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Original Article

Evaluation of Antimicrobial Efficacy of Homeopathic medicines Acid Carbolic, Kreosotum, *Echinacea*, and *Thuja occidentalis* Against *Enterococcus faecalis* Biofilm Formed on Tooth Substrate when compared to 3% Sodium Hypochlorite Solution

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Abstract

Introduction: The usual cause of failure of endodontic therapy is the persistence of microorganisms a major cause being *Enterococcus faecalis*. Sodium hypochlorite, the current gold standard among irrigant, has its own shortcomings and concurrent research for a better antibacterial agent is thus ensued in alternative medicine. The high cure rates of homeopathic medicines in many pathogenic manifestations are evident in literature. When homeopathic medicines are prescribed in ultradiluted form on the basis of symptom similarity, they stimulate body's own defense mechanisms. Hence, homeopathic phytotherapeutic substances which have shown substantial antibiotic, anti-inflammatory, and analgesic effects were tested in this study to assess their antimicrobial potential when compared to sodium hypochlorite.

Materials and Methods: A pure culture of *E. faecalis* was grown on brain heart infusion agar broth and incubated at 37°C overnight. Sixty newly extracted single-rooted teeth were divided into six groups (n = 10) after sectioning to a length of 13 mm. After sterilization, samples were inoculated with bacterial solution and were incubated. Group 1: Distilled water, Group 2: 3% sodium hypochlorite, Group 3: Acid carbolic, Group 4: *Echinacea angustifolia*., Group 5: Kreosotum, At the 2nd and 4th weeks, all groups were treated for 10 min with 3 ml of respective solutions. Quantitative analysis was performed.

Results: Among the tested homeopathic agents, acid carbolic (Group 3) and *E. angustifolia* (Group 4) showed an antibacterial efficacy very much similar to NaOCl in eliminating *E. faecalis* biofilm both at the 2 weeks and 4 weeks.

Conclusion: Homeopathic agents acid carbolic and *Echinacea* can be considered as potential endodontic irrigant.

Keywords: *Enterococcus faecalis*, Biofilm, Acid Carbolic, *Echinacea*, Sodium hypochlorite

INTRODUCTION

One of the cardinal goals of endodontic treatment is root canal disinfection and prevention of ensuing chances of reinfection. As an adjuvant procedure to instrumentation, root canal irrigation strives to eliminate the bacteria from the complex root canal intricacies.[1,2] However, the persistence of the microorganisms in the complex root canal system because of their incomplete elimination from the same due to inefficiency of irrigant can lead to the failure of endodontic treatment.[3,4] *Enterococcus faecalis*, a facultative bacterium, is one of the most prevalently isolated species from failed/infected root canals of both primary and permanent teeth with a prevalence rate of 10–76%.[5-7] An ideal irrigant thus should be able to dissolve necrotic debris, destroy bacteria, lubricate endodontic

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system, and remove the smear layer without affecting healthy tissues.[2,8] Therefore, success of an endodontic therapy largely depends on irrigation of root canal system.

Among the plethora of endodontic irrigant, sodium hypochlorite – NaOCl in the concentration range of 0.5–6% is the most commonly used irrigating solution.[8] However, the untoward effects of NaOCl have also been reported which include an unpleasant taste and odor, inability to abolish the smear layer, toxicity, possible paresthesia of mandibular nerve if extruded into periapical area, allergy, and an increase in coronal microleakage of adhesive restorations.[9-11] This scenario has led to much research in the realm of alternative medicine for an ideal endodontic irrigant.

Thus, homeopathy, one of the alternative medicine systems with its doctrine of "Similia Similibus Curentur," that is, likes are cured by likes and with reported high cure rates in pathogenic manifestations is undoubtedly a viable arena for the search of a better antibacterial agent.[12-16]

Aim

In view with the shortcomings of currently used irrigant in endodontics and the subsequent research for a better agent with higher antibacterial activity and biocompatibility, this study aimed to evaluate the antimicrobial efficacy of acid carbolic, Kreosotum, *Echinacea,* and *Thuja occidentalis* when compared to sodium hypochlorite.

SUBJECTS AND METHODS

For the study, 60 newly extracted non-carious teeth with a single root canal were chosen and divided into six groups (n = 10) for instrumentation. The teeth were immersed in 10% formalin for disinfection and fixation of the organic tissue for a period of 24 h, followed by cleaning of the external debris and calculus with an ultrasonic scaler. Teeth were kept in physiologic saline for the rest of the period. Each tooth was radiographed to establish the presence of a single patent canal, then sectioned with a diamond disc below the cement– enamel junction to achieve a standardized tooth length of 13 mm. The working length of each tooth was determined using a size 10 K-file. Root canals were then instrumented using step-back technique with hand instruments and the canals were enlarged to apical size 40 K-file. Canals were then irrigated with 3 ml of saline during the procedure to remove any debris. The samples were then sterilized by autoclave at 121°C for 20 min.

Bacterial Formation on Tooth Substrate

The specimens were immersed in a 5 ml brain heart infusion (BHI) broth culture of *E. faecalis* (American Type Culture Collection 29212) and cultured for 2 weeks at 37°C with 5% CO_2. Three times a week, the bacterial suspension was replenished with fresh medium, and contamination was tested by Gram staining and replating on blood agar plates. The same process was used to obtain a 4-week bacterial culture [Figures 1 and 2].

Biofilm Verification

Four teeth specimens were examined using a scanning electron microscope at magnification levels of ×1000, ×500, ×5000, and ×10,000 to verify biofilm formation following immersion in 5 ml *E. faecalis* culture and incubation in BHI broth for 2 weeks [Figure 3].

The samples were now divided into six groups based on the irrigant used in each. The irrigation protocol adopted was; irrigation with 15 ml of irrigant, using 30-gauge side vented needle (House Brand –EN122) at a flow rate of 2 ml/min.

Study Groups

Group 1: Distilled water
Group 2: 3% sodium hypochlorite
Group 3: Acid carbolic
Group 4: *Echinacea angustifolia*
Group 5: Kreosotum
Group 6: *T. occidentalis.*

Microbiological Analysis

The root canals were then ground to take out the dentine chips, and using the viable plate count method, colony-forming units (CFUs) were counted and mean CFU was calculated.[17]

Statistical analysis was done using IBM SPSS 20 (SPSS Inc., Chicago, USA). Kruskal–Wallis test with Bonferroni multiple comparison test was applied.

RESULTS

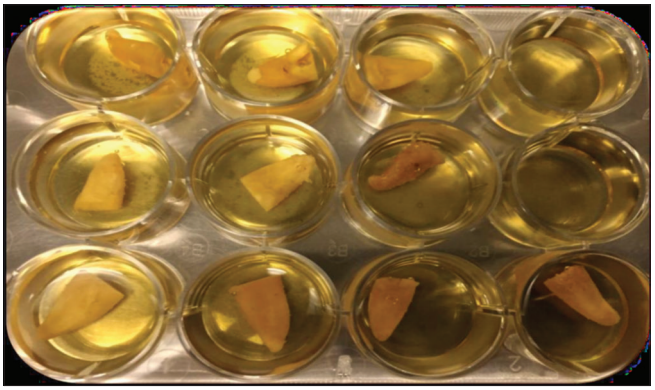


Figure 1: Roots immersed in brain heart infusion broth infected with *Enterococcus faecalis* (ATCC 2122)

Table 1: Number of colonies forming units in 10^6 pre-treatment values of each group

specimens	Group 1 distilled water	Group 2 NaOCl	Group 3 acid carbolic	Group 4 *Echinacea*	Group 5 Kreosote	Group 6 *Thuja*
1	6.6×10^6	6.6×10^6	6.6×10^6	6.6×10^6	6.6×10^6	6.6×10^6
2	6.6×10^6	6.6×10^6	6.6×10^6	6.6×10^6	6.6×10^6	6.6×10^6
3	6.6×10^6	6.6×10^6	6.6×10^6	6.6×10^6	6.6×10^6	6.6×10^6
4	6.6×10^6	6.6×10^6	6.6×10^6	6.6×10^6	6.6×10^6	6.6×10^6
5	6.6×10^6	6.6×10^6	6.6×10^6	6.6×10^6	6.6×10^6	6.6×10^6
6	6.6×10^6	6.6×10^6	6.6×10^6	6.6×10^6	6.6×10^6	6.6×10^6
7	6.6×10^6	6.6×10^6	6.6×10^6	6.6×10^6	6.6×10^6	6.6×10^6
8	6.6×10^6	6.6×10^6	6.6×10^6	6.6×10^6	6.6×10^6	6.6×10^6
9	6.6×10^6	6.6×10^6	6.6×10^6	6.6×10^6	6.6×10^6	6.6×10^6
10	6.6×10^6	6.6×10^6	6.6×10^6	6.6×10^6	6.6×10^6	6.6×10^6

Table 2: Mean colony-forming unit values after irrigation at 2 weeks

Irrigant	*n*	Colony-forming unit – 2 weeks			*P* value
		Mean	SD	Median (IQR)	
Group 1 Distilled water	10	5.41	0.09	5.4 (5.3–5.5)	<0.001
Group 2 Sodium hypochlorite 3%	10	1.00	0.00	1.0 (1.0–1.0)	
Group 3 Acid carbolic	10	1.00	0.00	1.0 (1.0–1.0)	
Group 4 Echinacea	10	1.51	0.13	1.50 (1.475–1.525)	
Group 5 Kreosotum	10	4.58	0.43	4.5 (4.2–4.975)	
Group 6 *Thuja occidentalis*	10	4.98	0.31	5.15 (4.65–5.20)	

n: Number of specimens, SD: Standard deviation

Table 3: Mean colony-forming unit values after irrigation at 4 weeks

Irrigant	*n*	Colony-forming unit – 4 weeks			*P* value
		Mean	SD	Median (IQR)	
Group 1 Distilled water	10	5.41	0.09	5.4 (5.3–5.5)	<0.001
Group 2 Sodium hypochlorite 3%	10	1.00	0.00	1.0 (1.0–1.0)	
Group 3 Acid carbolic	10	1.00	0.00	1.0 (1.0–1.0)	
Group 4 *Echinacea*	10	1.62	0.10	1.6 (1.5–1.7)	
Group 5 Kreosotum	10	4.70	0.44	4.5 (4.375–5.125)	
Group 6 *Thuja occidentalis*	10	5.14	0.18	5.2 (4.9–5.3)	

n: Number of specimens, SD: Standard deviation

Pre-treatment values obtained after inoculation with *E. faecalis* are given in Table 1. The results obtained after irrigating the samples with respective irrigant at 2 weeks are summarized in Table 2. The specimens treated with NaOCl showed no bacterial growth. Among the homeopathic irrigant, acid carbolic showed complete elimination of biofilm. *Echinacea* at this point showed significantly less bacterial growth (1.50 × 10 6 CFU/ml). The highest bacterial growth was seen in distilled water (5.41×10^6 CFU/ml) followed by *T. occidentalis* with a mean bacterial colony count of 4.98 × 10 6 CFU/ml followed by Kreosotum with a mean bacterial colony count of 4.58 × 10^6 CFU/ml [Figure 4].

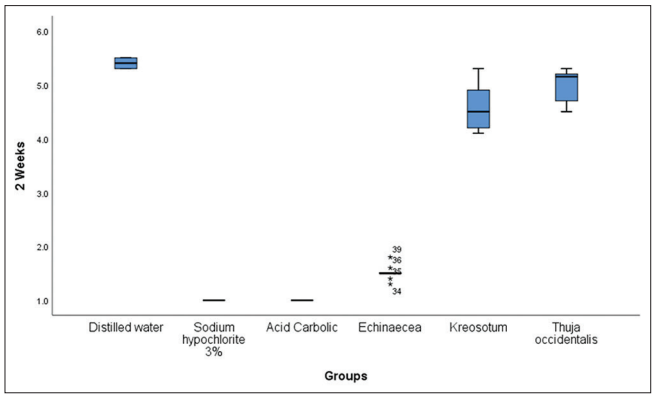
The remaining specimens in each group were similarly processed at the end of 4 weeks of incubation. The values obtained were like those seen at the 2 weeks evaluation. The specimens treated with NaOCl showed no bacterial growth. Among the homeopathic irrigant, acid carbolic showed complete elimination of biofilm. *Echinacea* showed significantly less bacterial growth (1.62×10^6 CFU/ml). The highest bacterial growth was seen in saline (5.41×10^6 CFU/ml) followed by *T. occidentalis* with a mean bacterial colony count of 5.14×10^6 CFU/ml followed by Kreosotum with a mean bacterial colony count of 4.70×10^6 CFU/ml. The results are summarized in Table 3 and Figure 5.

Bonferroni multiple comparison test was done to compare between the values at 2 weeks for each group was done, and it showed that the difference between each group was statistically significant. A similar comparison of the 4th week data also showed statistically significant differences [Graphs 1 and 2].

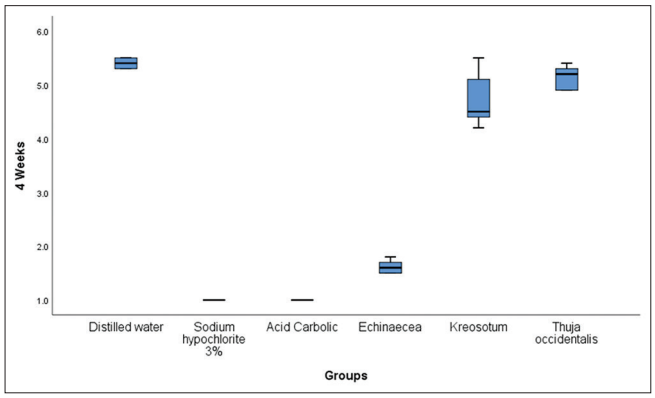
Percentage reduction of bacterial colony count after irrigation both at 2 weeks and 4 weeks period among groups was assessed using multiple comparison test. Statistically significant reduction in colony counts was seen in Group 2 sodium hypochlorite, Group 3 acid carbolic, and Group 4 *Echinacea* at both 2 weeks and 4 weeks. Results of percentage reduction of colony count are depicted in Bar Diagrams 1 and 2.

DISCUSSION

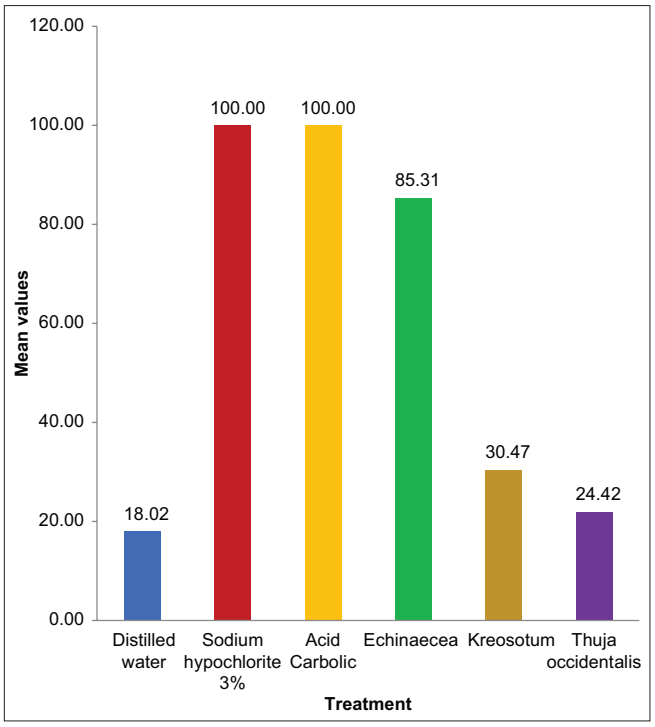
In endodontic therapy, complete control and elimination of infection remains challenging because of various intervening factors. It has been reported that 40% of a root canal system remain untouched even at the completion of cleaning and shaping procedure using rotary instrumentation which harbors bacteria causing reinfection.[18] A major micro organism among these are *E.faecalis* which are isolated from 63% of post treatment



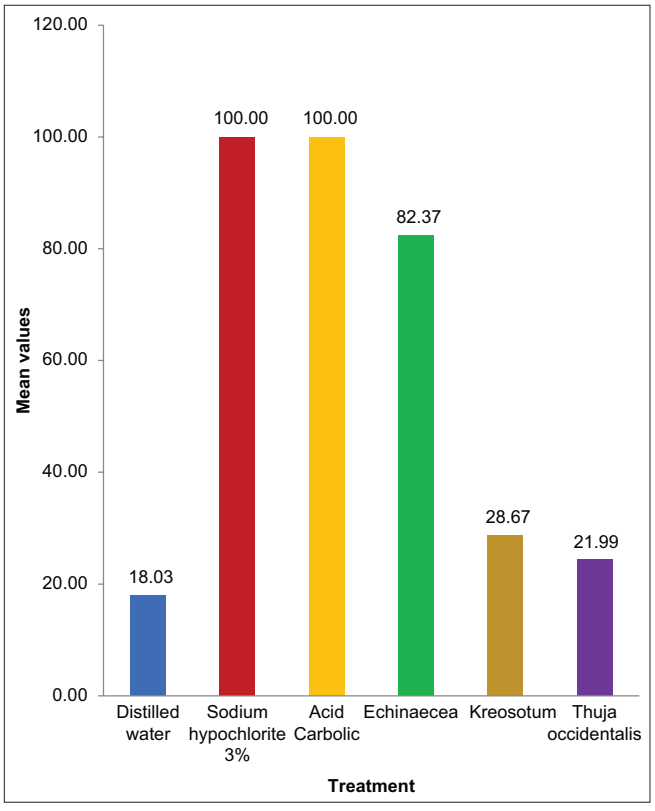
Graph 1: Comparison of colony-forming unit values after irrigation at 2 weeks



Graph 2: Comparison of colony-forming unit values after irrigation at 4 weeks



Bar Diagram 1: Percentage reduction of colony count among groups at 2 weeks



Bar Diagram 2: Percentage reduction of colony count among groups at 4 weeks

diseases. Thus, they entail a major role in etiology of persistent periradicular lesions after endodontic therapy. This underlines the need for endodontic irrigant as inevitable adjuncts to instrumentation in eliminating the microbes, especially in areas which are not accessible to mechanical instrumentation.[19] In the present day, endodontics, a plethora of endodontic irrigant, is being used among which NaOCl recommended as by the American Association of Endodontists as the gold standard for use as an endodontic irrigant.[20] However, as a reason of its high toxicity, corrosive nature, risk of causing emphysema, and severe hypersensitivity reactions, it's use is not approved by FDA.[21] Thus, in retrospection, neither sodium hypochlorite nor any other irrigant currently in use do accommodate the requirements of an ideal root canal irrigant. Thus, many researches have ensued which ponder on considering therapeutic agents in alternative and complementary treatment modalities as viable substitutes considering their minimum adverse effects.[22] Homeopathy is such a complementary medicine system, based on the key principle of "Like cures like" by utilizing phytochemicals and minerals as therapeutic agents. When homeopathic medicines are prescribed in ultradiluted form on the basis of symptom similarity, they stimulate body's own defense mechanisms to fight against pathogens. Homeopathy experiments on basophils and mast cells

showed that homeopathic medicines in ultradiluted form can influence degranulation of mast cells and basophil.[23] In the present study, the antimicrobial efficacy of four different homeopathic medicines when used as endodontic irrigant in the removal of *E. faecalis* biofilms (aged 2 weeks and 4 weeks) from root canals was compared with that of distilled water and 3% sodium hypochlorite. The homeopathic medicines used were acid carbolic, *Echinacea*, Kreosotum, and *T. occidentalis*.

In this study, the representative endodontic pathogen used was *E. faecalis*, because of its reported significance in persistent infections.[24,25] The virulence factor of *E. faecalis* in failed endodontically treated teeth may be related to its capability to invade dentinal tubules and adhere to collagen in the presence of human serum.[26] It can survive harsh environments such as extreme alkaline pH and temperature.[27] It has been reported that the resistance of these microorganisms is increased by the formation of biofilm. Hence, *E. faecalis* biofilm was established on a tooth substrate in this study.[14] Furthermore, a 2-week and 4-week incubation periods were adopted to confirm the biofilm maturation. This is in accordance with a previous report that a dense infiltration reaching 200 microns to 400 microns into the dentinal tubules is achieved during this time frame.[28,29] The present methodology has been adapted from a study by Prabhakar *et al.*[30] and has been corroborated in other similar studies.[31,32]

Being the recommended gold standard among endodontic irrigant, sodium hypochlorite (NaOCl) is capable of promoting biosynthetic changes in cells, metabolic alterations in them, and phospholipid destruction.[33] For killing the bacterial cells in <30 s, studies do report that 3% NaOCl is an efficient concentration.[34] Furthermore, Yesilsoy *et al.* reported that the toxicity of 3% NaOCl is much similar as that of its lesser concentrations.[35] Hence, in this study, 3% NaOCl was used. Although accepted by the American Association of Endodontists for routine use of NaOCl, due to its many short comings including high toxicity, FDA has not approved it.[21] In view with the concerns regarding usage of NaOCl, research in alternative medicine for a suitable irrigant has been the catchphrase of the moment. Homeopathy one of such alternative system has shown potential benefit in treating various diseases of epidemic proportions such as Swine flu, influenza-like illnesses, acute encephalitis syndrome, hemorrhagic dengue fever, diarrheal disorders, and chikungunya with already

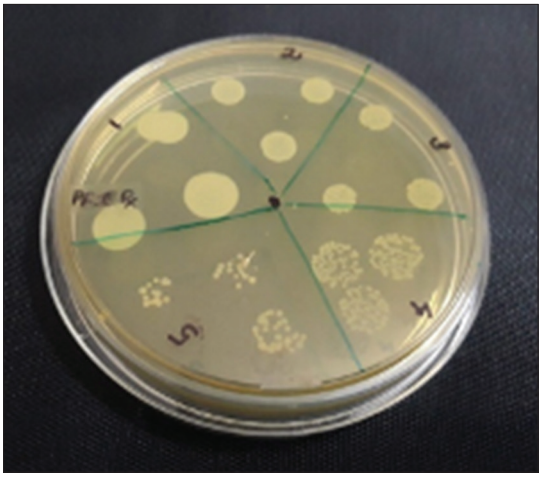


Figure 2: Pre-treatment colony-forming units

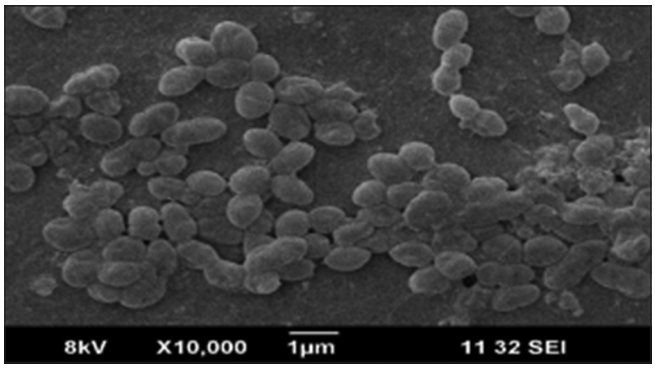


Figure 3: SEM image ×10,000

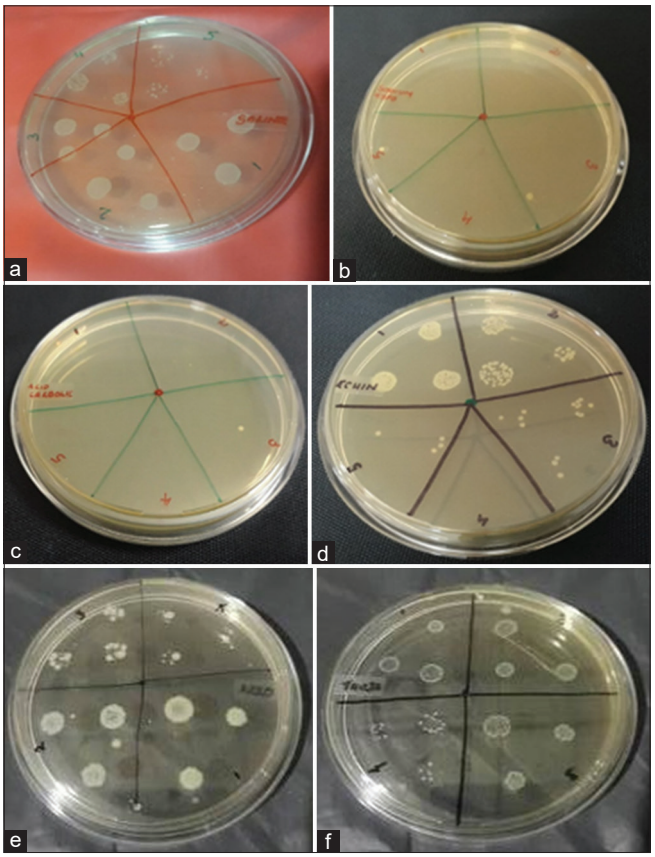


Figure 4: Colony-forming units post-irrigation = 2 weeks, (a) distilled water, (b) sodium hypochlorite (c) acid carbolic, (d) *Echinacea,* (e) Kreosotum, (f) *Thuja*

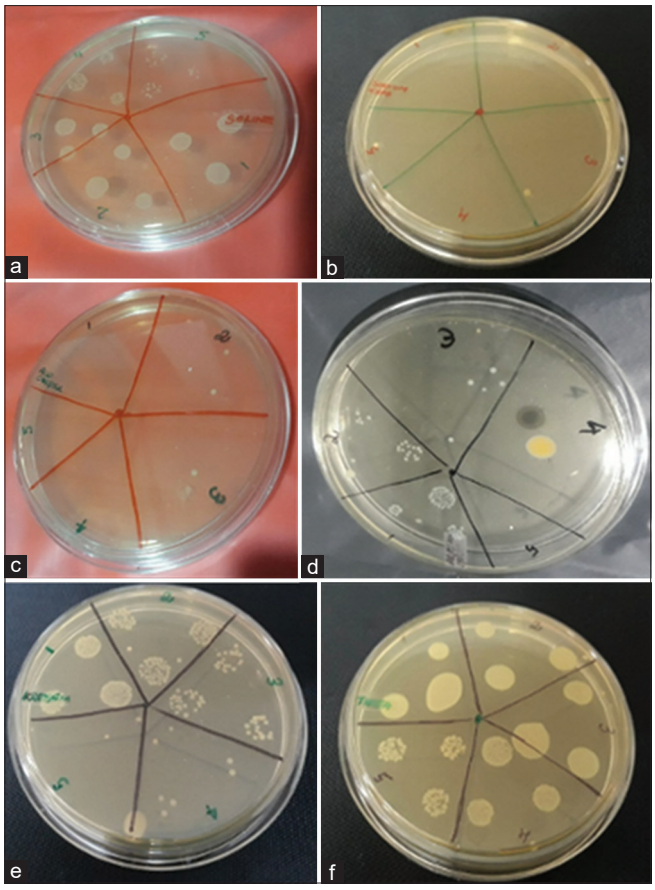


Figure 5: Colony-forming units post-irrigation = 4 weeks, (a) distilled water, (b) sodium hypochlorite, (c) acid carbolic, (d) *Echinacea*, (e) Kreosotum, (f) *Thuja*

existing and approved drugs.[36-44] At present, a previous advisory on the use of Arsenicum album 3°C as an immune booster for prophylaxis against COVID-19 infection is put forward by the Scientific Advisory Board of the Central Council for Research in Homeopathy.[45,46] In the present study, four homeopathic medicines, namely, acid carbolic, *Echinacea*, Kreosotum, and *T. occidentalis* were evaluated.

In the present study, acid carbolic (Group 4) when used as an endodontic irrigant, complete elimination of *E. faecalis* both at 2 weeks and 4 weeks, was observed. The antibacterial action of phenol is long reported in literature to be associated with cytological changes including membrane damage in bacteria.[47] Phenol has adequate penetration power into organic content. It has reported use in infected infections, as in omphalos of neonates. For cutaneous applications for itching, stings, bites, burns, etc., due to its anesthetic action and antibacterial activity to alleviate itching and to manage infections, the use of acid carbolic has been incorporated.[48-56]

Group 4 *Echinacea* showed statistically significant elimination of bacteria both at 2 weeks and 4 weeks. *Echinacea angustifolia* shows antibacterial activity against many microorganisms such as *Pseudomonas* species, *Saccharomyces cerevisiae*, and *Staphylococcus aureus*. A strong growth inhibition activity against *Haemophilus influenzae*, *Legionella pneumophila,* and *Clostridium difficile* has also been reported.[57-59]

In Group 5 (Kreosotum) and in in Group 6 (*T. occidentalis*), statistically significant elimination of bacteria either at 2 weeks or at 4 weeks was not observed. The antibacterial activity of Kreosote and *T. occidentalis* has been reported long back in literature and has a long history of use in treating psoriasis, cystitis, enuresis, uterine carcinomas, bronchial catarrh, amenorrhea and rheumatism.[60-64] The inferior antibacterial activity of Kreosotum agrees with a report by Lucas where Kreosotum extract only showed slight sensitivity against *E. faecalis* (7%) and *E. faecium* (1%).[65] Furthermore, the reduced effectiveness of *Thuja* in our study against a Gram-positive organism like *E. faecalis* can be attributed to the variation in availability of thujone, the major antibacterial component of *Thuja* according to each extraction procedure.[66]

In Group 1 sodium hypochlorite, there was complete elimination of the bacterial biofilm both at 2 weeks and 4 weeks. Elimination of *E. faecalis* can be ascertained to be due to the antibacterial effect of sodium hypochlorite. In Group 2 (distilled water), there was no elimination of *E. faecalis* post-irrigation either at 2 weeks or 4 weeks which can very well be attributed to the ability of *E. faecalis* to survive extreme alkaline pH and salt concentrations.[67-69]

A significant observation from this study is that both the homeopathic medications, namely, acid carbolic and *Echinacea* which were found effective against *E. faecalis* had significant antibacterial efficacy against both 2-week and 4-week biofilms. The fact that antibacterial efficacy of both of these medications was not influenced by the maturation of biofilm is an outcome of this study which is worth pondering

CONCLUSION

Thus, it can be concluded from the findings of the present study that the homeopathic agents acid carbolic and *Echinacea* can be considered as potential endodontic irrigant. An *in vitro* study, by its very nature, is never conducive when compared to that of a clinical scenario. Extensive clinical trials and further research are mandatory before any of these agents can be recognized as total replacements for the conventional and existing irrigant.

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