

An *in vitro* Comparative Study of Various Herbal and Synthetic Solutions on Gutta-percha Cones against *Enterococcus faecalis* and *Candida albicans*

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Abstract

Aim: The aim of the present study is to evaluate and compare the various herbal and synthetic solutions on the disinfection of gutta-percha cones. **Materials and Methods:** 160 gutta-percha cones (2% taper, size 80), microbial suspension of *Enterococcus faecalis* (ATCC2912), and *Candida albicans* (ATCC 1023). Chemical disinfectants (octenidine, 2% chlorhexidine, and 3% sodium hypochlorite.) Herbal disinfectants (*Aloe vera*, propolis 30%, and green tea extract). The data were collected and entered in Microsoft Excel 2010 (Microsoft corp.) and analyzed using the SPSS version 20.0 operating on Windows 10. All the descriptive data were presented as mean, standard deviation, frequency, and percentages represented as pie charts and bar diagrams. The continuous data were analyzed using student t-test for mean difference and the strength of association between the variable using Pearson's correlation was calculated. Results were presented in the form of tables and graphs. Shapiro–Wilk test, one-way analysis of variance test, and paired t-test were used. A $P < 0.05$ was considered statistically significant. **Results:** The result of four studies illustrate that herbal disinfectants are more effective against *C. Albicans* as compared to *E. faecalis*. **Conclusion:** In this *in vitro* study, all the tested solutions demonstrated disinfection action against *C. albicans* and *E. faecalis*. There was a significant difference found in the number of colony-forming units between Experimental and Control groups.

Keywords: *Candida albicans*, Endodontic treatment, *Enterococcus faecalis*, Herbal products.

INTRODUCTION

Endodontic treatment is one of the most predictable treatments in dentistry with success rates of 86% and 98% owing to the tremendous advancements that have taken place in recent years.^[1] According to concepts of infection control, every instrument and material placed within the root canal should be sterile.^[2] Pulpal and periapical diseases are inflammatory conditions of microbial etiology.^[3] However, microorganisms and their products are associated with endodontic treatment failure and perpetuation of periapical disease being considered as primary etiological agents of pulpal necrosis and apical periodontitis.^[4] Therefore, one of the main goals of endodontic treatment is the elimination of infection in the root canal system to obtain an environment conducive to the healing of apical periodontitis.^[5]

Enterococcus comprises a group of Gram-positive bacteria that are associated with endodontic infections in dentistry.^[6] *Enterococcus faecalis*, a commonly detected microorganism in endodontic treatment failures, is able to survive under

unfavorable conditions and organize in biofilm.^[7,8] The prevalence of *E. faecalis* in the root canal is higher in patients previously subjected to endodontic treatment or in cases of retreatment, which can be explained by low sensitivity to antimicrobial agents used as irrigating solution or intracanal medication.^[6]

Thorough biomechanical preparation and intracanal medication are essential for proper disinfection of root canal system during endodontic treatment. At the same time, it is also important to ensure that no exogenous bacteria enter the disinfected root canal subsequently thus maintaining the disinfection advent of the same; the present study was undertaken to

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evaluate and compare the antimicrobial activity and efficacy of various herbal solutions such as *Aloe vera*, green tea extract, and propolis (herbal) and synthetic solutions such as chlorhexidine gluconate and tetraclean (chemical) disinfectants on disinfection of gutta-percha cones before obturation against *E. faecalis* and *Candida albicans*.

MATERIALS AND METHODS

The present study titled “An *in vitro* comparative study of various herbal and synthetic solutions on gutta-percha cones against *E. faecalis* and *C. albicans* and a scanning electron microscopic evaluation of surface changes on gutta-percha cones after their disinfection” was conducted in Department of Conservative Dentistry and Endodontics, at Faculty of Dental Sciences, P.D.M. University, Bahadurgarh, Haryana, the Centre for Biotechnology, Maharshi Dayanand University, Rohtak, and All India Institute of Medical Sciences, New Delhi.

Study design

The design of the study was a comparative observational study.

Study center

The study was conducted in Department of Conservative Dentistry and Endodontics, at Faculty of Dental Sciences, P.D.M. University, Bahadurgarh.

Materials

160 gutta-percha cones (2% taper, size 80) were taken from freshly opened boxes under sterile condition. Test organisms: Microbial suspension of *E. faecalis* (ATCC2912) and *C. albicans* (ATCC 1023) of approximately 10^8 colony-forming units (CFU)/mL in Trypticase soy broth (Hi-Media Laboratories) was used for this study. Both the strains were grown in 8-mm culture tubes and incubated at 37°C for approximately 23 h. Chemical disinfectants are octenidine, 2% chlorhexidine, and 3% sodium hypochlorite. Herbal disinfectants are *A. vera*, propolis 30%, and green tea extract.

Methods

160 gutta-percha cones were divided into 2 groups: Group A and Group B with 80 cones in each group. The cones from Group A were contaminated with *C. albicans* and Group B were contaminated with *E. faecalis*. The contaminated cones from both groups were subdivided into 8 groups with 10 cones in each group.

- Group I cones were disinfected with octenidine, Group II with 3% sodium hypochlorite, Group III with 2% chlorhexidine, Group IV with *A. vera* juice, Group V with propolis 30%, and Group VI with green tea extract. Group VII consisted of gutta-percha cones without any disinfection in both Group A and Group B which served as positive control and Group VIII consisted of gutta-percha cones with contamination of *C. albicans* and *E. faecalis*, respectively, served as negative control group.
- The disinfection time was 1 min. All the cones were then incubated in thioglycolate media for 7 days. The thioglycolate media will be subculture and colony-forming units will be counted.

Artificial contamination of gutta-percha cones

160 GP cones were divided into 2 groups: Group A and Group B with 80 cones in each group. Group A with 70 cones was contaminated with 20 mL of microbial suspension of *C. albicans* for 30 min. Group B with 70 cones was contaminated with 20 mL of microbial suspension of *E. faecalis* for 30 min. They were subsequently transferred for air drying to sterile dishes containing sterile 4 × 4 gauze pads.

Remaining 10 cones of Groups A and B were uncontaminated and served as negative control group.

Disinfection of gutta-percha cones

After intentional contamination, GP cones were immersed in the respective disinfectant solutions for 1 min. Based on the disinfectant used, GP cones from both groups were subdivided into 7 groups with 10 cones in each group.

Group A:

- Group I A-10 contaminated cones (*C. albicans*) immersed in octenidine.
- Group II A-10 contaminated cones (*C. albicans*) immersed in 3% sodium hypochlorite.
- Group III A-10 contaminated cones (*C. albicans*) immersed in 2% chlorhexidine.
- Group IV A-10 contaminated cones (*C. albicans*) immersed in *A. vera* juice.
- Group V A-10 contaminated cones (*C. albicans*) immersed in propolis 30%.
- Group VI A-10 contaminated cones (*C. albicans*) immersed in green tea extract.
- Group VII A-10 contaminated cones (*C. albicans*) without any disinfectant and served as positive control.
- Group VIII A-10 uncontaminated cones served as negative control group

Group B:

- Group I B-10 contaminated cones (*E. faecalis*) immersed in octenidine.
- Group II B-10 contaminated cones (*E. faecalis*) immersed in 3% sodium hypochlorite.
- Group III B-10 contaminated cones (*E. faecalis*) immersed 2% chlorhexidine.
- Group IV B-10 contaminated cones (*E. faecalis*) immersed in *A. vera* juice.
- Group V B-10 contaminated cones (*E. faecalis*) immersed in propolis 30%.
- Group VI B-10 contaminated cones (*E. faecalis*) immersed in green tea extract.
- Group VII B-10 contaminated cones (*E. faecalis*) without any disinfectant and served as positive control.
- Group VIII B-consisted of 10 uncontaminated cones which served as negative control.

All the cones of Groups A and B were individually transferred to sterile test tubes containing 10 mL of thioglycolate media and were incubated at 37°C for 7 days. After 7 days, a micropipette was used to transfer the thioglycolate media to a

Petri dish containing brain–heart infusion (BHI) agar. A sterile cotton tip was used to spread the thioglycolate media in a thin layer over BHI agar. The plates were incubated for 48 h aerobically at 37°C, and the CFUs were counted with digital colony counter.

Statistical analysis

The data were collected and entered in Microsoft Excel 2010 (Microsoft corp.) and analyzed using the SPSS version 20.0 operating on Windows 10. All the descriptive data was presented in form of mean, standard deviation and frequency while data in form of percentages was represented in form of pie charts and bar diagrams. The continuous data were analyzed using student t-test for mean difference, and the strength of association between the variable using Pearson's correlation was calculated. Results were presented in the form of tables and graphs. Shapiro–Wilk test, one-way analysis of variance (ANOVA) test, and paired t-test were used. A $P < 0.05$ was considered statistically significant.

OBSERVATION AND RESULTS

In the present study, we investigated the antimicrobial activity and efficacy of various herbal solutions such as *A. vera*, green tea extract, propolis (herbal) and synthetic solutions such as chlorhexidine gluconate and tetraclean (chemical) disinfectants on disinfection of gutta-percha cones before obturation against *E. faecalis* and *C. albicans*.

160 GP cones were divided into 2 groups based on the contaminants used into Group A and Group B with 80 cones in each group. The cones from Group A were contaminated with *C. albicans* and Group B with contaminated with *E. faecalis*. The contaminated cones from both groups were subdivided into 8 groups with 10 cones in each group depending on the disinfectants used (octenidine, 3% sodium hypochlorite, 2% chlorhexidine, *A. vera* juice, propolis 30%, green tea extract, positive control, and negative control).

The data were collected and entered in Microsoft Excel 2010 (Microsoft corp.) and analyzed using the SPSS version 20.0 operating on Windows 10. All the descriptive data was presented in form of mean, standard deviation and frequency while data in form of percentages was represented in form

of pie charts and bar diagrams. The continuous data were analyzed using student t-test for mean difference and the strength of association between the variables using Pearson's correlation was calculated. Results were presented in the form of tables and graphs. Shapiro–Wilk test, one-way ANOVA test, and paired t-test were used. A $P < 0.05$ was considered statistically significant. The above table shows the comparison of mean colony values of different disinfectant solution groups contaminated with *C. albicans*. One-way ANOVA was applied which shows that there was statistically significant difference in the mean values ($P < 0.05$). One-way ANOVA was applied which shows that there is a statistically significant difference in the mean values ($P < 0.05$). Table 1 and Graph 1 shows the comparison of mean colony values of different disinfectant solution groups contaminated with *C. albicans*.

One-way ANOVA was applied which shows that there was a statistically significant difference in the mean values ($P < 0.05$). The mean value 146.90 was for positive control Group VIIA–without any disinfectant and mean value 1.630 of negative control Group VIIIA-uncontaminated cones shows the control values if no disinfectant was used after contamination and if no contamination was used.

In case of all other disinfectant solution groups, the mean value 2.280 for green tea extract group was minimum. Similarly, other lower mean value 6.06 for octenidine, mean value 8.70 for propolis 30%, and 11.69 for 3% sodium hypochlorite groups show better impact of disinfection, whereas higher mean colony values 71.10 for 2% chlorhexidine and 62.75 for *A. vera* juice shows lower impact of disinfection process.

Table 2 and Graph 2 shows the comparison of mean colony values of different disinfectant solution groups contaminated with *E. faecalis* and shows that one-way ANOVA was applied which shows that there is a statistically significant difference in the mean values ($P < 0.05$). The mean value 156.90 is of positive control Group VIIA–without any disinfectant and mean value 1.630 of negative control Group VIIIA–Uncontaminated cones shows the control values if no disinfectant was used after contamination and if no contaminated was used. In case of all other disinfectant solution groups, the mean value 2.92 for green tea extract group is minimum. Similarly, other lower mean value 5.62 for octenidine, mean value 9.09 for propolis

Table 1: Comparison of mean colonies among different groups (contaminated with *Candida albicans*)

Groups A Contaminated with <i>Candida albicans</i>	N	Mean Colonies	SD	F-test	P-value	Result
Group I A-octenidine.	10	6.060	0.438			
Group II A -3% sodium	10	11.690	0.420			
Group III A-2% chlorhexidine	10	71.100	1.545			
Group IV A- <i>Aloe vera</i> juice.	10	62.750	3.310			
Group V A-Propolis 30%.	10	8.700	0.570			
Group VI A-Green tea extract	10	2.280	0.322	8155.349	0.000	Sig
Group VIIA-W/O any disinfectant positive control	10	146.900	3.479			
Group VIIIA A-Uncontaminated cone-negative control	10	1.630	0.082			
Total	80	38.889	48.723			

30%, and 16.73 for 3% sodium groups show better impact of disinfection, whereas higher mean colony values 79.45 for 2% chlorhexidine and 68.80 for *A. vera* juice show lower impact of disinfection process.

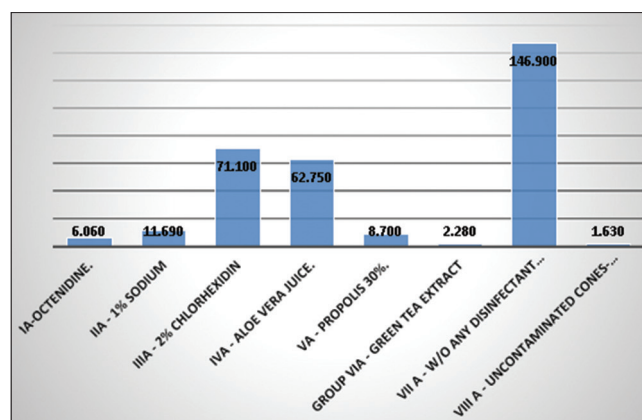
DISCUSSION

The placement of gutta-percha is the final step in the obliteration of the root-canal space; thus, sterility is of the utmost importance. Because it is difficult to determine number of accessory cones to be used during lateral condensation before initiating the procedure, an effective, quick-acting chemical agent which causes no or minimal changes in the surface of gutta-percha cones is mandatorily needed to prevent any possible surface contamination by microorganisms and success of root canal treatment in endodontic practice. In our study, we investigated the antimicrobial activity and efficacy of various herbal solutions such as *A. vera*, green tea extract, and propolis (herbal) and synthetic solutions such as chlorhexidine gluconate and tetraclean (chemical) disinfectants of gutta-percha cones before obturation against *E. faecalis* and *C. albicans*. 160 gutta-percha cones were selected and tested for the study and were divided into 2 groups based on the contaminants used into Group A and Group B with 80 cones in each group. The cones from Group A were contaminated with *C. albicans* and Group B with contaminated with *E. faecalis*. The contaminated cones from both groups will be subdivided into 8 groups with 10 cones in each group depending on the disinfectants used (octenidine, 3% sodium hypochlorite, 2% chlorhexidine, *A. vera* juice, propolis 30%, green tea extract, positive control, and negative control).

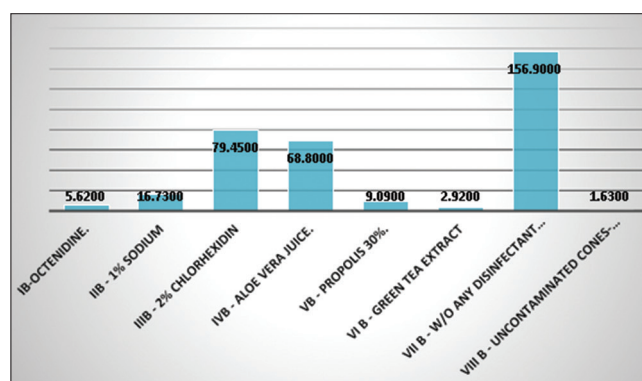
The agar diffusion method was used in this study as a preliminary assessment as it is one of the most often used methods for antimicrobial activity assessment. This method is accepted as an adequate way of comparing the antibacterial effect of different dental materials, medicaments, and root canal irrigants. The diffusion capacity of materials in agar is dependent on several factors such as contact between material and agar, molecular weight, concentration of test material, and agar gel viscosity. Furthermore, control and standardization of inoculation density, evaluation of results, selection of agar medium, incubation temperature of plates, and reading point of inhibition halos are also restricting factors affecting the dynamics and variability of diffusion tests in an agar medium.

The 3 herbal solutions used in this study are proven to be safe, containing active constituents that have beneficial property such as antimicrobial, antioxidant, and anti-inflammatory activity. Sheno *et al.*^[9] evaluated the efficacy of three herbal gels (lemon, tulsi, and green tea) in disinfecting GP cones. They concluded that all the gels showed inhibition zones nearly equal to 5.25% NaOCl. Thus, it would appear prudent to replace the traditional GP disinfecting solutions with these potential herbal solutions.

Within the limitations of this study, green tea extract showed the best results among the three herbal-tested solutions. All the tested solutions demonstrated disinfection action against



Graph 1: Comparison of mean colonies among different groups (contaminated with *Candida albicans*)



Graph 2: Comparison of mean colonies among different groups (contaminated with *Enterococcus faecalis*)

C. albicans and *E. faecalis*. There was a significant difference found in the number of CFU between Experimental and Control groups.

Green tea extract showed statistically significant activity against *E. faecalis* and *C. albicans*. Although less effective, these experimental solutions are less tissue toxic when compared with sodium hypochlorite. Further research is required to conclusively establish the efficacy of these herbal extracts as disinfectants.

Green tea was most effective as a disinfectant solution followed by octenidine. Green tea exhibited 2 in 1 quality with good disinfection action against *C. albicans* and *E. faecalis*, followed by octenidine and propolis 30%. Hence, herbal alternatives prove to be advantageous considering the several undesirable characteristics of NaOCl (Short *et al.*^[10] and Valois *et al.*^[11]). Furthermore, as the herbal alternatives are cheaper and easily available, they can be preferred over the synthetic solutions.

SUMMARY

Within the limitation of this study, the following observations were made:

- In this *in vitro* study, all the tested solutions demonstrated disinfection action against *C. albicans* and *E. faecalis*.

Table 2: Comparison of mean colonies among different groups (contaminated with *Enterococcus faecalis*)

Groups B Contaminated with <i>Enterococcus faecalis</i>	N	Mean	SD	F	P-value	Result
Group I B-Octenidine	10	5.6200	0.41042			
Group II B-3% sodium hypochlorite	10	16.7300	0.37431			
Group III B-2% chlorhexidine	10	79.4500	1.27911			
Group IV B- <i>Aloe vera</i> juice	10	68.8000	2.25093			
Group V B-Propolis 30%	10	9.0900	0.60268	14530.21	0.000	Sig
Group VI B-Green tea extract	10	2.9200	0.33599			
Group VII B-W/O any disinfectant Positive Control	10	156.9000	3.07137			
Group VIII B-Uncontaminated cone-negative control	10	1.6300	0.08233			
Total	80	42.6425	52.21282			

- There was a significant difference found in the number of CFU between Experimental and Control groups.
- A statistically significant correlation was observed between the mean colonies among different groups for both groups contaminated with *C. albicans* and *E. faecalis* (one-way ANOVA; $P = 0.000$) (Tables 1 and 2).
- The result of four studies illustrates that the herbal disinfectants are more effective against *C. Albicans* as compared to *E. faecalis*.
- In the present study, changes in the surface topography of gutta-percha were observed with all disinfectants as crystal deposits, granular deposits, or surface irregularity.
- A statistically significant correlation was observed in our study between the surface topographic changes and disinfectant solutions (Chi-square test; $P = 0.000$).
- All disinfectants showed surface topographic changes with *A. vera* juice showing no changes in 7 and mild changes in 3 followed by green tea extracts, octenidine, propolis 30%, and 2% chlorhexidine with no surface changes in 6, 3, 2, and 1, respectively. 3% NaOCl showed topographic changes in all samples.
- Green tea was most effective as a disinfectant solution followed by octenidine whereas *A. vera* juice showed minimum surface topographic changes in GP cones followed by green tea extracts.
- Green tea exhibited 2 in 1 quality with good disinfection action against *C. Albicans* and *E. faecalis*, followed by octenidine and propolis 30%. Hence, herbal alternatives prove to be advantageous considering the several undesirable characteristics of NaOCl.
- Furthermore, as the herbal alternatives are cheaper and easily available, they can be preferred over the synthetic solutions.

CONCLUSION

It can be concluded that

- In this *in vitro* study, all the tested solutions demonstrated disinfection action against *C. albicans* and *E. faecalis*. There was a significant difference found in the number of CFU between Experimental and Control groups. A statistically significant correlation was observed between the mean colonies among different groups for

both groups contaminated with *C. albicans* and *E. faecalis* (one-way ANOVA; $P=0.000$) (Tables 1 and 2). The results of our study illustrate that the herbal disinfectants are more effective against *C. Albicans* as compared to *E. faecalis*. Green tea exhibited 2 in 1 quality with good disinfection action against *C. Albicans* and *E. faecalis*, followed by octenidine and propolis 30%. Hence, herbal alternatives prove to be advantageous considering the several undesirable characteristics of NaOCl.

REFERENCES

1. Prada I, Micó-Muñoz P, Giner-Lluesma T, Micó-Martínez P, Collado-Castellano N, Manzano-Saiz A. Influence of microbiology on endodontic failure. Literature review. Med Oral Patol Oral Cir Bucal 2019;24:e364-72.
2. Tilakchand M, Naik B, Shetty AS. A comparative evaluation of the effect of 5.25% sodium hypochlorite and 2% chlorhexidine on the surface texture of gutta-percha and resilon cones using atomic force microscope. J Conserv Dent 2014;17:18-21.
3. Kakehashi S, Stanley HR, Fitzgerald RJ. The effects of surgical exposures of dental pulps in germ-free and conventional laboratory rats. Oral Surg Oral Med Oral Pathol 1965;20:340-9.
4. Nair PN, Sjogren U, Krey G, Kahnberg KE, Sundqvist G. Intraradicular bacteria and fungi in root-filled, asymptomatic human teeth with therapy-resistant periapical lesions: A long-term light and electron microscopic follow-up study. J Endod 1990;16:580-8.
5. Guerreiro-Tanomaru JM, Morgental RD, Faria-Junior NB, Berbert FL, Tanomaru-filho M. Antibacterial effectiveness of peracetic acid and conventional endodontic irrigants. Braz Dent J 2011;22:285-7.
6. Lins RX, Marceliano-Alves MF, Lind BB, Pinto SS, Hirata Junior R. Effectiveness of peracetic acid in rapid disinfection of gutta-percha and resilon cones exposed to *Enterococcus faecalis*. Dent Press Endod 2017;7:85-91.
7. Nascimento CA, Tanomaru-Filho M, Faria-Junior NB, Faria G, Guerreiro-Tanomaru JM. Antimicrobial activity of root canal irrigants associated with cetrimide against biofilm and planktonic *Enterococcus faecalis*. J Contemp Dent Pract 2014;15:603-7.
8. Pang NS, Jung IY, Bae KS, Baek SH, Lee WC, Kum KY. Effects of short-term chemical disinfection of gutta-percha cones: Identification of affected microbes and alterations in surface texture and physical properties. J Endod 2007;33:594-8.
9. Shenoi PR, Morey ES, Makade C, Gunwal MK, Wanmali SS. To evaluate the antimicrobial activity of herbal extracts and their efficacy in disinfecting gutta percha cones before obturation-an *in vitro* study. J Med Sci Clin Res 2014;2:2676-84.
10. Short RD, Dorn SO, Kuttler S. The crystallization of sodium hypochlorite on gutta-percha cones after the rapid-sterilization technique: An SEM study. J Endod 2003;29:670-3.
11. Valois CR, Silva LP, Azevedo RB. Structural effects of sodium hypochlorite solutions on gutta-percha cones: Atomic force microscopy study. J Endod 2005;31:749-51.