

**COMPARATIVE EVALUATION OF GARLIC (*Allium sativum*) EXTRACT
IN CHAIR SIDE DISINFECTION OF GUTTA PERCHA CONES: AN IN
VITRO STUDY**

ABSTRACT

Background: Microbial contamination of gutta percha (GP) cones can be harmful for the success of endodontic therapy. A rapid chair side disinfection is preferred to avoid secondary infection.

Aim: The purpose of this study was to compare the efficacy of 20% and 100% garlic extract with 5.25% Sodium hypochlorite (NaOCl) and 2% Glutaraldehyde in rapid disinfection of GP

Materials and Methods: GP were immersed in prepared suspensions of *Enterococcus faecalis* and *Staphylococcus aureus*. The cones were disinfected in 5.25% NaOCl, 2% glutaraldehyde, 20% garlic extract and 100% garlic extract for 1 and 5 minutes. The cones were then transferred to sterile test tubes containing 1ml sterile saline and vortexed vigorously for 5 minutes. 100µl of the saline suspension plated onto Brain heart infusion agar by spread plate method. The plates were

incubated for 24-48 hours at 37°C and the total colony forming units/ml were calculated.

Results: Statistical analysis was done using one-way ANOVA followed post hoc Tukey test. There was no statistically significant difference among 5.25% NaOCl, 100% garlic extract and 2% glutaraldehyde.

Conclusion: Within the limitations of the study, 100% garlic extract possesses superior antibacterial activity when compared with 2% glutaraldehyde and 20% garlic extract and similar effect as 5.25% NaOCl.

Key words: Disinfection, Enterococcus faecalis, Garlic, Staphylococcus aureus, Sodium hypochlorite.

INTRODUCTION:

The fundamental goals of endodontic treatment are the effective entrance and locating the canals, cleaning and shaping of the canals, and eventually obturation of the root canal. All of the phases are crucial to the treatment's prognosis. The final stage of endodontic therapy is obturation, which eliminates the root canal space. This is accomplished by using a root-filling substance (GP) in conjunction with a sealer.⁽¹⁾ Gutta-percha points are the most commonly used material for

obturation of the root canal system.⁽²⁾ They are biocompatible, dimensionally stable, radiopaque, and thermoplastic in nature.⁽²⁾

In addition to materials and procedures, there are a number of other elements to consider for a successful root canal treatment, such as sterilisation of endodontic equipment and contamination prevention during or before the procedure. During packing and storage, GP points should be handled aseptically. Regardless of the measures taken, once they are exposed to the dental operational environment, they may get contaminated. According to studies, 5 to 8% of the points from sealed GP packages are contaminated by bacteria.⁽³⁾

The Staphylococcus genus is reported to be the most prevalent micro-organism infecting GP sites during packing and following handling with gloves, according to Klager P and Gomes et al. The recovery rate of Staphylococcus genus from contaminated root canals is around 15.7 percent, according to Guimaraes et al; this data underlines the need for GP cleaning before obturation.⁽⁴⁾ *Enterococcus faecalis* another organism that is found to be the most resistant intra canal pathogen in failed root canal treatments that serves as a gold standard bacteria in endodontic researches.⁽⁵⁾

Dry heat sterilisation and wet heat sterilisation are the two most common methods of sterilisation. Traditional techniques of heat sterilisation, such as moist or dry

heat sterilisation, cannot be employed due to the thermoplastic nature of GP. To reduce secondary infection and root canal failures, a fast chairside chemical cleaning is recommended for best infection management.⁽⁴⁾

Based on various studies, several chemical agents have been proposed as GP disinfectants, including sodium hypochlorite (NaOCl), glutaraldehyde, alcohol, iodine compounds and hydrogen peroxide.^(1,4) One of the widely used chemical disinfectant for GP is 5.25% sodium hypochlorite.⁽⁴⁾ It produces residual disinfection and has an effective bactericidal activity. 5.25% sodium hypochlorite is a broad-spectrum antimicrobial agent and is recognized as an efficient, inexpensive and reliable chair side disinfectant.^(1,4) Glutaraldehyde is also a commonly used disinfectant to sterilize GP points.⁽¹⁾ Solution of 0.1% to 2% of glutaraldehyde concentration used as a biocide disinfection and as a preservative for long term.⁽⁶⁾

There are also several herbal extracts possessing antibacterial properties that can be used for disinfection. Herbal extracts are effective because they interact with specific chemical receptors.⁽⁷⁾ Anushri claims that the strength of Yashoda and Puranik herbal extracts varies. As a result, the quest for alternative goods continues, and natural phytochemicals extracted from plants used in traditional medicine are being viewed as viable alternatives to synthetic chemicals. Herbal

medications are also increasingly being utilised as sedatives, plaque reduction, and gum health.

Allium sativum (garlic), a medicinal plant with sulphides, produces antibacterial qualities from the allicin ingredient when chopped and crushed. *Allium sativum* kills streptococcus pyogenes and corynebacterium diphtheriae in 2 to 3 minutes, according to Bakri and Meredith. Garlic extract is an excellent root canal irrigant against intra canal pathogens, according to several research.^(8,9) A thorough literature search showed that efficiency of garlic extract for GP points disinfection has not been analysed. Hence the current in vitro study was undertaken to assess the efficiency of garlic extract for GP points disinfection.

Thus, the aim of the study is to evaluate and compare the efficacy of 5.25% NaOCl, 2% glutaraldehyde and 20% & 100% garlic extract in rapid chair side disinfection of GP points.

MATERIALS AND METHODS:

In the present study gutta percha cones (size 80, dentsply) were used to check the efficacy of four solutions used as disinfectants. Total 100 gutta percha cones were taken and divided into two groups as 1 and 2 based on the efficacy of the disinfectants against the organisms *E. faecalis* and *S. aureus* with 50 samples each. The samples were contaminated with *E. faecalis* in group 1 and with *S. aureus* in

group 2. GP cones from groups 1 and 2 will be further subdivided into 6 groups like Group A, B, C, D, E and F. Groups A to D were treated with the disinfecting solution for 1min and 5min separately with 5 samples each. Grouping for both the organisms given as,

Group 1: *E. faecalis*(n=50)

Group 1A: 5.25% NaOCl for 1minute (n=5) and 5minutes (n=5)

Group 1B: 2% glutaraldehyde for 1minute (n=5) and 5minutes (n=5)

Group 1C: 20 % garlic extract for 1minute (n=5) and 5minutes (n=5)

Group 1D: 100 % garlic extract for 1minute (n=5) and 5minutes (n=5)

Group 1E: Positive control, Gentamycin (n=5)

Group 1F: Negative control, no treatment(n=5)

Group 2: *S. aureus* (n=50)

Group 2A: 5.25% NaOCl for 1minute (n=5) and 5minutes (n=5)

Group 2B: 2% glutaraldehyde for 1minute (n=5) and 5minutes (n=5)

Group 2C: 20 % garlic extract for 1minute (n=5) and 5minutes (n=5)

Group 2D: 100 % garlic extract for 1minute (n=5) and 5minutes (n=5)

Group 2E: Positive control, Gentamycin (n=5)

Group 2F: Negative control, no treatment(n=5)

Preparation of inoculum suspension

The test bacterial strains were grown on Brain Heart Infusion Agar and then sub cultured in BHIB broth medium. The cell cultures were harvested, washed twice with Phosphate buffered Saline (PBS) and adjusted to $\sim 10^8$ CFU/mL (0.5 McFarland standard) optically read at 490nm in UV spectrophotometer adjusted to 0.2 OD.

Artificial contamination of gutta-percha cones

Microbial suspension of *E. faecalis* (ATCC 29212) and *S. aureus* (ATCC 25923) of 10^8 CFU/ml in BHIB) was used for this study. Gutta-percha cones from the study Groups were immersed in respective microbial suspensions in sterile saline incubated at 37°C for 30 minutes at 80rpm in a shaker incubator. The cones were then transferred to sterile paper pads and allowed to air dry for 10 minutes. Following artificial contamination, all the GP cones from Group A to E were then subjected to the disinfection protocol with the disinfection solution for 1min and 5min as mentioned in the table 1 and 2. Group F which is the negative control was contaminated with the organisms *E. faecalis* and *S. aureus* but not subjected to any of the disinfection solution.

Preparation of garlic extract

Garlic extract was used as disinfectant on GP cones against *Staphylococcus aureus* and *Enterococcus faecalis*. The extract was prepared in two concentrations as 20% and 100% in saline. 100g of chopped garlic were rinsed in sterile water (Autoclaved) and allowed to boil in 100mL sterile saline for 30min. 20% of the extract was prepared by taking 20mL of the extract and adding it to 80mL of sterile saline.

Disinfection of gutta-percha cones

After fake contamination, gutta-percha cones from Groups 1A to 1F and Groups 2A to 2F were individually placed on aluminium foil and aseptically submerged in the corresponding disinfectant solutions contained in a petri dish for 1 minute and 5 minutes. The cones were then vortexed rapidly for 5 minutes in sterile test tubes each containing 1ml sterile saline. By using the spread plate approach, 100L of the saline solution would be plated onto brain heart infusion agar. After that, the plates were incubated aerobically at 37°C for 24-48 hours, and the total colony forming units/mL were estimated using the formula

$$\text{Total colony forming unit (CFU/mL)} = \frac{\text{total colony counted}}{\text{Dilution} \times \text{volume}}$$

The data obtained from the study were tabulated using Microsoft Excel software and analysed using one-way ANOVA followed post hoc Tukey test using SPSS software system.

RESULTS:

Comparison among the test groups and time comparison were depicted in the table 1 and 2. On observation the mean total colony forming unit was found to be lesser for 5.25% sodium hypochlorite than other tested groups. According to the results obtained for disinfection of GP cones there was no statistical significance difference between 5.25% NaOCl, 2% Glutaraldehyde and 100% garlic extract at both the time periods for both the groups 1 and 2 ($p > 0.001$). And against both the organisms 100% garlic extract was found to be more effective at 5mins than at 1min and the difference was statistically significant ($p < 0.001$)

DISCUSSION AND CONCLUSION

The persistence of microorganisms in the root canal is considered as a main cause of failure in endodontic treatment.⁽¹⁾ The dentists occasionally come across the case of recurrent infections ⁽⁵⁾Contamination of equipment and GP cones during treatment is one of the main causes of this recurrence. As a result, the gutta percha cones utilised in the procedure must be aseptic and sterile. GP cones are created in a sterile environment, but they become contaminated throughout the

obturation stage owing to inappropriate physical handling.⁽¹⁰⁾ In order to overcome this, GP cones disinfection is considered to be vital for optimum infection control during the treatment.⁽¹⁰⁾

The goal of this study is to see how effective various irrigating solutions are after experimentally infecting GP cones with two distinct bacteria, *S.aureus* and *E.faecalis*. Because they are commonly isolated from infected root canals, these two bacterial strains were chosen.⁽¹¹⁾ They are classic representatives of facultative aerobic gram positive and gram-negative bacteria.⁽¹¹⁾

Enterococcus faecalis is the most prevalent bacterium linked with post-treatment infection of the root canal system, according to Nabeshima and Machado. This strain may also be demonstrated to persist for lengthy periods of time in dentinal tubules. *E.faecalis* having a higher potential for long-term survival without food, as well as a better adaption to the endodontic system.⁽⁵⁾ *Staphylococcus aureus* is a facultative anaerobic coccus and is found in the saliva and skin. *S.aureus* was selected as one of the test organisms, as it is the most common microorganism contaminating GP cones in their boxes and after handling with gloves.⁽⁴⁾

5.25 percent sodium hypochlorite, 2 percent glutaraldehyde, 20 percent garlic extract, and 100 percent garlic extract were utilised in this investigation to disinfect

the infected GP cones. Because of its antibacterial properties, sodium hypochlorite is a frequently used irrigating solution that may also be used as a disinfection solution.^(1,4) In terms of its antimicrobial activity, sodium hypochlorite is a broad-spectrum antibacterial agent that is effective against both Gram-positive and Gram-negative bacteria, yeast, fungi and viruses.^(1,10) It is mostly used in concentrations that vary from 0.5% to 5.25%.⁽¹⁰⁾ Based on that, the antimicrobial activity of NaOCl was related to its concentration, i.e., higher concentrations have better antimicrobial action.⁽¹²⁾

All glutaraldehyde solutions tested are used in endodontic practice for rapid decontamination of gutta-percha cones.⁽¹⁾ Thus, contributing to the maintenance of the aseptic chain, an essential factor for successful root canal treatment.^(1,6) A 2 percent glutaraldehyde aqueous solution exhibits a broad spectrum of action as well as a high rate of destruction against the majority of bacteria. In this investigation, 2 percent glutaraldehyde was utilized for disinfection, and its efficacy was shown to be less than that of 100% garlic extract.

Garlic (*Allium sativum*) offers a wide range of medicinal properties with low toxicity. Garlic was shown to be as efficient as 5.25 percent sodium hypochlorite against *E. faecalis* and *S. aureus* in the current investigation. The reason for this is because garlic includes an active component called allicin, which is formed by the action of the enzyme allinase on allin.⁽¹³⁾ and allicin reacts with

thiol groups of enzymes in susceptible bacteria to form S-allylmercaptocysteine, thus causing their inhibition. This disrupts the metabolic activity of the bacteria and causes cell lysis.⁽¹⁴⁾ In the present study 100% garlic extract was found to be more effective than 20 % garlic extract. This might be due to the dilution of the solution.

There have previously been no research that looked into the efficiency of garlic extract as a disinfectant for GP cones. According to the results, when the GP cones were treated with sodium hypochlorite and then 100 percent garlic extract and 2 percent glutaraldehyde, the mean bacterial count for both E.faecalis and S.aureus was found to be lower. When compared to 2 percent glutaraldehyde and 20% garlic extract, 100 percent garlic extract had greater antibacterial action. When comparing the disinfection of GP cones with 100 percent garlic extract, disinfection for 5 minutes is shown to be more successful than disinfection for 1 minute. It suggests that 100% garlic extract can be used for better disinfection of gutta percha cones during the root canal treatment.

According to the findings of the study, the efficiency of 100 percent garlic extract at both time periods and against both species is comparable to that of 5.25 percent NaOCl. Furthermore, 100 percent garlic extract outperforms 2 percent glutaraldehyde in terms of antibacterial activity. However, more research is needed to see how garlic extract affects the surface topography of GP cones.

Within the restrictions of the current investigation, it can be concluded that immersing GP cones in a solution of 100% garlic extract for 5 minutes is an effective disinfection approach. As a result of the findings, immersion in 100 percent garlic extract can be recommended as a suitable technique of gutta percha disinfection. More research is needed to see if garlic extract affects the physical characteristics of gutta percha.

NOTE:

The study highlights the efficacy of " traditional medicine " which is an ancient tradition, used in some parts of India. This ancient concept should be carefully evaluated in the light of modern medical science and can be utilized partially if found suitable.

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GROUPS	<i>E.Faecalis</i> (mean total colony forming unit * 10 ² CFU/ml)		<i>S.aureus</i> (mean total colony forming unit * 10 ² CFU/ml)	
	1min	5min	1min	5min
5.25%NaOCl	28 ± 8.60	11± 5.70	25± 6.28	8± 2.00
Glutaraldehyde	49± 23.18	34± 11.42	81± 26.33	26± 8.86
20% garlic extract	284± 9.03	188± 9.67	139± 18.43	106±16.60
100% garlic	43± 12.25	21± 4.18	54± 13.38	10± 2.92

extract				
p value	0.000*	0.000*	0.000*	0.000*

TABLE 1 :Mean Total Colony Forming units for Group 1 and 2 for both the time periods

GROUPS	<i>E.faecalis</i>		<i>S.aureus</i>	
	1min	5min	1min	5min
NaOCl × Glutaraldehyde	0.143	0.002*	0.001*	0.040*
NaOCl × 20% garlic extract	0.000**	0.000**	0.000**	0.000**
NaOCl × 100% garlic extract	0.389	0.263	0.083	0.987

Glutaraldehyde × 20% garlic extract	0.000**	0.000**	0.000**	0.000**
Glutaraldehyde × 100% garlic extract	0.913	0.101	0.114	0.75
20% garlic extract × 100% garlic extract	0.000**	0.000**	0.000**	0.000**

* Denotes significance at 5% level

** Denotes significance at 1% level

TABLE 2 : Post hoc tukey test results for comparison among the groups