



RESEARCH ARTICLE

TO EVALUATE THE EFFECTIVENESS OF *TAGETES ERECTA* AND 5.25% SODIUM HYPOCHLORITE AT DIFFERENT TIME INTERVALS AGAINST *ENTEROCOCCUS FAECALIS* CONTAMINATED ROOT CANALS-AN INVITRO STUDY

¹, *Poojitha Viswanath, ²Prathaban Munisamy, ³Deepa Thangaraj,
¹Abitha Banu and ¹Loganathan

¹PG Student, KSR Institute of Dental Science and research, Tiruchengode, Tamil Nadu, India

²PG and Research, Department of Microbiology, K S Rangasamy Arts and Science College, Tamil Nadu, India

³Reader, KSR Institute of Dental Science and Research, Tiruchengode, Tamil Nadu, India

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ABSTRACT

Aim: The purpose of this study was to evaluate the antimicrobial efficacy of *Tagetes erecta* and 5.25% sodium hypochlorite at different time intervals against *Enterococcus Faecalis* in contaminated root canals.

Materials and Methods: Forty five biomechanically prepared single rooted teeth were selected and were inoculated with *Enterococcus faecalis* and randomly divided into 4 groups and control group containing 9 samples each. Group 1 samples were treated with 5.25% sodium hypochlorite (NaOCl) for 1 min, Group 2 specimens were treated with *Tagetes erecta* for 1 min. Group 3 samples were treated with sodium hypochlorite for 5 min and group 4 samples were treated with *Tagetes erecta* for 5 min and saline as final irrigant in all groups. The antimicrobial efficacy were tested by collecting dentinal chips from the inner walls of the canal and the number of colony forming units of viable *E.faecalis* obtained on agar plates were statistically analysed by One way Anova and Post hoc Tukey's analysis with a significance level at $p < 0.01$

Results: There was no statistically significant difference between 5.25% sodium hypochlorite and *Tagetes erecta* in 5 min time interval.

Conclusion: The antimicrobial efficacy of *Tagetes erecta* is more or less equal to Sodium Hypochlorite as root canal irrigants.

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INTRODUCTION

It makes impossible to shape and clean the root canal system completely due the complex anatomy of root canal system (Nair, 2006). Irrigation is an essential part of a root canal treatment as it allows for cleaning beyond the root canal instruments in irregularities of the root canal wall including oval extensions, isthmuses and apical deltas (Wu et al., 2007). The goal of irrigation is to remove pulp tissue and/or microorganisms (planktonic or biofilm) from the root canal system. It should also remove smear layer and dentine debris that occur following instrumentation of the root canal. Studies shows that within oval canals only 40% of the apical root canal wall area can be contacted by instruments when a rotating technique is used (Wu et al., 2003).

*Corresponding author: Poojitha Viswanath,

PG Student, KSR Institute of Dental science and research, Tiruchengode, Tamil Nadu, India.

An irrigant serves to flush out debris from the instrumented root canals, dissolve organic tissue remnants, disinfect the root canal space, provide lubrication during instrumentation and remove smear layer without causing irritation to the biological tissues (Cheung, 1993). The most commonly investigated agent, which has been accepted as the gold standard for the irrigation of root canal space, is sodium hypochlorite (NaOCl) because of its clinical efficacy in endodontic therapy. It has solvent activity for both necrotic and vital tissues. However there are still some concerns with respect to the toxic effects, bad smell and taste, corrosive potential and allergic reactions (Tirali et al., 2012). *Tagetes erecta*, popularly known as marigold, is grown as an ornamental plant. Flowers of this plant are used loose or in garlands for social and religious purposes in Eastern countries. The flowers are usually thrown away after their religious uses. This plant belongs to the family Asteraceae (Compositae) and the English name is marigold. Different parts of this plant including flower is used in folk medicine.

Table 1. Comparison of mean distribution of *Tagetes erecta* and sodium hypochlorite at different time intervals

Groups	N	Time interval	Mean±SD	p value
<i>Tagetes erecta</i>	9	1 min	353.44±17.42	<0.001
Sodium Hypochlorite	9	1 min	271.77±18.49	
<i>Tagetes erecta</i>	9	5 min	38.55±4.97	
Sodium Hypochlorite	9	5 min	27.33±3.80	
Negative control	9		590.00±64.42	

*One way ANOVA

Table 2. Post hoc analysis for *Tagetes erecta* and sodium hypochlorite at different time intervals

Groups	Sodium Hypochlorite 1 min	<i>Tagetes erecta</i> 5 min	Sodium hypochlorite 5 min	Negative control
<i>Tagetes erecta</i> 1 min	<0.001	<0.001	<0.001	<0.001
Sodium Hypochlorite 5 min		<0.001	<0.001	<0.001
<i>Tagetes erecta</i> 1 min				<0.001
Sodium Hypochlorite 5 min				<0.001

In traditional and homeopathic medicine it has been used for skin complaints, wounds and burns, conjunctivitis and poor eyesight, menstrual irregularities, varicose veins, hemorrhoids, duodenal ulcers, etc. The flowers are especially employed to cure eye diseases, colds, conjunctivites, coughs, ulcer, bleeding piles and to purify blood (Padalia, 2015). The null hypothesis is that there is no antimicrobial efficacy for *Tagetes erecta* against *E.faecalis* as root canal irrigants. Hence the aim of this study was to evaluate the effectiveness of two irrigation solutions at different time intervals for the elimination of *E.faecalis* in permanent teeth in vitro.

MATERIALS AND METHODS

The study protocol was analyzed and approved by the Institutional Review Board of KSR Institute of Dental Science and Research.

Flower Extract

250 grams of dried and ground petals were dried in a hot air oven and the materials were mixed with methanol and stirred at 350 rpm, 37°C for 24 hrs using an orbital shaker. The sample was filtered and solvent was then evaporated under vacuum using the rotary evaporator. The concentrated/dried extract was transferred into a small vial and stored at 4°C for further analysis. Extract was mixed in DMSO solution in to find the minimal inhibitory concentration.

Agar Diffusion Assay Method

Solid agar was punched with 7 mm diameter wells. Inoculum of *E faecalis* is spread on agar plates using sterile swabs. Wells were filled with 20 µl, 40 µl, 60 µl, 80 µl and 5.25% sodium hypochlorite. The results showed that 60 µl of solution had maximum zone of inhibition when compared with other concentrations. Forty five extracted single rooted human teeth were selected in this study, crown was decoronated upto cement enamel junction to standardize working length of 18 mm and apical foramina were sealed with composite. The specimens are autoclaved for 15 minutes under 121 degree celsius. The 0.01 ml *E.Faecalis* bacteria were inoculated in root canals and bacterial suspension everyday for 1 week. They were grouped into positive and negative controls, and divided into 4 groups.

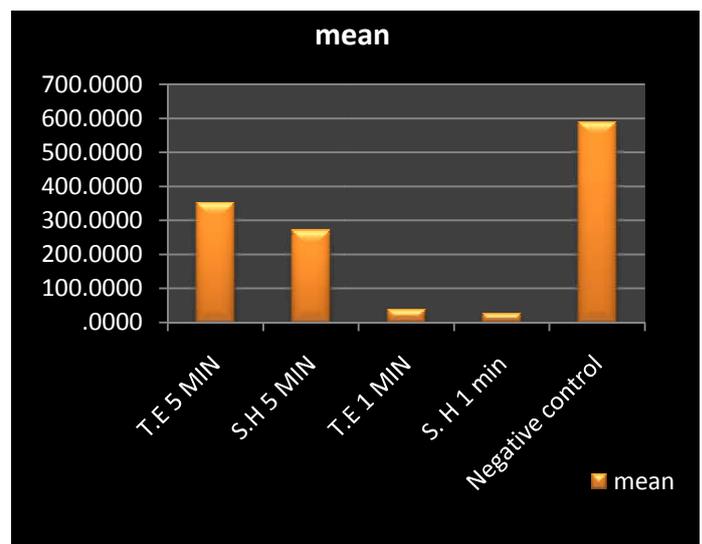
Group 1 - 5.25% NaOCl irrigation done for 1 min(n=9)
Group 2 - *Tagetes erecta* irrigation done for 1 min(n=9)

Group 3 - 5.25% NaOCl irrigation done for 5 min(n=9)
Group 4 - *Tagetes erecta* irrigation done for 5 min(n=9)

Canals were dried and refilled with normal saline (transfer fluid), and dentinal chips were collected from the inner walls by using N0. 1 gates glidden drills. Sterile paper points were used to collect the transfer fluid, placed into a test tube. Nutrient agar culture plates were incubated at 37 °c for 48 hours. CFU for each plate were calculated.

RESULTS

The statistical analysis were done by using One way Anova and Post hoc Tukey's analysis. The mean difference of *Tagetes erecta* (38.5556 ± 4.9) and sodium hypochlorite (27.3333 ± 3.8) showed there is no statistically difference between sodium hypochlorite and *Tagetes erecta* as antimicrobial irrigants when used in 5 min interval. Graph 1 represents the statistical difference between irrigants compared with negative control.

**Graph 1. Sodium hypochlorite and *Tagetes erecta* used as irrigants in 5 min and 1 min interval**

DISCUSSION

E. faecalis is a gram positive facultative anaerobe. It is a known endodontic pathogen. It is isolated frequently from failed root canal treated teeth. *E. faecalis* reduces the action of lymphocytes and hence result in endodontic failure. They

also show genetic polymorphisms. It has serine protease, collagen-binding protein (Ace), which helps the bacteria to attach to dentin and they are also small enough to invade and survive within dentinal tubules. Even a small proportion of the flora in untreated canals makes it a persistent organism causes persistent periradicular lesions after root canal treatment. It is commonly found in a high percentage of root canal failures and it is able to survive in the root canal as a single organism or as a major component of the flora. The bactericidal effect of NaOCl is due to release of hypochlorous acid and active chlorine. The depth of penetration of NaOCl is limited to 100 µm whereas, the depth of penetration of *E. faecalis* is up to 300-400 µm (Stuart, 2006). Studies by Estrela *et al.*, have shown antibacterial efficacy of NaOCl is questionable (Estrela, 2003). Use of good aseptic technique, increased apical preparation sizes, and inclusion of 2% chlorhexidine in combination with sodium hypochlorite are currently the most effective methods to combat *E. faecalis* within the root canal systems of teeth. (Wang, 2007) Bacteria deep in root dentin are protected from instrumentation and irrigation, due to limitation of depth of penetration of irrigants. This lead to the search of newer disinfectants against the *E. faecalis*. It has been proved earlier that essential oils were alternative for the treatment of localized infections in severe hospital acquired strains (Warnke, 2009). Many herbal medicines have a potential use in endodontics. Although herbal intracanal irrigants have been used in permanent teeth. In this study the mean value of sodium hypochlorite is higher when compared with *Tagetes erecta* in both 1 min and 5 min intervals. There was no statistically significant difference between *Tagetes erecta* and sodium hypochlorite in 5 min interval. The antibacterial activity of *Tagetes erecta* might be due to polyphenols, like flavonoids substances which has showed the antimicrobial against strains of negative and positive gram bacteria (Behidj-Benyounes, 2014). Eventhough antimicrobial efficacy of *Tagetes erecta* has been proved in various other microorganisms. (Padalia, 2015). To our knowledge no study has been done in *Tagetes erecta* as root canal irrigants.

Conclusion

- Within limitations of this study, *Tagetes erecta* can be used as an alternative irrigant by increasing its concentration and time duration.
- Further invivo studies might be helpful in determining the real potential of *Tagetes erecta* as root canal disinfectant.

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