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IP Indian Journal of Conservative and Endodontics

Journal homepage: <https://www.ijce.in/>

Original Research Article

Comparative evaluation of various herbal agents for the disinfection of guttapercha cones – An in vitro study

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ARTICLE INFO

Article history:

Received 21-03-2023

Accepted 27-04-2023

Available online 25-05-2023

Keywords:

Guttapercha

Lemon grass oil

PanchaTulasi

Ozonated olive oil

disinfectants

ABSTRACT

In clinical practice, the dentist often encounters with the problem of post obturation infection, one possible reason for this could be the introduction of contaminated G.P into the root canal space. Herbal agents are gaining popularity these days as they are natural alternatives and are economical. So herbal agents like PanchaTulasi, Ozonated olive oil and Lemon grass oil are selected as experimental chair side disinfectants as GP cannot be sterilized owing to its inherent properties. The estimated sample size was 45 and they are grouped and artificially contaminated with *E faecalis* and *S aureus* and treated with respective disinfectants followed by incubation and checked for their efficacy using a digital microbiological colony counter. Lemon grass oil was found to be the most effective in both the groups followed by Panchatulasi in *S aureus* group and ozonated olive oil in *E faecalis* group.

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1. Introduction

The success of endodontic therapy can be achieved by maintaining an effective aseptic chain right from access opening to permanent coronal restoration.¹ Every tool and material used in the root canal, including obturating materials, must be sterile for unsurpassed infection control, keeping in mind the external bacterial contamination

Gutta-percha (G.P) is a solid core filling material introduced by Bowman in 1867,² and down the line it is successfully used in obturation material.¹ In clinical practice, the dentist often encounters with the problem of post obturation infection, One possible reason for this could be the introduction of contaminated G.P into the root canal space.^{3,4} Although G.P cones are manufactured under aseptic conditions, once exposed they can be contaminated through dental aerosols, physical handling with gloved hands etc.¹

According to literature staphylococcus genus is a commonly isolated species contaminating G.P cones in the boxes as well as after handling with gloves *E. faecalis* serves as a gold standard bacterium in endodontic research and found to be the most resistant intracanal pathogen in failed root canals. Hence these two organisms are selected for the study owing to their common occurrence G.P cannot be sterilized using conventional moist or dry heat sterilization due to its thermoplastic behavior, additional chair side disinfection or decontamination is critical to maintain aseptic chain during root canal treatment.^{1,3,4}

Herbal medicaments are gaining more popularity as they are natural form of alternatives and also they are easily accessible and economical.³ Few such herbal agents are Lemon grass oil, Ozonated olive oil and PanchaTulasi. Lemon grass essential oil is a proven anti-inflammatory and antioxidant mainly composed of citral which is a racemic mixture of 2 monoterpene aldehydes.⁵ Ozonated oil has a potent antiviral, antifungal and bactericidal

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activity and additionally it exhibits high biocompatibility.⁶ PanchaTulasi is a well-known herbal agent exhibiting good disinfective property due to the presence antimicrobial components like tannins, alkaloid's, anthraquinones etc. along with bioactive components.¹

Thus, the present study aims to evaluate and compare the disinfective efficacy of Lemon grass oil, Ozonated olive oil and Panchatulasi against *Staphylococcus aureus* and *E. faecalis*.

2. Materials and Methods

For the study, a total of 45 guttapercha (*Dentsplysironamaillefer*) cones of ISO size 50 were chosen. GP cones which are damaged, bent and available in pre opened packages are not included in the study

2.1. Artificial contamination of GP cones

40 GP cones were divided into two groups: Group A and Group B with 20 cones in each group. Group A with 20 cones are contaminated with 20 ml of microbial suspension of *S. aureus* for 30 minutes (Figure 1A). Group B with 20 cones were contaminated with 20 ml of microbial suspension of *E. faecalis* for 30 minutes (Figure 1B). Remaining 10 GP cones were not contaminated as they were used as the negative control without any disinfectant



Fig. 1: A: GP cones contaminated with *S.aureus*; B: GP cones contaminated with with *E faecalis*

2.2. Disinfection of GP cones

After artificial contamination, GP cones were immersed in the respective disinfectant solutions for one minute. Based on the disinfectant used, GP cones from both the groups are subdivided into four groups with 5 cones in each group.

Group I A: Five contaminated cones (*S. aureus*) immersed in Lemon grass oil. (Figure 2A)

Group II A: Five contaminated cones (*S. aureus*) immersed in Ozonated olive oil. (Figure 2A)

Group III A: Five contaminated cones (*S. aureus*) immersed in PanchaTulasi. (Figure 2A)

Group IV A: Five contaminated cones (*S. aureus*) without any disinfectant were taken as a positive as a control.

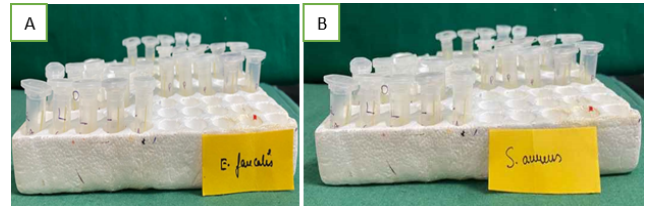


Fig. 2: A: Samples of the Efaecalis group disinfected with each herbal agent; B: samples of the Saureusgroup disinfected with each herbal agent

Group I B: Five contaminated cones (*E. faecalis*) immersed in Lemon grass oil. (Figure 2B)

Group II B: Five contaminated cones (*E. faecalis*) immersed in Ozonated olive oil. (Figure 2B)

Group III B: Five contaminated cones (*E. faecalis*) immersed in PanchaTulasi.(Figure 2B)

Group IV B: Five contaminated cones (*E. faecalis*) without any disinfectant serve as a positive control.

Group V: Consists of 5 uncontaminated cones which serve as negative control.

All the cones were transferred individually to sterile test tubes containing 10 ml of thioglycolate media and incubated at 37°C for seven days(Figure 3A).After seven days, a micropipette was used to transfer the thioglycolate media to a Petri dish containing Brain Heart Infusion (BHI) agar(C).A sterile cotton tip was used to spread the thioglycolate media in a thin layer over BHI agar. The plates were then incubated for 48 hours aerobically at 37°C (Figure 3B) and the Colony Forming Units (CFU) were counted with digital colony counter.

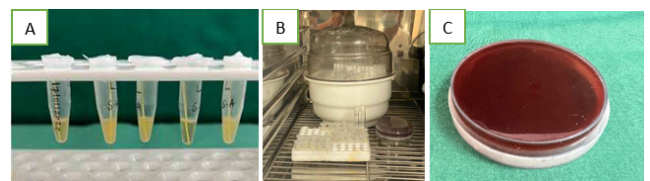


Fig. 3: A: samples transferred to the test tube for incubation in thioglycolate media; B: samples incubated in microbiological incubator; C: BHI agar

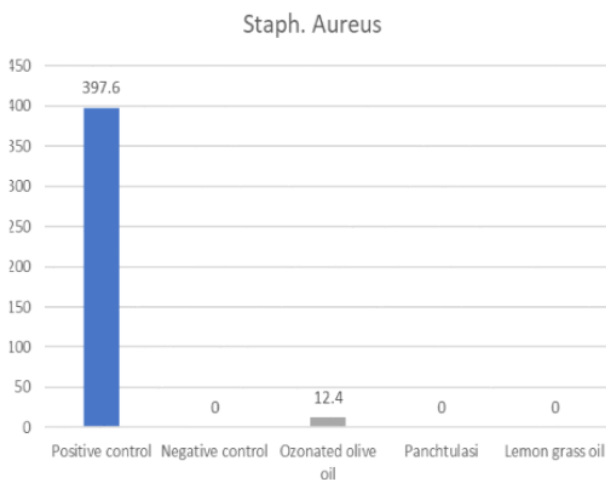
2.3. Statistical analysis

The values obtained were statistically analysed using computer software G Power 3.1.9.2. One way analysis of variance (ANOVA) followed by Tukey Post hoc test was used to analyze the data. P value <0.05 was considered as

statistically significant.

3. Results

Mean colony forming units were compared in all the groups and there was a statistically significant difference present among the groups ($p < 0.01$). As seen in there is statistically significant difference in the mean and SD when comparing group 1A and IIIA with 2A in E faecalis group and 1B and IIB with IIIB in S aureus group. And post hoc table represents the comparison between each group and proved that P value is highly significant. Graphs show the difference in the growth using each herbal agent and represent a statistically significant difference in mean values in both Efaecalis (Graph 1) and S aureus group (Graph 2). Lemon grass oil was found to be the most effective in both the groups followed by Panchatulasi in S aureus group and ozonated olive oil in E faecalis group.

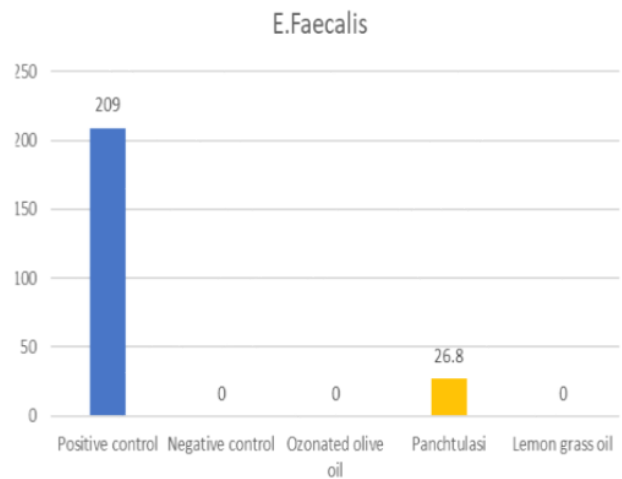


Graph 1: Comparison of the disinfective efficacy of the three herbal agents against S aureus in terms of CFU. In S.aureus group lemon grass showed less growth followed by panchatulasi and ozonated olive oil.

4. Discussion

GP cones may become contaminated by a number of microorganisms even though they are created in an aseptic setting or even just by handling. Research has shown that between 5% and 20% of newly opened GP packs contain microorganisms.¹ Chemical disinfectants are required since high temperatures are ineffective for sterilising GP owing to its inherent properties. Furthermore, this should be a quick, economical, and effective way.³

In the present study GP cones were artificially contaminated with E faecalis and staphylococcus genus. E. faecalis was chosen for the present study because of its superior virulence property to represent the other



Graph 2: Comparison of the disinfective efficacy of the three herbal agents against E faecalis in terms of CFU. In E faecalis group lemon grass oil showed less growth followed by ozonated olive oil and panchatulasi.

potential microorganisms that may contaminate GP cones. The Staphylococcus aureus is one among the most prevalent microorganisms infecting GP cones in their boxes and following glove handling. According to De Lima Guimaraes et al., 15.7% of patients had the Staphylococcus genus, which supports the need for GP disinfection.¹

Herbal agents are gaining popularity as they serve as natural disinfectants and also due to the fact that they are economical and easily accessible. Mukka et al in 2017 compared various herbal agents like aloe vera juice, amla juice and panchatulasi and concluded that good disinfection is achieved with panchatulasi this may be due to its high content of bioactive and antibacterial components, such as flavonoids, alkaloids, anthraquinones, saponins, resins, polysaccharides, steroidal terpenes, cardiac glycosides, and steroidal rings etc.

In the current study Lemon grass oil, Ozonated olive oil and Panchatulasi are used for disinfection of GP cones contaminated with S.aureus and E.faecalis.

Shin et al (1970) in his clinical testing revealed the antimicrobial nature of ozone on the planktonic forms of E faecalis. Ozone has been employed in medicine for the last ten years because of its great biocompatibility as well as due to its antiviral, antifungal, and bactericidal properties (Valacchi et al., 2005). Ozonated olive oil has been used extensively to support tissue healing and regeneration because it has a potent antibacterial and oxygenating effect on a range of microorganisms additionally having a disinfectant effect.⁷

Saddiq et al and Falaw et al reported the antimicrobial activity of lemon grass oil which mainly contains citral which is a racemic mixture of 2 monoterpene aldehydes.

De silva et al also proved its 100% toxicity against fungus. Which is comparable to the findings of the present study, It is anti-inflammatory, and antioxidant; hence recommended for use in mouth rinses, tooth pastes, and in diseases of oral cavity.⁵ However, there is relatively little literature describing its application, and much more research is needed.

In the present study Lemon grass oil had shown excellent disinfection of gutta-percha cones followed by PanchaTulasi and ozonated olive oil.^{8–12}

5. Conclusion

Gutta-percha disinfection, although seems to be a minor step it plays an important role in the success of the root canal treatment. Within the limitations of this study all the herbal agents demonstrated disinfection against *S.aureus* and *E.faecalis*. lemon grass oil exhibited good disinfection followed by panchatulasi and ozonated olive oil. There was significant difference between the number of colony forming units between the experimental groups and control groups.

6. Conflicts of Interests

The authors have no financial interests or conflicts of interests.

7. Source of Funding

None.

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Cite this article: Chowdary GA. Comparative evaluation of various herbal agents for the disinfection of guttapercha cones – An in vitro study. *IP Indian J Conserv Endod* 2023;8(2):86-89.