducted to evaluate the antimicrobial efficacy of herbal root canal irrigants (neem, garlic extract, Tridax procumbens) and sodium hypochlorite against Enterococcus faecalis.
Materials and Methods: Before being inoculated onto Mueller-Hinton agar plates, the E. faecalis bacterial culture was grown overnight in the brain heart infusion (BHI) broth. Antibacterial inhibition was measured using agar well diffusion. The appropriate wells in agar plates were filled with a solution of sodium hypochlorite and herbal irrigants (neem, garlic extract, T. procumbens, and A. marmelos extracts), which were then incubated for 24 hours at 37°C. The bacterial inhibition zone in each well was measured and noted. The results were tabulated and analysed using statistics.
Results: Sodium hypochlorite indicated the maximum inhibitory zone against E. faecalis, subsequently neem, garlic extract and the lowest by Tridax procumbens.
Conclusion: Neem, garlic extract, and Tridax procumbens were tested herbal remedies that demonstrated an inhibitory zone against E. faecalis. These irrigants are therefore suitable for use as root canal irrigating solutions.

Key Words: *Antibacterial, irrigant, neem, garlic extract, root canal, sodium hypochlorite*

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INTRODUCTION

Bacteria and their byproducts are primarily to blame for the aetiology of pulpal necrosis and periapical pathosis [1]. The main requirements for successful outcomes are the elimination of irritants and the prevention of reinfection of the root canal after treatment [2]. In failed root canals, apical periodontitis, and asymptomatic persistent infections, facultative anaerobic gramme positive Enterococcus faecalis is typically the only source of infection. E. faecalis is capable of withstanding common endodontic disinfectants as well as the nutrient-poor conditions found inside a root canal treated tooth [1].

Persistent endodontic infections are caused by the retention of microorganisms in dentinal tubules. Certain parts of the root canal are not impacted by mechanical instrumentation. Mechanical instrumentation alone cannot completely disinfect the root canal because of its complex anatomy. [1] Using irrigating solutions with strong antimicrobial action in addition to mechanical preparation is essential to further reduce microorganisms. Root canal irrigation assists in the eradication of bacteria in locations where instrumentation is not available. Chemical root canal irrigants include sodium hypochlorite (NaOCl) and chlorhexidine (CHX) 2%, both of which have been used successfully. The ideal root canal irrigants should be nontoxic, biocompatible, and have a pleasant taste and smell [1, 2 & 3].

Because of minimum toxicity, and long-lasting broad spectrum action, chlorhexidine has been used as an irrigant. It works well as both an irrigant and a medication in endodontics. Both Gram-positive and -negative microorganisms are inhibited by CHX. CHX has drawbacks such as a bad taste and smell and tissue toxicity. Chlorhexidine has a long-lasting antibacterial effect, but it also discolours teeth and has poor tissue dissolving abilities [1, 3 & 4]. NaOCl irritates periapical tissue, triggers allergic reactions, is toxic to tissues, stains tools, is difficult to eliminate smear layers, and has an unpleasant taste and smell [3]. Studies reported that NaOCl was significantly efficient in reducing E. faecalis biofilms [5]. Sodium hypochlorite is toxic to vital tissues, resulting in hemolysis, skin ulceration, and necrosis [6].

The increased resistance to antibiotics of microbes and toxicity of antimicrobial agents has engendered a need for alternative, nontoxic, effective, and readily available herbal agents [1, 7 & 8]. Numerous herbal extracts, including neem, tulsi extracts, proplis, garlic, aloe vera, turmeric, triphala, and Amla (Emblica officinalis), are showing promise for use as endodontic irrigants because of their antimicrobial, anti-inflammatory, and therapeutic properties [2, 3, 4, 7, 8, 9, 10, 11 & 12].

Azadirachta indica (Neem) is the most commercially exploited traditional and medicinal plant of India. Use of neem as an endodontic irrigant might be advantageous because it is biocompatible, antimicrobial, antiadherent, and antioxidant [2].

Garlic (*Alliumsativum* L.) has many properties, such as antimicrobial features; the common organisms inhibited by garlic include *Staphylococcus aureus*, *Streptococcus mutans*, and *Escherichia coli*. Garlic (Allium sativum L) has been found to have several pharmacological properties such as antimicrobial, antithrombotic, antiplatelet, and anticancer activity [1, 13].

Tridax procumbens (T) has been reported to exert antimicrobial properties suggesting their potential to be used as endodontic irrigants [5].

However, there isn't much information or proof about these herbal extracts as an antibacterial properties in endodontics. Thus, the goal of the present research was to identify whether herbal root canal irrigants like neem, garlic extract and Tridax procumbens had any antimicrobial effects against *E faecalis* when compared with sodium hypochlorite.

MATERIALS AND METHODS

The current research was carried out in Conservative dentistry and Endodontics department.

Washed, 25 gm of fresh neem leaves was added to 50 mL of absolute ethanol and macerated for 1 to 2 minutes. Mixture was filtered for coarse residue using muslin cloth. This process was repeated again for coarse residue with 25 mL ethanol.

Fresh garlic was washed and prepared for extraction. A total of 100 g of cleaned garlic bulbs and 125 mL of distilled water was added to a juicer and crushed. The mixture was filtered using a double filter paper and the supernatant was then centrifuged at 10,000 rpm for 20 min. Final concentration of garlic extract filtrate, in solution was found to be 249 mg/mL. It was stored at -20°C until required [1].

T. procumbens extracts was made into a solution by dissolving it in 10% dimethyl sulfoxide (DMSO) (S.D. Fine Chem Pvt. Ltd, Chennai, India).

On the same day that the extracts were prepared, and antimicrobial testing was conducted.

Microbiological analysis

For intracanal irrigation, forty eight samples were categorised into 4 groups with 12 samples each: Group I: 2.5% sodium hypochlorite, Group II: neem, Group III: garlic extract, and Group IV: T. procumbens. The Microbiological Laboratory served as the location for the microbiological investigation.

A pure culture of E. faecalis (American Type Culture Collection [ATCC] 29212) was incubated at 37C overnight, and adjusted to an optical density (OD600) of 1 with sterile broth.

The antibacterial inhibition zones around neem, garlic extract, T. procumbens, and sodium hypochlorite medications were identified using the agar disc diffusion method. The whole process was conducted with asepsis method. Each medication was placed in the appropriate well of the BHI agar plates, which had been prepared. The plates were kept in a 37°C incubator for 24 hours. The bacterial inhibition zone around each well was measured after the plates had been incubated [3].

The tabulate data were statistically evaluated using SPSS software version 23.0 IBM Corp.'s USA, with ANOVA test at P less than 0.05.

RESULT

Sodium hypochlorite (27.57±1.145 mm) had the maximum mean inhibitory zone against *E. faecalis*, followed by neem (22.31±1.131mm), garlic extract (18.45±1.083 mm), and Tridax procumbens (11.45±1.083 mm) (Table 1).

Table 1: Inhibitory zone (mm) against *Enterococus Feacalis* by various root canal irrigants

Group	Mean± SD zone of inhibition in mm	SE
Group I: 2.5 % Sodium hypochlorite	27.57±1.145	0.254
Group II: Neem	22.31±1.131	0.145
Group IIII: Garlic extract	18.45±1.083	0.178
Group IV: Tridax procumbens	11.45±1.083	0.162

SD- Standard deviation, SE- standard error, $p<0.05$

DISCUSSION

Root canal infection and/or reinfection occur mainly due to microorganisms present in the canal system. [2] *E. faecalis* are commonly found in failed root canals [8].

Octavia et al evaluated the efficiency of garlic extract against the viability of *E. faecalis* from clinical isolates of nonvital primary root canals. They concluded that, garlic extract was effective in decreasing the viability of *E. Faecalis* [10].

Eswar et al, compared the efficiency of garlic extract with 2% chlorhexidine (CHX) and calcium hydroxide $Ca(OH)_2$ in disinfection of dentinal tubules contaminated with *Enterococcus faecalis* by using real-time polymerase chain reaction (PCR) and concluded that, 2% CHX had the maximum efficiency against *E. faecalis*, followed by garlic extract and $Ca(OH)_2$ [1].

Mustafa found that, neem leaf extract had comparable zones of inhibition with that of chlorhexidine and sodium hypochlorite [14].

Bansal et al assessed the antimicrobial efficacy of different herbal products, that is, propolis, garlic, neem, aloe vera, and rosemary, against Bacillus subtilis, Staphylococcus aureus, and Enterococcus faecalis using agar diffusion test. They found that, Propolis and rosemary showed maximum zone of inhibition against B. subtilis. Garlic, neem, and aloe vera showed maximum zone of inhibition against S. Aureus [6].

Jain et al evaluated the antibacterial efficacy of Garlic was peeled, Amla and Ginger chopped into small pieces and Neem, Tulsi and Aloe vera leaves. They found that, Positive control 0.2% Chlorhexidine produced significantly larger inhibition zones against the test bacteria. However, the negative controls produced no observable inhibitory effect [12]. Surender et al evaluated the antimicrobial efficacy of Tridax procumbens, Aegle marmelos, and 5% sodium hypochlorite against Enterococcus faecalis biofilm formed on the tooth substrate and found that, T. procumbens, A. marmelos had statistically significant antibacterial activity [5].

The persistence of *E. faecalis* located in the root canals is due to the presence of proteinases, gelatinases, and collagen proteins that enhance dentine bonds. These bacteria can survive for long periods in conditions of starvation until adequate nutrition becomes available. *E. faecalis* adheres to the root canal walls, accumulates, and forms biofilms, such that the bacteria are 1000 times more resistant than planktonic forms to phagocytosis, antibodies, and antimicrobials [10].

In present study tested herbal products showed antibacterial inhibitory zone against E faecalis. Hence these can be used as an alternate irrigants. Further studies are needed to validate the results.

CONCLUSION

Sodium hypochlorite is considered as a strong antimicrobial agent. In the present study, herbal extract; neem, garlic extract and Tridax procumbens showed antibacterial effect against E faecalis indicating they can be used as an alternative root canal irrigants.

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