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EVALUATION OF CHANGES IN MECHANICAL PROPERTIES OF GUTTA PERCHA DIPPED IN THREE HERBAL SOLUTIONS AND ANTIMICROBIAL EFFICACY OF THESE SOLUTIONS AGAINST *E. FAECALIS*: AN INVITRO STUDY

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Abstract

Objectives: The following study comparatively evaluates changes in mechanical properties of Gutta Percha cones in herbal solutions using aloe vera gel, lemon grass oil and pancha tulsi, keeping sodium hypochlorite as the control group and antimicrobial efficacy of these solutions against the most predominant microorganism of root canal, *E. Faecalis*.

Materials and Methods: 200 F3 Gutta Percha cones were used for the study. GP cones were treated with the herbal solutions and NaOCl, group 1- lemon grass oil, group 2- pancha tulsi, group 3- aloe vera gel and group 4- sodium hypochlorite, which was then subjected to test for tensile strength and percentage elongation under Universal Testing Machine. Antimicrobial efficacy was checked with the help of formation of zones of inhibition on the agar culture plates incubated with *E. faecalis* by dispensing herbal solutions and NaOCl. Decontamination of GP was done by noting the absence or presence of turbidity.

Results: The Mechanical strength of GP did not alter much in group 1 as compared to that of NaOCl. Antimicrobial efficacy was seen in lemon grass oil with highest zones of inhibition.

Conclusion: All extracts proved to be potential rapid chairside disinfectants of GP cones and can be used as root canal irrigants, with LG showing the highest antimicrobial activity and good in tensile strength and percentage elongation.

Keywords: Disinfection; Gutta percha; Lemon grass; Aloe vera; tensile strength; sodium hypochlorite; pancha tulsi; decontamination

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Introduction

To achieve a bacterial free environment from the first entering into the root canal to the final filling in the coronal region of the tooth is the major goal of endodontic therapy. For endodontic procedures to be successful, minimizing or eliminating microorganisms is crucial. GP can become contaminated throughout the storage process by handling, aerosols, and physical causes. Because this could result in an irreversible change to the structure, the usual technique that uses moist or dry heat to sterilize GP is ineffective. Since it is impossible to determine in advance how many GP points will be required, immediate chairside chemical disinfection is required. Many solutions other than sodium hypochlorite have been explored as disinfection for GP cones. Such solutions can destroy germs in a matter of seconds or over extended periods of time. For a quick high level of disinfection of GP cones, NaOCl at a concentration of 5.25% is an efficient agent. All vegetative forms are quickly destroyed by 2% CHX. Some microorganisms in biofilms on GP cones can be eliminated by 2% peracetic acid solution after 1 minute of exposure¹.

The procedure for disinfecting GP uses 1% Milton's solution or 0.5% Dakin's solution of sodium hypochlorite for one minute each³. Sodium hypochlorite comes with certain drawbacks like disagreeable taste, toxicity, and inefficient to dissolve the smear layer on its own because it only dissolves organic material.

In endodontics, herbs are generally used to disinfect root canals. There has been an increase in study into herbal irrigants because the majority of synthetic intracanal medicines have side effects. The most frequent reason why root canal treatments fail is due to the bacteria *E. faecalis*⁴. In vitro, colony-forming units in *E. faecalis* biofilms were decreased by essential oil of *L. sidoides* at concentrations of 2.5 and 10% after exposure for 30 and 60 minutes. *S. mutans*, *E. faecalis*, and

S. aureus were three bacteria against which the antibacterial activity of methanolic extracts of Neem, Tulsi, Bakul, and Giloy were tested.

In contrast to untreated cones, Valois et al. (2005)⁵ found that 5.25% NaOCl treatment for 1 minute enhanced the flexibility of gutta-percha cones. Some studies showed surface changes when GP cones were immersed in 5.25% NaOCl for 5 minutes. NaOCl is a potent oxidizing agent and efficient decontaminant that drastically changes the topography of the cones and accelerates their deterioration. As a result, the adherence of GP cones to endodontic sealer is reduced. With 5.25% NaOCl, the firmness of GP cones is reduced, and cuboidal crystals were formed on the surface of the material. These changes may have an impact on the root canal's ability to seal, as well as the effectiveness of endodontic treatment⁶.

So, the need of this study was to assess the antimicrobial efficacy and mechanical properties of GP in various herbal solutions keeping sodium hypochlorite as control group. As we know that synthetic products like hypochlorite pose serious effects on the gutta percha cone and their sealing ability to the root canal walls so herbal irrigants can become the coveted choice for irrigation in the root canal system.

Materials and Methods

200 F3 Gutta Percha cones were taken. All the herbal solutions were commercially taken and used in this study. For ANTIMICROBIAL EFFICACY, *E. faecalis* was incubated overnight in nutrient broth and lawn cultured on petri-dish. Each petridish was punched with sterile template on its surface to make 4 wells 5×5mm.(fig.1) Each well was marked and 50 microlit herbal solutions were dispensed. Strains tested using agar diffusion technique (Kirby Bauer method), incubated at 37°C for 24 hrs. and observed for clear zones. (fig. 2). 100 samples were divided into 4 subgroups with 25 GP each.

The GP cones were then contaminated with *E. faecalis* for 1 min and was cut into two halves (fig. 3). First half is placed in test tubes containing BHI and other half remaining in herbal solutions for 1 min. The remaining half of contaminated cones were then treated with herbal solutions:-

- Group A: 25 cones immersed in LG oil for 1min.

- Group B: 25 cones immersed in pancha tulsi for 1min.
- Group C: 25 cones immersed in aloe vera gel for 1min.
- Group D: 25 cones in sodium hypochlorite (5.25%) for 1 min.



Fig. 1 dispensing 4 test solutions in the wells prepared in the agar plate



Fig 2. Measurement of zones of inhibition on agar plates



Fig. 3 contamination of GP cones in *E. faecalis*

Treated cones were placed on absorbent paper to remove excess solution before immersing in BHI. All test tubes were incubated for 24 hrs. and recorded for turbidity. Growth noted at 24 hours is considered positive; observe for 72 hours (fig. 4).

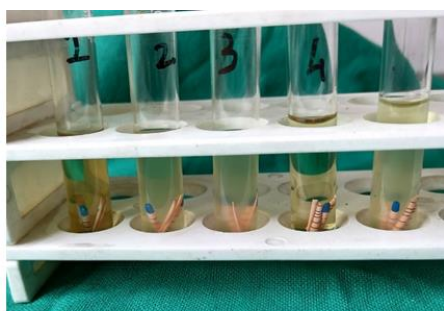


Fig. 4 turbidity test after 24 hr of incubation (positive/ negative)

For mechanical properties, samples will be divided into 4 subgroups with 25 GP each.

- Group A: 25 cones immersed in LG oil for 1min.
- Group B: 25 cones immersed in pancha tulsi for 1min.
- Group C: 25 cones immersed in aloe vera gel for 1min.
- Group D: 25 cones in sodium hypochlorite (5.25%) for 1 min.

The cones were transferred individually and rinsed in 5ml of distilled water for 1 min and allowed to dry in sterile petridishes containing sterile filter pads. The tensile strength of GP after

disinfection was measured using computer controlled universal testing machine. (fig. 5) Each cone was standardized to a length of 14mm and 2 mm from both sides will be inserted in the holders and load is applied at a speed of 1mm/min. Percentage elongation was be measured using same method.



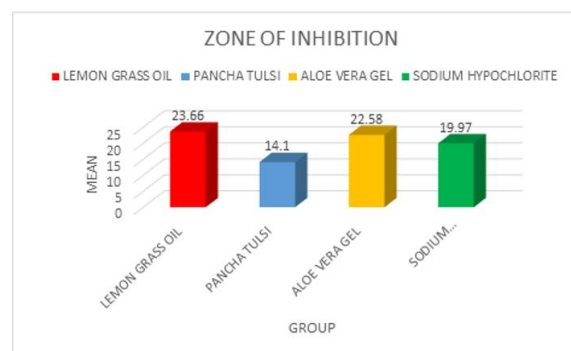
Fig 5. Checking for tensile strength in UTM

The data was compared for differences using one-way analysis of variance, followed by multiple comparisons using post-hoc bonferroni test and post hoc turkeys test. Test for turbidity was done using Chi-square test. The level of statistical significance was set at 0.05.

Results

Results showed that Lemon Grass oil group A, showed largest zones of inhibition (mean \pm SD) (23.66 \pm 1.02) (graph 1). Results revealed that efficacy of

herbal extracts differed significantly across the 4 compared groups using one way anova test ($p \leq 0.05$). For pairwise comparison, it is found to be statistically significant using post hoc bonferroni test. The highest turbidity was found in pancha tulsi 28% after 24 hours followed by aloe vera gel 24%, sodium hypochlorite 12% and the lowest turbidity after 24 hour was found in lemon grass 0.00%, using chi square test ($p \leq 0.05$) (table 1) After 72 hrs, highest turbidity was found in pancha tulsi 36% followed by aloe vera gel 32%, sodium hypochlorite 12% which was similar to lemon grass 12%. It was found to be statistically non-significant ($p \geq 0.05$) using chi-square test.

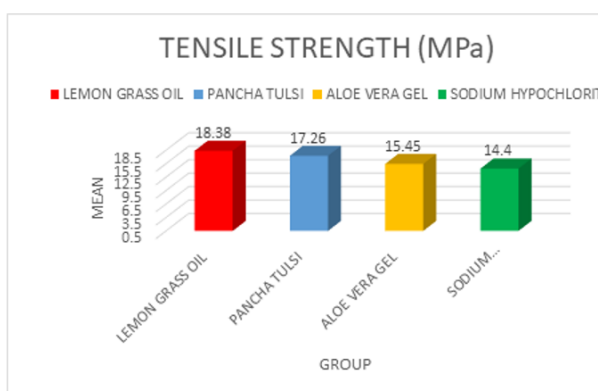


Graph 1: Zone of Inhibition after 24 hrs.

Table 1: Presence and Absence of Turbidity Among Groups after 72 hrs

Group	Turbidity			p-Value	Result
	NEGATIVE N (%)	POSITIVE N (%)	Total		
LEMON GRASS OIL	22(88)	3(12)	25(100.0)	0.074	Non-significant
PANCHA TULSI	16(64)	9(36)	25(100.0)		
ALOE VERA GEL	17(68)	8(32)	25(100.0)		
SODIUM HYPOCHLORITE	22(88)	3(12)	25(100.0)		

As for the tensile strength of GP after 1 min of disinfection in herbal solutions, showed that Lemon Grass oil had minimum of no change in tensile strength and had an increase in percentage elongation, followed by pancha tulsi, aloe vera gel which had a significant difference from the control group (D) with 5.25% NaOCl. (graph 2, table 2& 3) 5.25% of NaOCl decreases tensile strength and left a cuboidal crystal on the surface of GP cone after 1 min of immersion. The results are considered as statistically significant at $P \leq 0.05$.



Graph 2- Difference between Tensile Strength when Treated with the Solutions

Table 2: Multiple comparison Using One Way Anova

MULTIPLE COMPARISON FOR TENSILE STRENGTH					
GROUP	N	Mean± Std. Deviation	p-Value	Result	Post hoc pairwise comparison
LEMON GRASS OIL	25	18.38±0.27	0.000	Highly significant	a>b>c>d
PANCHA TULSI	25	17.26±0.46			
ALOE VERA GEL	25	15.45±0.26			
SODIUM HYPOCHLORITE	25	14.40±0.15			

Table 3: Multiple comparison Using One Way Anova

MULTIPLE COMPARISON FOR PERCENTAGE ELONGATION					
GROUP	N	Mean± Std. Deviation	p-Value	Result	Post hoc pairwise comparison
LEMON GRASS OIL	25	31.28±1.94	0.000	Highly significant	a>b>c>d
PANCHA TULSI	25	23.20±3.34			
ALOE VERA GEL	25	10.39±1.86			
SODIUM HYPOCHLORITE	25	2.77±1.07			

Discussion

To provide a bacterial free environment is the main aim of a successful endodontic therapy. For endodontic procedures to be successful, minimizing or eliminating microorganisms is crucial¹. As we already know that NaOCl is the gold standard material used for irrigation and decontamination of GP cones. Sodium hypochlorite possess many bactericidal effects, if acts on the cell membrane causes rupture of the membrane, loss of permeability and coagulation of the cytoplasm.

The drawbacks that were seen in Sodium hypochlorite were then overcome by introduction of other irrigating solutions that showed similar or better results than NaOCl and had less cytotoxic effects. A new agent called MTAD was introduced to eliminate the negative effects of NaOCl and to stand out as an ideal irrigant. It is an aqueous mixture of 3% broad-spectrum antibiotic doxycycline, 4.2% demineralizing citric acid, and 0.5% detergent polysorbate 80 (Tween80). Additionally, it has several drawbacks like a high price, a short shelf life, and poor compatibility with tooth pulp cells⁷.

Herbs may have antibacterial, antiseptic, antiviral, antifungal, and immunomodulatory activities, according to scientific research. Although herbal disinfectants for GP cones have rarely been tested, they are environmentally

friendly. Additionally, unlike sodium hypochlorite, herbal solutions have no impact on the mechanical characteristics of GP.

The GP cone's mechanical properties were discovered to adhere to Hook's law and were consistent with a partially crystalline polymeric substance⁸. Researches have shown, the wax/resin components of GP cones are associated with the flexibility of the cone, whilst the ZnO content of GP cones is associated to elastic modulus and percentage lengthening (elongation). The GP component of GP cones was discovered to be associated with tensile strength. NaOCl⁹ may reduce the chemical stability of the chain polymers, resins, and waxes used to create GP cones because it is a potent oxidising agent. The mechanical properties of a GP cone would be adversely affected by such a chemical instability¹⁰.

In this study we have discussed the use of herbal solutions as an effective disinfectant for the root canals as well as aids in decontamination of the GP cones used in root canal treatment. Herbal solutions do not affect the mechanical properties of GP cones and hence does not interfere with the bonding and sealing ability of root canal sealers with GP cones. Many herbal solutions have now come to play, and aloe vera gel, lemon grass oil and pancha tulsi was used in this study, keeping sodium hypochlorite as the control.

In the antimicrobial testing, *E. faecalis* was cultured on nutrient agar and zones of inhibition were seen on the agar culture plates. Four wells were made in the culture plates and 50 microlit of herbal solutions were dispensed in the wells and were incubated for 24 hrs at 37°C. After 24 hrs well dispensed with lemon grass oil showed the biggest zone of inhibition with a mean value of 23.66mm.

In this study GP cones when treated with herbal solutions subjected under UTM to test for change in tensile strength showed that cones treated with lemon grass oil had

the greatest tensile strength with mean value of 18.3896 MPa and a percentage elongation of approximately 31.28%. Tensile strength of group treated with pancha tulsi is 17.26 MPa and percentage elongation 23.20% and group treated with aloe vera gel has a tensile strength of 15.45 MPa and a percentage elongation of 10.92%. The least tensile strength with 14.40 MPa along with minimum percentage elongation of GP was seen in cones treated with sodium hypochlorite was 2.77%.

The antibacterial properties of aloe vera are linked to compounds including, cinnamic acid, pyrocatechol, ascorbic acid, and p-coumaric acid. It has been used to treat a wide range of illnesses, from gastric ulcers as well as in cosmetics. The chemicals cinnamic acid, pyrocatechol, ascorbic acid, and p-coumaric acid have been particularly recognised as having a well-established antibacterial activity.

Another important advantage is that it demonstrates quick and efficient disinfections of GP cones. There is no alteration in the tensile strength of GP and no surface changes are seen with improved bonding capability to root canal as compared to NaOCl.

This might have been because LG oil has a high citral epoxide activity level. Cymbopogon citratus, or LG, has also been shown to have antibacterial and antifungal properties. Citral epoxide exhibits significant action, and Saddiq et al.¹¹ and Falcao et al.¹² reported that it was effective in combating *S. aureus*. De Silva et al.¹³ showed that citral was 100% harmful against the development of fungus because it produces large complex transfer with fungus cells acting as electron donor, which in turns results in fungal death. Naik et al.¹⁴ revealed, even at low concentrations, LG is more efficient against Gram-positive micro-organism than Gram-negative micro-organism. It also demonstrated that many types of

bacteria with antibiotic resistance can be successfully treated.

Turbidity test showed that when GP cones contaminated with *E. faecalis* and dipped in herbal solutions for 30 sec before placing in sterile BHI broth showed turbidity which was noted as positive and negative. The test tubes with negative turbidity showed that decontamination of GP cones in the solutions were effective against *E. faecalis* and test tubes with positive turbidity showed that the solution was ineffective. BHI broth is kept as positive control. GP cones treated with lemon grass oil before placing it in sterile BHI broth, after 24 hours 100% negative turbidity which was indicative of that maximum decontamination of GP cones. Followed by NaOCl with 88% of negative turbidity, then followed by aloe vera with 76% of negative turbidity and then by pancha tulsi with 72% of negative turbidity. Aloe vera and pancha tulsi almost showed similar turbidity effects.

Later after an observation period of 72 hrs, GP cones treated with lemon grass oil showed positive results in 12% which was similar to NaOCl. While with aloe vera, increased to 32% and with pancha tulsi, increased to 36%. Although with NaOCl remained the same after 72 hrs as well.

Conclusion

To conclude, the study shows that herbal solution of lemon grass oil offers better antimicrobial properties which can be made use in irrigation of the root canal system. Lemon grass oil offers effective decontamination against *E. faecalis* contaminated GP cones. The biggest disadvantage of herbal solutions is that they have short shelf life and to prepare a fresh solution every time is a cumbersome task. So, more studies should be conducted to overcome this drawback. There have been abundance of studies where herbal solutions are used as irrigant in in-vivo cases but only in cases of primary

dentition showing equal or similar level of disinfection as that achieved by sodium hypochlorite. There are various studies to evaluate antimicrobial properties of herbal solutions, which can affect these results.

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