A comparative evaluation of the antibacterial efficacy of *Thymus vulgaris*, *Salvadora persica*, *Acacia nilotica*, *Calendula arvensis*, and 5% sodium hypochlorite against *Enterococcus faecalis*: An *in-vitro* study

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**Abstract**

**Objectives:** The aim of this study is to evaluate and compare the antibacterial efficacy of *Thymus vulgaris*, *Salvadora persica*, *Acacia nilotica*, *Calendula arvensis*, and 5% sodium hypochlorite against *Enterococcus faecalis*.

**Methodology:** Herbal extracts of *T. vulgaris*, *S. persica*, *A. nilotica* and *C. arvensis* were prepared. Tryptone soya broth was used to grow *E. faecalis* and agar plates were prepared. The tested solutions (Group A: 5% NaOCl, Group B: 20% *T. vulgaris*, Group C: 12.5% *S. persica*, Group D: 10% *A. nilotica*, Group E: 10% *C. arvensis*) were added to the wells made on agar media. Agar diffusion test was performed. Plates were incubated at 37°C for 24 h. Bacterial zones of inhibition were recorded.

**Results:** The data were analyzed statistically by Analysis of Variance (ANOVA) and post hoc comparison by Tukey’s t-test. The highest zone of inhibition against *E. faecalis* was shown by 5% NaOCl, followed by 10% *C. arvensis*, 20% *T. vulgaris* and 10% *A. nilotica* showed similar comparable antibacterial activity. The least zone of inhibition was showed by *S. persica*.

**Conclusion:** 5% NaOCl showed the maximum antibacterial activity, and herbal products demonstrated significant antibacterial activity against *E. faecalis* and can be employed as an alternative to NaOCl.

**Keywords:** *Acacia nilotica*; *Calendula arvensis*; *Enterococcus faecalis*; *Salvadora persica*; sodium hypochlorite; *Thymus vulgaris*

**INTRODUCTION**

The initiation, propagation, and persistence of pulpal and periradicular pathosis can be attributed to the bacteria, their metabolic products, enzymes, and various toxins.[1] Complete eradication of intracanal microorganisms creates an environment favorable for healing, prevents re-infection, and thus helps achieve long-term success.[1] Successful endodontic therapy relies not only on adequate biomechanical preparation and three-dimensional obturation of the root canal space but also on thorough irrigation of the root canals, its complex internal anatomy and other irregularities that might be inaccessible to instrumentation.[2] *Enterococcus faecalis*, a facultative anaerobe, is attributed to 4%–40% of primary endodontic infections and nine times more likely the cause of...
secondary infections. This highly resistant microorganism has the capacity to inactivate antimicrobial agents, tolerate and thrive in extreme environmental conditions due to biofilm formation. Hence, effective chemomechanical debridement and disinfection of the root canal space play a pivotal role in the predictable long term success of the root canal treatment. Routinely used irrigants such as sodium hypochlorite and chlorhexidine have effective antimicrobial action. However, they have certain disadvantages such as tissue toxicity, unpleasant taste, and odor, corrosion of instruments, inability to remove the smear layer, reduction in the elastic modulus, and flexural strength of dentin. The use of phyto-medicines extracted from natural plants provides several advantages over conventional synthetic drugs. The healing potential of herbal plants is an ancient belief; however, it has gained interest and importance in recent times. These herbal products are not only safe, easily available, and cost-effective, but also have increased shelf-life and lack of microbial resistance so far.

**METHODOLOGY**

**Procurement of the microorganism**
A pure culture of *E. faecalis* (ATCC 29212; Himedia, Mumbai, India) was inoculated on Tryptone soya agar plates (Himedia, Mumbai, India) and was incubated at 37°C overnight for 24 h.

**Procurement of the herbal powders**
The herbal powders were procured from Bagwan Ayurvedic Medical Stores, Karad, Maharashtra, India.

**Preparation of the herbal extracts**

*Thymus vulgaris*: 300 ml of sterilized distilled water was added to 30 g of thyme powder and was heated below the boiling point and stirred for 2 h at 90°C. The extract was filtered by muslin cloth, then by filter paper No. 1 (Whatman No. 1) and was stored at 5°C in the refrigerator. A 20% concentration of the extract was used in the study.

*Acacia nilotica*: The method of extraction employed for it was maceration, for which the powder was kept in suspension in 50%-90% ethanol for 7 days. After filtration and evaporation of ethanol, the extract was oven-dried at 60°C until it became dry. It was re-dissolved in ethanol to obtain a concentration of 10%.

*Salvadora persica*: The extract was prepared using the maceration method. 800 g of *S. persica* chewing sticks were ground to powder form. 120 ml of 60% ethanol was added to 40 g of powder in a sterile well-capped vial. It was left for 3 days at room temperature and then filtered using No. 1 filter paper. The extract was incubated at 37°C until it became dry. It was stored in sterile screw-capped vials in the refrigerator until needed.

12.5% alcoholic extract was obtained by serial dilutions with sterilized Ringer’s Lactate. Initially, 1 g of alcoholic extract of *S. persica* was dissolved in 2.5 ml of Ringer’s Lactate to give 100% concentration. 2.5 ml of 100% alcoholic extract and 2.5 ml of Ringer’s Lactate gave 50% concentration. 2.5 ml of 50% alcoholic extract and 2.5 ml of Ringer’s Lactate gave 25% concentration. 2.5 ml of 25% alcoholic extract and 2.5 ml of Ringer’s Lactate gave 12.5% concentration. Thus, 12.5% of alcoholic concentration was used for the study.

*Calendula arvensis*: Hexanolic extract was prepared by Soxhlet extraction of 200 g of Calendula powder in about 700 ml of hexanol for 24 h at a temperature of 55°C. The filtered solvent was evaporated in vacum until dryness using rotator evaporator. The extract was kept at 4°C. It was dissolved in dimethylsulfoxide (DMSO) solvent to get a concentration of 10%.

5% NaOCl (Prime Dental limited, Mumbai, India) was used in the study.

The cultured *E. faecalis* was suspended in 5 ml Tryptone soya broth (Himedia, Mumbai, India). It was incubated for 4 h at 37°C. The turbidity was adjusted to 0.5 MacFarland standard, and 50 µl of the inoculum was spread over petri plates with the help of L-arm loop.

**Antibacterial assay**
Wells of 5 mm diameter were punched using a sterile cork bore and 50 µl of the irrigants were added to these wells with the help of a micropipette as follows:
- Group A: 5% NaOCl
- Group B: 20% *T. vulgaris*
- Group C: 12.5% *S. persica*
- Group D: 10% *A. nilotica*
- Group E: 10% *C. arvensis*.

The plates were incubated at 37°C for 24 h. After 24 h, the diameter of the bacterial zone of inhibition was recorded to the nearest size in millimeters [Figure 1 and Table 1]. The experimental procedure was done four times, and the mean values were calculated [Table 2].

The data were analyzed statistically using one-way Analysis of Variance (ANOVA) [Table 2] and post hoc Tukey’s t-test [Table 3].

Results can be condensed as follows:

5% NaOCl > 10% *C. arvensis* > 20% *T. vulgaris* = 10% *A. nilotica* > 12.5% *S. persica*.

Table 2 shows the mean values for the zone of inhibition (in mm) for all the five groups. The inter-group comparison among the mean scores of the zone of inhibition of the various irrigating solutions by one-way
The present study evaluated and compared the antibacterial activity of the root canal system becomes an utmost important phase of endodontic treatment to disinfect the root canal. Owing to the limitations of mechanical instruments to reach far and beyond in narrow isthmuses, accessory canals, and dentinal tubules, it becomes essential that an irrigant with good penetrability and bactericidal activity is used. Irrigation of the root canal is an essential adjunct to mechanical debridement as it flushes out the debris, infected material, disinfects the canal system, dissolves the tissues and also cleans the complex internal anatomy of the root canal space inaccessible to instrumentation. Thus, the use of irrigants ensures the elimination of bacteria and organic tissue remnants, thereby preventing reinfection.

The mean values for the zone of inhibition was: Group A: 5% NaOCl - 18.75 mm, Group B: 20% T. vulgaris - 12.50 mm, Group C: 12.5% S. persica - 11.75 mm, Group D: 10% A. nilotica - 12.25 mm and Group E: 10% C. arvensis - 13.75 mm [Table 2].

Table 2: Mean and standard deviations for all the groups

| Sample          | Mean | Standard deviation |
|-----------------|------|--------------------|
| Sodium hypochlorite | 4    | 18.75              |
| T. vulgaris      | 4    | 12.50              |
| S. persica       | 4    | 11.75              |
| A. nilotica      | 4    | 12.25              |
| C. arvensis      | 4    | 13.75              |

Post hoc Tukey’s t-test; *Significant at P<0.05. T. vulgaris: Thymus vulgaris, S. persica: Salvadora persica, A. nilotica: Acacia nilotica, C. arvensis: Calendula arvensis

Table 3: Pairwise comparison

| Groups (I)          | Groups (J)          | Mean difference (I-J) | Significant |
|---------------------|---------------------|-----------------------|-------------|
| Sodium hypochlorite | T. vulgaris         | 6.250                 | 0.001*      |
|                     | S. persica          | 7.000                 | 0.001*      |
|                     | A. nilotica         | 6.500                 | 0.001*      |
|                     | C. arvensis         | 5.000                 | 0.006*      |
| T. vulgaris         | Sodium hypochlorite | -6.250                | 0.001*      |
|                     | S. persica          | 0.750                 | 0.967       |
|                     | A. nilotica         | 0.250                 | 1.000       |
|                     | C. arvensis         | -1.250                | 0.825       |
| S. persica          | Sodium hypochlorite | -7.000                | 0.001*      |
|                     | T. vulgaris         | -0.750                | 0.967       |
|                     | A. nilotica         | -0.500                | 0.993       |
|                     | C. arvensis         | -2.000                | 0.468       |
| A. nilotica         | Sodium hypochlorite | -6.500                | 0.006*      |
|                     | T. vulgaris         | -0.250                | 1.000       |
|                     | S. persica          | -0.500                | 0.993       |
|                     | C. arvensis         | -1.500                | 0.714       |
| C. arvensis         | Sodium hypochlorite | -5.000                | 0.001*      |
|                     | T. vulgaris         | 1.250                 | 0.825       |
|                     | S. persica          | 2.000                 | 0.468       |
|                     | A. nilotica         | 1.500                 | 0.714       |

Post hoc Tukey’s t-test; *Significant at P<0.05. T. vulgaris: Thymus vulgaris, S. persica: Salvadora persica, A. nilotica: Acacia nilotica, C. arvensis: Calendula arvensis

The page contains a table of bacterial zones of inhibition and discussion on endodontic treatment. The text discusses the importance of irrigation in endodontic treatment, highlighting its role in eliminating microorganisms and other contents of the pulpal spaces, which might act as possible sources of infection or reinfection. It further emphasizes the need for an irrigant that can penetrate well and have bactericidal activity. The study evaluated and compared the antibacterial activity of several herbal irrigants against E. faecalis and found that an irrigant with good penetrability and bactericidal activity is essential.

**DISCUSSION**

Microorganisms essentially lead to the development of pulpal and periradicular diseases and are associated with endodontic treatment failures. Endodontic treatment aims to eliminate the microorganisms and other contents of the pulpal spaces, which might act as possible sources of infection or reinfection. Hence, chemomechanical debridement of the root canal system becomes an utmost important phase of endodontic treatment to disinfect the root canal. Owing to the limitations of mechanical instruments to reach far and beyond in narrow isthmuses, accessory canals, and dentinal tubules, it becomes essential that an irrigant with good penetrability and bactericidal activity is used. Irrigation of the root canal is an essential adjunct to mechanical debridement as it flushes out the debris, infected material, disinfects the canal system, dissolves the tissues and also cleans the complex internal anatomy of the root canal space inaccessible to instrumentation. Thus, the use of irrigants ensures the elimination of bacteria and organic tissue remnants, thereby preventing reinfection.

The present study evaluated and compared the antibacterial efficacy of T. vulgaris, S. persica, A. nilotica, C. arvensis and 5% sodium hypochlorite against E. faecalis. The mean values for the zone of inhibition were as follows: Group A: 5% NaOCl - 18.75 mm, Group B: 20% T. vulgaris - 12.50 mm, Group C: 12.5% S. persica - 11.75 mm, Group D: 10% A. nilotica - 12.25 mm, and Group E: 10% C. arvensis - 13.75 mm. Table 3 shows the comparison of mean values between the five tested groups.
The result of the present in vitro study is in accordance with the study done by Garg et al.

E. faecalis, a facultative anaerobic Gram-positive bacteria, is the most common Enterococcus species cultured from nonhealing endodontic cases.[11] It is the most frequently isolated microorganism in failed root canal cases.[3] Its virulence helps it to endure extreme environmental conditions and persist as a single organism or as a component of the flora.[18] It has the ability to form biofilm, which renders it resistant against antimicrobial agents.[3,11] Hence, E. faecalis was chosen as the test organism for this study.

The agar diffusion method allows direct comparison of the materials against the organisms, indicating the potential of the test materials to eliminate microorganisms in the local microenvironment of the root canal system.[3,12] The results may vary depending upon the ability of the material to diffuse across the medium and not only on the toxicity of the material for the particular organism.[3,12] In the present study, the experiment was repeated four times in accordance with a study by Balakrishnan et al., thereby making the results more reliable.

Sodium hypochlorite was used in this study as it is considered to be the gold standard for irrigation. It is both an oxidizing and hydrolyzing agent and also possesses bactericidal and proteolytic properties.[13] In the present study, 5% NaOCl had considerable antibacterial efficacy against E. faecalis. It created the largest bacterial zones of inhibition in the agar media.

The antibacterial activity of the hexanolic extract of Calendula powder, dissolved in DMSO can be attributed to flavonoids which have spasmylytic, anti-inflammatory, antihelminthic and antimicrobial property.[14] It inhibits the bacterial enzymes due to the reaction of the addition of their amine or thiol group.[14] It also contains carotenoids which has anti-fungal property and sesquiterpene glycosides (based on its alloaromadendrene, eudesmane and cubebane skeletons).[14] DMSO, which was used as a solvent for Calendula; is a clean, safe, highly polar, aprotic solvent that helps in bringing out the pure properties of all the components of the herb being dissolved.[14,15]

Known primary constituents of thyme include essential oil (borneol, carvacrol, cymol, linalool, and thymol), tannin, flavonoids (apigenin and luteolin), saponins, and triterpenic acid.[14] They have the ability to disintegrate the outer membrane of Gram-negative bacteria, releasing lipopolysaccharides and increasing the permeability of the cytoplasmic membrane to ATP.[17,18] Its extracts have phenols and tannins, which affect the enzymatic system of bacteria, especially those that are needed for plasmid replication.[16]

The antimicrobial function of A. nilotica is believed to be due to tannins, phenolic compounds, essential oil, and flavonoids.[3] It is effective against E. faecalis. It contains anti-inflammatory agents that inhibit the synthesis of prostaglandin, which is one of the most important mediators of inflammation.[19]

S. persica contains trimethylamine, salvadorime chloride, and fluoride in large amounts.[20] It showed some antimicrobial activity, which makes it possible to be used as an irrigant solution in endodontic treatment against the endodontic pathogens.[20]

Overall the results of the present study revealed that the herbal irrigants do exhibit antibacterial activity against endodontic pathogens and can be used as a substitute for
sodium hypochlorite, although the latter remains the gold standard.

**CONCLUSION**

Under the limitations of this in vitro study, it can be concluded that 5%NaOCl showed the maximum antibacterial activity against *E. faecalis*. The herbal products also demonstrated significant antibacterial efficacy and hence can be employed as an alternative to NaOCl. Since herbal products are easily extracted and are cost-effective, this study opens new avenues for the use of herbal products as root canal irrigants. More preclinical and clinical trials should be carried out, demonstrating the antimicrobial activity of the various abundant herbal products available in nature and also discover new natural compounds.

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**Conflicts of interest**

There are no conflicts of interest.

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