How efficacious are *Neem*, *Tulsi*, *Guduchi* extracts and chlorhexidine as intracanal disinfectants? A comparative *ex vivo* study

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Abstract

Introduction: In endodontics, despite careful instrumentation and antimicrobial irrigation, root canals still harbor cultivable microorganisms. Such cases require intra canal medicament that eliminates the microbial inhabitants from the canals. Recent trend advocates the use of herbal extracts due to easy availability, cost-effectiveness, low toxicity, and lack of microbial resistance. Hence, in the present study, *Neem*, *Tulsi,* and *Guduchi* extracts were used as intracanal medicaments. **Aim:** This study aimed to evaluate and compare the antibacterial efficacy of *Neem*, *Tulsi*, *Guduchi* extracts, and chlorhexidine against *Enterococcus faecalis*, when used as intracanal medicaments. **Materials and Methods:** One hundred and twenty-five extracted human teeth, inoculated with *E. faecalis,* were divided into four experimental groups and a control group (n = 25 in each group). The experimental groups were treated with chlorhexidine, *Neem*, *Tulsi,* and *Guduchi* extracts and their antibacterial property was evaluated by estimating microbial counting (CFU/ml). **Results:** The reduction in bacterial count for chlorhexidine, *Neem*, *Tulsi,* and *Guduchi* groups was 60.76%, 51.98%, 37.73%, and 34.93%, respectively. Statistically significant difference in reduction of bacterial count was observed in all the groups, when compared with the control group. **Conclusion:** Among all the herbal extracts, *Neem* was found to be the most potent medicament followed by *Tulsi* and *Guduchi*. However, chlorhexidine was found to be at epic.

Keywords: Antibacterial, chlorhexidine, *Enterococcus faecalis*, *Guduchi*, intracanal medicaments, *Neem*, *Tulsi*

Introduction

Endodontic therapy or root canal therapy is a sequence of procedures for treating the infected pulp of a tooth, resulting in the elimination of infection and the protection of the decontaminated tooth from future microbial invasion.[1] The microenvironment of root canal presents excellent conditions to establish microbial growth. The major cause of disease after root canal treatment is the persistence of microorganisms in the apical third of the root canal of teeth, especially *Enterococcus faecalis*.[2]

E. faecalis plays a major role in the etiology of persistent periradicular lesion after root canal treatment.[2,3] It is frequently found in high percentage of root canal failures and is able to survive in the root canal as single organism or as a major component of the mixed flora.[4-8]

The goal of the endodontic treatment is to debride and disrupt the microbial ecosystem associated with the disease process and to neutralize any antigen that may be left in the canal after elimination of the microorganisms. Therefore, the infected root canal is subjected to combined chemo-mechanical treatment involving instrumentation plus copious irrigation with the antimicrobial agents or disinfectants followed by suitable intracanal medicaments.[9]

Currently used intracanal medicaments are phenolic compounds such as camphorated monochophenols, cresatin, formocresol, gluteraldehyde, halides, calcium hydroxide, and some antibiotic pastes. These compounds are potent antibacterial agents under laboratory test conditions, but their efficacy in clinical use is unpredictable and have certain

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demerits such as toxic and allergic reactions that cause tissue injury.[10]

The search for the effective antimicrobial agent led to the use of chlorhexidine digluconate (CHX) within the root canals. For endodontic purposes, 2% CHX can be used in a liquid or in a gel presentation. CHX when used as intracanal medicament has shown potent results against common endodontic pathogens, especially *E. faecalis*.[11,12]

With a rising interest toward holistic approach, herbal remedies have steadily regained popularity from the 1960s to present.[13] In endodontics, because of the cytotoxicity of most of the commercial products used as intracanal medicaments and their inability to eliminate bacteria from dentinal tubules, recent trend of holistic approach to use biologic medication extracted from natural plants has increased rapidly. The major advantages of using herbal alternatives are easy availability, cost-effectiveness, increased shelf life, low toxicity and decreased microbial resistance.[14]

Tinospora cordifolia (*Guduchi*) has been reported to contain tenosporin, coloumbin, and tinosporic acid. It is well known in ayurvedic literature to treat various ailments such as fever, inflammation, skin infection, and urinary infections.[15]

Neem has been proven to possess several pharmacological and medical applications in ancient literature. It is mentioned as a powerful antimicrobial agent that inhibits the increase and establishment of microorganism, causing infectious diseases. It also promotes an anti-adherence activity by altering bacterial adhesion and ability of organism to colonize.[16]

Tulsi has been used as a medicinal plant traditionally in day-to-day practice in Indian homes for various ailments. The essential oil extracted from *Tulsi* leaves contains eugenol, which is a phenolic compound that may be attributed to its anti-diabetic and anticancer properties and most importantly for its antimicrobial properties.[17,18]

Owing to the potential side effects, safety concerns, and ineffectiveness of conventional allopathic formulations, consumption of preparations from medicinal plants has increased over the last few decades. Hence, in the present study, *Neem*, *Tulsi,* and *Guduchi* extracts were used as intracanal medicaments, as they were earlier proven to be potent antibacterial agent.

Materials and Methods

The present study was conducted in the Department of Paedodontics and Preventive Dentistry, Subharti Dental College and Hospital, Meerut, in collaboration with the Department of Microbiology, Subharti Medical College and Hospital, Meerut.

One hundred and twenty-five freshly extracted caries-free human permanent teeth with single canal (extracted for orthodontic purposes or periodontal problems) were included in the study and were called as samples.

Inclusion criteria

Teeth with a single straight canal were included in the study.

Exclusion criteria

Extracted teeth with caries, fractured segment, curved canals, and calcified canals were excluded from the study.

The samples were stored in physiologic saline solution and the tooth length was standardized by measuring it from the root apex to the cemento-enamel junction up to 15 mm [Figure 1]. Biomechanical preparation was done in all the sectioned teeth upto number 50 k file and then canals were irrigated with sterile physiologic saline.

The external surfaces of the roots were coated with nail polish except the cervical access and apical foramen. After setting of the nail polish, the root canals were filled with 17% ethylene diamine tetra acetic acid (EDTA)_ and left for 3 min in order to remove the smear layer. The samples were then alternatively irrigated with 3% sodium hypochlorite and 3% hydrogen peroxide and finally with 5 ml of physiologic saline solution.

Microbiological evaluation and grouping of samples

All the roots were sterilized by autoclaving at 121°C for 20 min. *E. faecalis* MTCC 439 used in this study was standardized to 1.5×10^8 microorganisms/ml to form an inoculum.

The tooth apices were sealed with Cavit™ G temporary cement. Ten microliters of the bacterial inoculum was then injected into the prepared canal with the help of an automatic micropipette. Sterile cotton soaked with the inoculum was placed in the cervical access of the canal and then sealed with Cavit™ G temporary cement.

The roots were then placed on a gauze pad in sterile Petri plates and incubated at 37 ± 1ºC for 72 h. After 72 h, the coronal orifices of canals were again opened and microbiological sampling was carried out to establish the level of contamination (CFU/ml) prior to application of medicaments.

The canals were dried using paper points. Samples were then randomly divided into four test groups on the basis of intracanal

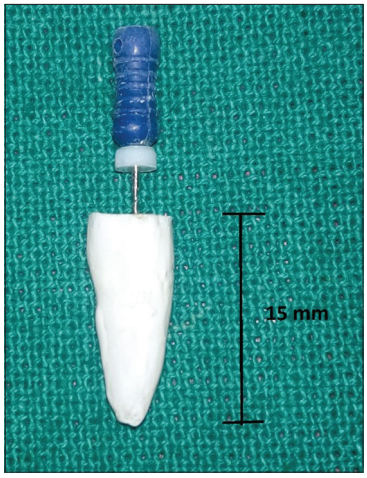

Figure 1: Prepared sample with standardized measurement

medicament to be placed and a control group (n = 25) as follows:

Control	Chlorhexidine	*Neem*	*Tulsi*	*Guduchi*
Group 1	Group 2	Group 3	Group 4	Group 5

The test medicaments, i.e., aqueous extract of *Neem*, *Tulsi*, *Guduchi,* and CHX, were placed into the canals using micropipette tip. A sterile cotton plug was placed at the orifice and the specimens were coronally sealed with Cavit™ G temporary cement.

The specimens were then incubated again at 37 ± 1°C for 48 h under anaerobic conditions in a desiccating chamber to determine the microbiological count at the end of 48 h. After 48 h, canals were re-entered and irrigated with sterile physiologic saline.

The samples were then instrumented with no. 50 K-file to create dentinal shavings and irrigated with physiologic saline solution. Sterile paper points were placed into the canal for 60 s to collect samples for microbial testing [Figure 2]. Paper points were then placed in Eppendorf tubes containing 1 ml of sterile physiologic saline solution [Figure 3].

Test tubes containing microbiological samples were incubated for 30 min at 37ºC and shaken vigorously for 60 s in a vortex mixer. Ten microliters from each test tube was then taken with automatic micropipette and culture was performed on agar petri plates to estimate the level of CFU/ml.

Statistical analysis

Data collected were analyzed using Statistical Package for the Social Sciences (SPSS, V 19.0 IBM, India). For pairwise comparison between groups, Mann–Whitney test was applied. For inter- and intragroup comparison, one-way analysis of variance (ANOVA) was applied. For comparison between premedication and postmedication control group, *Z*-test was applied.

Results

On comparison of the control, *Neem*, *Tulsi*, *Guduchi,* and CHX groups after 48 h of incubation period, it was observed that the mean bacterial count was found to be 71,076, 27,824, 34,016, 44,224, and 46,496, respectively. In all the experimental groups, the mean bacterial score was statistically significant. Highest scores of bacterial counts were present in control group while the least counts were found in CHX group [Figure 4]. It was also observed that the maximum difference was in group 2 (CHX), i.e., 60.76%, while minimum difference was observed in group 5 (*Guduchi*), i.e., 34.52% [Figure 5].

Among all the possible groups including control group with test group, it was observed that a high statistically significant difference was present in bacterial scores (CFU/ml) in all the groups. After 48 h of incubation period, all the possible groups were at 0.05 level of significance, i.e., $P < 0.05$ [Table 1].

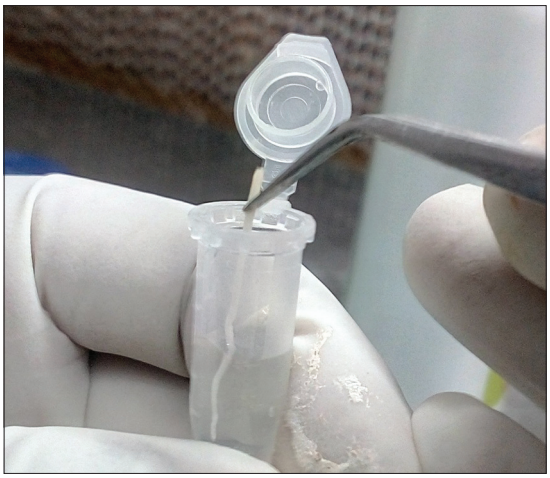

Figure 2: Bacterial sample collection with paper point

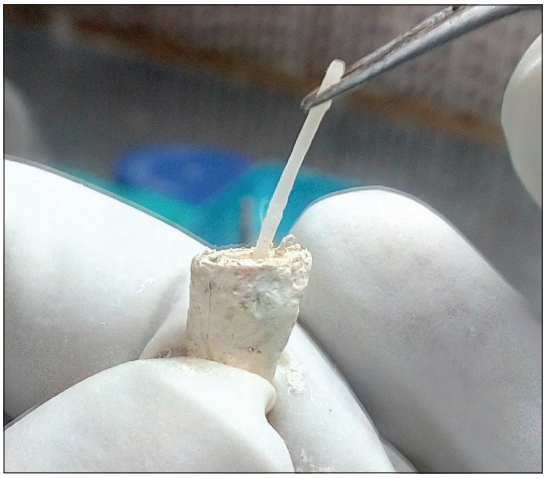

Figure 3: Paper point placed in Eppendorf tube

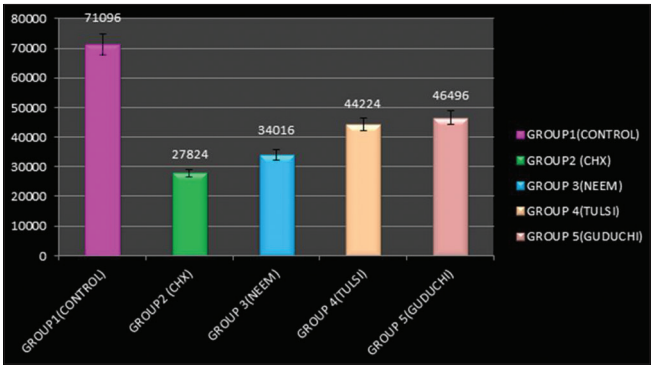

Figure 4: Mean scores of bacterial counting (CFU/ml) in control group and different test groups

When comparing the significant difference in bacterial scores (CFU/ml) among all groups (including and excluding control group), ANOVA revealed that a high significant difference in bacterial count was observed at 0.05 level of significance, i.e., $P < 0.05$.

On comparison between bacterial count (CFU/ml) in premedication score and after 48 h scores of incubation

and their significant difference, it was observed that in Group 1 (control) the mean bacterial count (CFU/ml) was reduced by 28.91% after 48 h of incubation period and was found to be statistically significant [Table 2].

Discussion

The principle of a treatment to reach favorable outcomes in endodontic infection management requires the recognition of the problem and the removal of the etiological factors. The microenvironment of root canal presents excellent conditions to establish microbial growth. The most common species isolated from the root canals is *E. faecalis*. Development of certain microbial combinations contributes to persistent clinical signs and symptoms.[6]

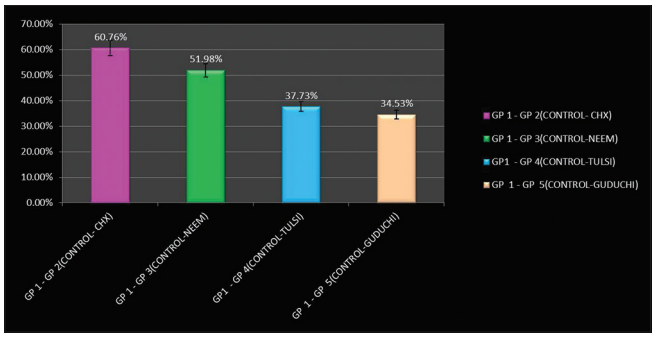

Figure 5: Percentage reduction from control group to the different test groups for estimating bacterial count after 48 h of incubation period

Table 1: Pair wise comparison in bacterial scores (CFU/ml) among all possible groups including control group with test group

Pair of all possible groups	Probability of Mann-Whitney test	*P*/significance
Group 1-Group 2 (control and CHX)	0.0000*	<0.05 (significant)
Group 1-Group 3 (control and *Neem*)	0.0002*	<0.05 (significant)
Group 1-Group 4 (control and *Tulsi*)	0.0031*	<0.05 (significant)
Group 1-Group 5 (control and *Guduchi*)	0.0004*	<0.05 (significant)
Group 2-Group 3 (CHX and *Neem*)	0.0000*	<0.05 (significant)
Group 2-Group 4 (CHX and *Tulsi*)	0.0000*	<0.05 (significant)
Group 2-Group 5 (CHX and *Guduchi*)	0.0006*	<0.05 (significant)
Group 3-Group 4 (*Neem* and *Tulsi*)	0.0001*	<0.05 (significant)
Group 3-Group 5 (*Neem* and *Guduchi*)	0.0012*	<0.05 (significant)
Group 4-Group 5 (*Tulsi* and *Guduchi*)	0.0000*	<0.05 (significant)

*Significant difference at 0.05 level of significance, i.e., $P<0.05$.
CHX: Chlorhexidine

Despite careful instrumentation and antimicrobial irrigation, published studies suggest that more than 1/3rd of all root canals still harbor cultivable microorganisms at that time.[20-22] An intracanal medicament with good antimicrobial properties could help to eliminate the microbial inhabitants from the canals.

E. faecalis is implicated in root canal failures and persistent infections.[4] *E. faecalis* has an ability to survive in harsh environments including extreme alkaline pH and salt concentrations. Mostly in root canal infections, mechanical preparation and irrigation alone cannot eliminate all the bacteria from the infected root canal. In these cases, the use of intracanal medication is essential to help disinfect the infected root canal system.[19]

An ideal root canal disinfectant should have several properties such as be able to disinfect dentin and its dentinal tubules, offer antibacterial sub-stativity, inactivate endotoxins, non-antigenic, nontoxic, and non-carcinogenic, have no adverse effects on dentin, would not affect the sealing ability of filling materials, be relatively inexpensive and convenient to apply, and should cause no tooth discoloration.[10]

CHX is a wide-spectrum antibacterial agent, which is active against Gram-positive and Gram-negative bacteria as well as yeasts.[23] Owing to its cationic nature, it is capable of electrostatically binding with the negatively charged surfaces of bacteria, damaging the outer layers of the cell wall and rendering it permeable.[24-26] Owing to the potential side effects, safety concerns, and ineffectiveness of conventional allopathic formulations, consumption of preparations from medicinal plants has increased over the last few decades. Hence, in the present study, *Neem*, *Tulsi,* and *Guduchi* extracts were used as intracanal medicaments.

After 72 h of incubation, the count of *E. faecalis* MTCC 439 was 10^5 CFU/ml which was in accordance with the previous studies in which the bacterial count was found to be 10^5 CFU/ml. At 48 h, the mean bacterial count in control group was reduced to 71,096. This may be due to the unfavorable condition of *in vitro* environment.[9]

The present study provided a standardized experimental setup which allowed for validated comparison of different herbal

Table 2: Comparison between premedication score and after 48 h scores of incubation and their significant difference (by *Z*-test single-sample proportion)

Premedication score (bacterial count) (CFU/ml)	After 48 h scores (bacterial count) (CFU/ml)	Difference in bacterial count between 0 and 48 h, *n* (%)	Probability of *Z*-score (single-sample proportion)	Significance
100,000	71,096	28,904 (28.91)	0.0036(p-value)	$P<0.05$ (significant)

medicaments. The ready-made powdered extracts of the plants were used in our study (Indian Herbs Specialities Pvt. Ltd., Saharanpur, Uttar Pradesh) instead of preparing extracts using raw methods. Nowadays, using latest techniques and equipment, pharmaceutical companies have been able to provide powdered extracts with nearly 100% purity. In this study, powdered extracts with 99.98% of powder purity were used. This in turn simplified the whole procedure and saved the valuable time consumed in the experiment. However, the active biocompound responsible for such antimicrobial activities in the herbal extracts has not been identified.

To ensure proper cleansing of the root canal, different irrigation regimens have been used to enhance the effectiveness of NaOCl in disinfecting the root canal system. Grossman (1943) suggested the alternate use of NaOCl and hydrogen peroxide for the irrigation of the root canal. This association caused effervescence, which improved the debridement and disinfection of the root canal.[27] Hence, in the present study, samples were irrigated alternatively with hydrogen peroxide and sodium hypochlorite. 17% EDTA in each canal for 3 min was used to make the canal free from biofilm and smear layer to prevent cross contamination as stated by Teixeira *et al*. Another reason to use EDTA additionally was that NaOCl alone is not effective in the removal of intracanal smear layer, especially in the apical third of the canal.[28] To prevent cross contamination with desired bacterial growth, all the samples were sterilized in autoclave at 121°C, 15 lbs pressure for 20 min. An external coating with nail polish was done in all the samples to seal the dentinal tubules so as to prevent the seepage of bacteria.

In evaluating the antibacterial property of *Neem*, *Tulsi*, *Guduchi,* and CHX, it was observed that CHX showed the maximum antibacterial efficacy. After 48 h, the bacterial count was reduced to 27,824 CFU/ml, i.e., 60.76% decrease as compared to control group. When compared with control group, the values were found to be statistically significant. These results are in accordance with that of Ramani *et al*. in which CHX was found to be a potent antibacterial agent against *E. faecalis*.[9] When compared with other test groups, the results were again found to be statistically significant.

Antibacterial efficacy of *Neem* was found to be statistically significant when compared with that of the control group. The mean bacterial counts were 34,016 CFU/ml, i.e., 51.98% reduction with the control group. When compared with *Tulsi* and *Guduchi* groups, the results were also statistically significant. These results are in accordance with that of Bohra *et al*. (2011) and Nayak *et al*. (2011) who concluded that *Neem* has potent antibacterial efficacy against *E. faecalis*.[16,29] Microbial inhibition potential of *Neem* leaf extracts observed in this study opens perspectives for its use as an intracanal medication. However, clinical trials are needed to evaluate the biocompatibility and safety of *Neem* before it can conclusively be recommended as an intracanal medicament.

Tulsi was found to be the second most potent herbal medicament. When compared with control group, the overall bacterial count was reduced to 37.73% (i.e., 44,224 CFU/ml) with the control group. When *Tulsi* group was compared with *Neem* and *Guduchi* groups, the results were found to be statistically significant. According to Singh *et al*., the antibacterial properties of *Tulsi* are due to the linolenic acid. Presence of linolenic acid in the oil imparts antibacterial activity against many bacteria.[30]

In the present study, *Guduchi* also showed potent antibacterial efficacy and the results were found to be statistically significant when compared with control group, although its antibacterial property was found to be least efficacious. The mean bacterial count was found to be 46,496 CFU/ml, i.e., 34.53% reduction with the control group. When CHX, *Neem,* and *Tulsi* groups were compared, antibacterial potency of *Guduchi* was found to be statistically significant and it is in accordance with the study of Jeya chandran *et al*.[31]

In the present study, *Neem* was found to be the best antibacterial herbal medicament followed by *Tulsi* and *Guduchi*. However, contrary to this, Mistry *et al*. in 2014 conducted a study in which *Tulsi* and *Guduchi* were found to be more potent than *Neem* and CHX. The variation in the result might be due to difference in methodology and the type of extracts used in the study. Further, in the present study, bacterial counts were considered while Mistry *et al*. evaluated the zone of inhibition by agar diffusion method to check the antibacterial efficacy against *E. faecalis*.[32]

Conclusion

Based on the results of the present study, the following conclusions were drawn:

1. All the tested medicaments showed marked antibacterial efficacy and the differences were statistically significant when compared with the control group
2. When the three herbal medicaments were compared, *Neem* was found to be with the most potent antibacterial efficacy while *Guduchi* was least efficacious
3. Among the four tested medicaments, CHX showed the maximum antibacterial properties while *Guduchi* was found to be the least effective intracanal medicament
4. In decreasing order, the antibacterial efficacy of the four intracanal medicaments was as follows:

   CHX > *Neem* > *Tulsi* > *Guduchi*

Based on the results of the study, it can be said that herbal extracts of *Neem*, *Tulsi*, *Guduchi,* and CHX can be used as effective antibacterial intracanal medicaments against *E. faecalis*. However, further studies with larger sample size and in clinical situation are needed to validate the results of the present study.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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