



Endodontics

EFFECTIVENESS OF DIFFERENT HERBAL EXTRACT DISINFECTANT SOLUTIONS IN ELIMINATING MICRO ORGANISMS AND ITS EFFECT ON SURFACE TOPOGRAPHY OF GUTTA-PERCHA CONES: A SEM-EDX STUDY

Dr. S. Anitha Rao*

Professor & HOD, Department of Conservative Dentistry and Endodontics, Mamata Dental College, Khammam-507002. *Corresponding Author

Dr. Chandrasekhar Veeramachaneni

Professor, Department of Conservative Dentistry and Endodontics, Mamata Dental College, Khammam-507002

Dr. Shaik Parveen Fathima

Postgraduate Student, Department of Conservative Dentistry and Endodontics, Mamata Dental College, Khammam-507002.

ABSTRACT

Introduction: Gutta-percha is one of the widely used and accepted root canal filling material. Although gutta-percha cones are manufactured under aseptic conditions when taken from sealed package are exposed to dental operating environment and become contaminated by variety of micro-organisms. So, rapid chair-side asepsis of gutta-percha cones is of prime importance to have an aseptic chain during root canal procedures. **Aim:** This research aimed to evaluate the anti-microbial activity and surface topographical changes of Gutta-Percha (GP) cones after disinfection with chemical & natural disinfectant s prior to obturation. **Methodology:** 120 gutta-percha cones will be taken out of which 80 will be assessed for microbiological assesment and 40 will be used for SEM-EDS analysis. Microbiological assessment: 80 gutta-percha cones will be divided into two groups. Group-A (n=40) contaminated by *Staphylococcus aureus*. Group-B (n=40) by *Enterococcus faecalis* and will be sub-divided into 4 subgroups based on disinfectant used. Sub-group 1: *Achyranthes aspera* (*Uttareni*) for 1min. Sub-group 2: *Acalypha indica* (*Kuppinta*) for 1min. Sub-group 3: 3% NaOCl for 1 min. Sub-group 4: (control) no disinfection. All the disinfected samples will be then incubated for 24 hours to detect bacterial growth. SEM-EDX Analysis: 40 Gutta-percha cones were subjected to SEM/EDS to assess the topography and chemical elements present on their surface. **Results:** Data obtained for surface changes was analysed using Chi-square test; indicates significant difference at $p \leq 0.05$ Kruskal Wallis test; Post hoc Bonferroni test; indicates a significant difference at $p \leq 0.05$ for anti-microbial activity. **Conclusion:** According to the result of our study, that *Acalypha indica* and *Achyranthes aspera* both showed better antibacterial activity against *E. faecalis* and *S. aureus* and causes less surface topographical alteration of GP cones when compared to 3% Naocl.

KEYWORDS :**INTRODUCTION:**

Endodontic treatment aims at complete elimination of all the necrotic material and microbial load from the infected root canal followed by replacing it with a root canal obturating materials such as gutta-percha (GP) and resin sealer.¹ For optimum infection control, every instrument and material placed within root canals should be sterile.²

Gutta-percha cones are, at present, the most commonly used material for the obturation of the root canal system. They are biocompatible, dimensionally stable, radiopaque, and thermoplastic. Moreover, they are easily removed from the root canal when necessary.³ Even though gutta-percha cones (GPCs) are manufactured under aseptic conditions, they can get contaminated during the storage process, while handling, by aerosols, and physical sources etc. Therefore, before inserting GPCs into the root canal, disinfection is required. Because of their thermoplastic properties, the conventional heating method cannot be used to sterilize them.⁴ As a result, before using GP points as a filling material, a fast chairside disinfection protocol (CDP) of GP points is required.⁵ Hence, chemical disinfection can be the choice for disinfection of gutta-percha cones.

There are various chemical agents that have been employed for chemical disinfection of gutta-percha cones, one among them is sodium hypochlorite which is widely used⁶. The disinfecting efficiency of NaOCl depends on the concentration of undissociated hypochlorous acid (HClO) in solution. HClO exerts its germicidal effect by an oxidative action on sulphhydryl groups of bacterial enzymes. Therefore, The immersion of GP cones in a solution of 3% NaOCl for 1 min is an efficient method to promote their disinfection.²⁴

One of the potential drawback is the changes in the physical properties of gutta-percha cones after disinfection with chemical agents.⁷ Herbal solutions which are commonly available in Indian market have shown dramatic results as antimicrobials, anticancer, antidiabetic, immune modulatory, in respiratory diseases, in liver disorders and as cosmetics agents. The popularity of herbal medications has increased due to the search for cheaper, more accessible and natural form of alternatives. However, data regarding their use as disinfection agents in endodontic practice is still lacking.⁸

Staphylococcus genus is determined to be the most prevalent bacteria

infecting gutta percha cones in their packaging and after handling with gloves, according to numerous investigations. Another organism that has been proven to be the most resistant intracanal pathogen in unsuccessful root canals and used as a gold standard bacterium in endodontic research is *Enterococcus faecalis*.⁵ Chethana has shown that herbal extracts of lemongrass produced the highest inhibition zones for EF, SA and *Candida albicans*., followed by obicure tea extract, NaOCl, and basil oil.²⁶

Achyranthes aspera L. (Amaranthaceae) is one of the plant used for medicinal purposes.¹⁰ Methanolic extracts of leaves of *Achyranthes aspera* have shown different activities against 22 microorganism (bacterial and fungal) .¹¹ The antibacterial activity against *S. aureus* decreased in the order of root > leaf = inflorescences > stem.²⁵

Acalypha indica plant is used to treat dysentery, diarrhea, malnutrition, and malaria. These beneficial activities of the plant are allied to various chemical constituents and essential oil present in it. This plant extract reported a wide antibacterial spectrum against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus cereus*, *Streptococcus faecalis*, and *Pseudomonas aeruginosa*.¹²

Till date there are no studies conducted to check the disinfection property of these plant extracts. As a result, plant extract such as *Achyranthes aspera* and *Acalypha indica* were used in the present study. The aim of the present study was to examine the efficacy of *Achyranthes aspera* and *Acalypha indica* in disinfection of gutta percha cones and also to analyse their effect on surface characteristics of GP cones.

MATERIALS AND METHODOLOGY:

This in vitro study was approved by institutional review board of Mamata Dental College, Khammam, Telangana, India (EC/IRB NO. MDC – R – 088462).

PREPARATION OF PLANT EXTRACT:

Commercially available organic leaf powder of *Achyranthes aspera* and *Acalypha indica* were taken and weighed 50 gms. Ethanol extract were prepared by dissolving the powder in increasing polarity of solvents ether, chloroform and methanol. Incubated for 24 hours and filtered using whatman no.1 filter paper.

MICROBIOLOGICALASSESSMENT:

From a sealed packet, A total of 80 GP cones (ISO size 20, 6% taper ; Dentsply Maillefer, Ballaigues, Switzerland) were selected and distributed into two groups containing 40 GP cones in each group. Group A, (40 cones) was contaminated by immersing in 20ml of microbial solution of *S.aureus* ATCC 2592 (fig.2a) for 30 minutes at 37°C. Likewise, Group B (40 cones) was contaminated with *E.faecalis* 29212.

All the test tube (with contaminated GP cones) were incubated at 37°C for 1 hour and recorded for the formation of turbidity. After artificial contamination, These 80 GP cones from Group A and Group B were divided into four subgroups based on disinfection solutions with 10 cones in each subgroups:

Sub-group 1 : Gutta-percha cones were disinfected with *Achyranthes aspera* (*Uttareni*) for 1min.

Sub-group 2: Gutta-percha cones were disinfected with *Acalypha indica* (*Kuppinta*) for 1min.

Sub-group 3: Gutta-percha cones were disinfected with 3% NaOCl for 1min

Sub-group 4 (Control): Gutta-percha cones were kept without disinfection.

After respective disinfection, the cones were transferred into fresh brain heart infusion broth and incubated for 24 hours and recorded for the formation of turbidity and checked by visual method and plated out on Muller Hinton Agar.

Plates were incubated at 37°C for 24 hours. After 24 hours of incubation, the number of colonies formed in each plate was recorded.

Number of *Staphylococcus aureus* and *Enterococcus faecalis* bacteria grown on an agar plate was subjected to quantitative analysis using Colony counter (Figure 1).

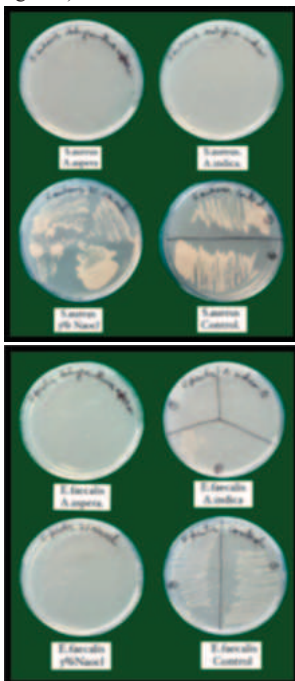


Figure 1: Bacterial growth i.e *S.aureus* and *E.faecalis* colonies with 4 different disinfectant solutions

Evaluation Of Surface Topographic Changes: Sem-edx Analysis:

40 GP points (Protaper Universal gutta percha points; Dentsply Sirona of F3-size) which were not contaminated but disinfected for a contact time of 1 min each using all the 3 disinfectant solutions observed for surface topographic changes under scanning electron microscope (SEM) at 300X magnification and energy-dispersive spectroscopy (EDS) to assess, chemical elements present on their surface.

In group 1 (*Achyranthes aspera*): 10 GP points were selected and were

immersed and soaked in *Achyranthes aspera* solution (3V Products, Chennai, India) for 1 minutes and the cones were placed under scanning electron microscope (SEM) and pictures were taken.

In group 2 (*Acalypha indica*): 10 GP points were selected and were immersed and soaked in *Acalypha indica* solution (Diets Organic, Chennai, India) for 1 minutes and the cones were placed under scanning electron microscope (SEM) and pictures were taken.

In group (3% Sodium hypochlorite): 10 GP points were selected and were immersed and soaked in 3% Sodium hypochlorite (Prime dental Products Pvt Ltd, INDIA) for 1 minutes and the cones were placed under scanning electron microscope (SEM) and pictures were taken.

In group 4 (control) 10 GP points were directly taken from the sealed pack with the help of locking tweezers and pictures were captured under scanning electron microscope (SEM).

RESULTS:

Gutta-percha cones were selected from each group and the percentage was calculated by number of cones that showed changes divided by total number of cones.¹⁶ The results were tabulated as shown in (table 1), (figure 4a-d).

Formula for Calculating the Percentage of GP Cones Undergone Changes % of GP undergoing changes = GP cones undergone changes $\times$ 1020066002667000 / Total no of GP cone

Comparison Of Changes Seen On Surface Of Gutta-percha Cones Table 1: Distribution Of Groups Based On Changes Seen On Gutta-percha Cones

Group		No change	Mild	Moderate	Severe	p-value
Achyranthes aspera	n	0	3	4	3	0.002*
	%	0.00%	30.00%	40.00%	30.00%	
Acalypha Indica	n	0	4	4	2	
	%	0.00%	40.00%	40.00%	20.00%	
3% NaOCl	n	0	2	4	4	
	%	0.00%	20.00%	40.00%	40.00%	
Control	n	6	4	0	0	
	%	60.00%	40.00%	0.00%	0.00%	

Table 1 compares the changes seen on the surface of gutta-percha among four groups. There was a significant difference in the changes seen on the surface of gutta-percha among the four groups. The control group showed a significantly lower change on the surface of gutta-perch cones as compared to the other three groups.

Comparison Of S. Aureus CFU Among Four Groups**Table 2: Descriptive Statistics For S.aureus By 4 Comparison Group**

Group	Mean $\pm$ SD	Mean Rank	p-value
Achyranthes aspera	5000 $\pm$ 10540.93	13.90	<0.001*
Acalypha indica	9500 $\pm$ 10658.85	17.50	
3% NaOCl	6500 $\pm$ 10814.09	15.10	
Control	100000 $\pm$ 0.00	35.50	

Table 2 compares the *S. aureus* CFU among four groups. The highest *S. aureus* CFUs were seen in the control group and lowest in the *Achyranthes aspera* group. There was a significant difference in the *S. aureus* CFU among the four groups. The control group showed a significantly greater no of *S. aureus* CFUs as compared to the other three groups.

DISCUSSION:

Maintaining the aseptic chain during cleaning, shaping, and obturation is the most important step during the endodontic treatment.¹⁵

Gutta-percha is the most frequent solid material used for filling of root canals and, due to its constitution it doesn't resist the conventional methods of sterilization by moist or dry heat.⁶

Thus, chemical disinfection of guttapercha cones becomes an important step as the physical sterilization process cannot be undergone.

As root canal filling cones must remain in the root canal over long period of time, they must be able to withstand rigorous sterilization procedures.¹⁸

Moorer et al. showed that gutta-percha cones exhibit antimicrobial activity due to their zinc oxide content, however, this activity may be too weak to be effective clinically.³

Therefore, a rapid chair-side disinfection technique is needed to disinfect the GP cones once they are removed from the sealed packaging and before placing them into the root canals. Chemical disinfectants are used for 1-5 min to disinfect GP. NaOCl is considered to be the most rapidly effective agent. Various herbal extracts were put to trial.⁷

Ingle stated that dropping a cone into a good germicidal solution for a fair period of time will evidently decontaminate the cone. The use of sodium hypochlorite as a disinfecting agent has been recommended by several studies. However, at higher concentration (5.25%) they produce chloride crystals on the surface of gutta percha cones that could impair the obturation and hermetic seal.⁹

The antibacterial activity of NaOCl is mainly related to the liberation of active chlorine and its action on bacterial essential enzymes, promoting lipid peroxidation of the bacterial cytoplasmic membrane.¹⁹

In a study by Valois et al., it was concluded that 5.25% NaOCl had resulted in surface deterioration of GP cones because of loss of GP cone components by the oxidizing agent (NaOCl). Deep irregularities which are formed through deterioration of GP cones can create interfacial gaps between the GP cones and the root canal wall, increasing the risk of leakage.¹⁵

These results also corroborate with the findings of Grecca et al. (2011), which observed the presence of marked irregularities on the cones surface, compatible with residual crystals of sodium chloride, after promoting the treatment of gutta percha cones with 2.5% NaOCl solution for 10 min.¹⁷

When the disinfected specimens were compared with the non-disinfected specimens, it was possible to observe the presence of large surface irregularities and residual crystals, possibly resulting from the action of the disinfectant compounds over the gutta-percha cones (Prado et al., 2011).²⁰

In order to avoid the disadvantage of Sodium hypochlorite, plant based medicine can be considered as alternative for various medical health practices throughout the globe because of their safety, availability and cost effectiveness. Plant derived extract and extracts are used for various disease and helps in maintaining good health too.¹⁵ So, in this study we have included two different herbal extracts i.e. *Achyranthes aspera* and *Acalypha indica*.

Besides chemical disinfectants, herbal disinfectants are safe and effective. Scientific literature suggests that herbs possess antimicrobial, antiseptic, antiviral, antifungal, and immunomodulatory properties. As a result, they can be utilised safely in the food and pharmaceutical industries with less or no adverse effects. Herbal agents are environment friendly; however they have rarely been tested as preoperative disinfectant for GP cones.

Athiban PP et al., proved the antimicrobial efficacy of Alovera gel against three microbes by forming effective inhibition zones which was almost equal to 5.25% NaOCl.²¹

Shenoi PR et al., evaluated the efficacy of three herbal gels in disinfecting GP cones. They concluded that all the gels showed inhibition zones nearly equal to 5.25% NaOCl.¹⁴

Thus, herbal products are effective substitutes the chemical disinfectants. So, in this study we have include two different Indian plant extract such as *Achyranthes aspera* and *Acalypha indica* to disinfect GP in a stipulated time period of one minute.

Several investigators have reported that the methanolic extracts of leaves. *Achyranthes aspera* has significant antimicrobial activity against the Gram-Positive (*S. aureus* *Bacillus subtilis*), Gram-negative bacterial (*K. pneumoniae*, *E. coli*) and fungal species (*Aspergillus niger*, *Calbicans*).¹⁵

Methanolic extracts of leaves of *Achyranthes aspera* have shown

different activities against 22 micro-organisms (bacterial and fungi).¹¹

Contamination of gutta percha cones can occur if they are handled incorrectly during retreatment or if the master cone is not checked for fit before the final rinse. Microorganisms have been found in 5%-19% of freshly opened gutta percha sachets, according to studies.⁵

S. 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 gutta-percha cones in their boxes and after handling with gloves.²

E. faecalis are facultative gram positive cocci shaped bacteria that were chosen as the test bacterial contaminant because it has been shown to be a predominant bacterial species in persistent apical periodontitis and it is the most common bacteria associated with post-treatment infection of root canal system.⁴

Gomes et al. were able to confirm this. *E. faecalis* is a gold standard to represent other potential microorganisms because it is the most resistant intracanal pathogen in failed root canals. As a result, *E. faecalis* was chosen for this study.⁹

Sterilization procedures with contact between gutta-percha and the chemical agents can cause extreme surface alterations of the cones. GPCS present irregular regions on their surfaces and root canal sealers are known to fill these irregularities. However, these gap areas could create a large interface with root canal walls, resulting in the leakage of molecules that will serve as nutrients for the microorganisms present the root canal system.¹⁴

LIMITATIONS:

Root canal infections are polymicrobial in nature. However, this is only an antibacterial study.

The other limitations of this study include shorter incubation time. A longer incubation time may be needed for some species of bacteria, and this cannot be overlooked. Hence, more sensitive tests such as PCR testing are to be carried out before confirming sterility of GP cones.

CONCLUSION:

Herbal extracts are eco-friendly and cost-effective, and may serve as a promising tool for rapid chair-side disinfection of GP cones before obturation.

Further research in this area should be conducted to investigate these extracts for using it as GP disinfectants for clinical relevance.

Within the limitations of the present study it can be concluded that *Acalypha indica* and *Achyranthes aspera* both showed better antibacterial activity against *E. faecalis* and *S. aureus* and causes less surface topographical alteration of GP cones when compared to 3% NaOCl.

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