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RESEARCH ARTICLE

COMPARATIVE EVALUATION OF ANTIBACTERIAL EFFICACY OF HERBAL IRRIGANTS LIKE MORINDA CITRIFOLIA JUICE, PROPOLIS AND GREEN TEA AGAINST CONVENTIONAL IRRIGANTS LIKE 3% SODIUM HYPOCHLORITE AND 2% CHLORHEXIDINE AGAINST ENTEROCOCCUS FAECALIS: AN IN VITRO STUDY

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ABSTRACT

Introduction: For many years, sodium hypochlorite and 2 % chlorhexidine have been used for eradication of E faecalis. But side effects caused by sodium hypochlorite and constant increase in antibiotic resistant strains has shifted attention towards herbal alternatives.

Aim: To evaluate and compare the antibacterial efficacy of herbal irrigants like Morinda citrifolia, Propolis, green tea with gold standard Sodium Hypochlorite and Chlorhexidine

Methodology: Muller Hinton agar plates were prepared and incubated for 24 hours at 37°C. 5 Holes of 6mm diameter were punched out on muller hinton agar plate followed by streaking with strain of Enterococcus faecalis on the plate. 5 holes were marked at the bottom of the petri dish for different groups as, Group 1-Morinda Citrifolia, Group 2- Propolis, Group 3- Green tea, Group 4- Sodium Hypochlorite, Group 5- Chlorhexidine. 100 µl of each irrigant was pipetted in its respective well and incubated at 37°C for 24 hours and plates were examined for the presence and size of zone of inhibition. The readings were subjected to statistical analysis using one way ANOVA and Tukey's test.

Results: Chlorhexidine exhibited highest antibacterial efficacy as compared to other irrigants. Amongst herbal irrigant Propolis showed comparatively higher antibacterial efficacy followed by Morinda Citrifolia and least with Green Tea. Propolis showed no statistically significant results as compared to Sodium Hypochlorite.

Conclusion: According to the results of this study, propolis can be used as an effective antimicrobial agent similar to that of sodium hypochlorite, although long-term in vivo studies are warranted.

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INTRODUCTION

Microorganisms and their products are considered to be the major cause of pulp and periradicular pathosis. The various microorganisms found in association with endodontic infections are Enterococcus, Streptococcus, Actinomyces, Peptostreptococci, Eubacterium, Bacteroids, etc. E faecalis of the enterococci group is the most frequently found species in tooth with persistent infection after root canal treatment (Bhardwaj, 2013). Mechanical instrumentation and chemical irrigation plays important role in disinfection of root canal. Various irrigating solutions have been advocated for elimination of microorganisms.

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The gold standard among those are sodium hypochlorite (NaOCl), 2% chlorhexidine solution (Stuart, 2006). Sodium Hypochlorite is the most commonly used irrigant because of its superior tissue dissolving property and excellent antibacterial efficacy. But it has few shortcomings like high toxicity, reduction in elastic modulus and flexural strength of dentin, unpleasant taste (Bhargava, 2015). A2% chlorhexidine gluconate might be used as a irrigating solution which has bis-bis guanide with amphiphatic and antiseptic properties. It is antimicrobial and biocompatible. However its use as an endodontic irrigant should be restricted because it can discolour the tooth some patients may have side effects such as loss of taste, burning sensation, dryness of mouth and discolouration of tounge (Matthias Zehnder, 2006). All these factors led to the search of herbal irrigants like Neem, Green tea polyphenols, Morinda citrifola and Propolis. Herbal

products have wide range of antimicrobial, anti- inflammatory, anti-oxidant property and are biocompatible. Herbal irrigants shows low toxicity and lack of microbial resistance and easy availability (Prabhakar, 2010). *Morinda citrifolia* commonly known as "Indian mulberry" or "noni" plant is innate to tropical countries is the important folk medicine. Its juice has a broad range of therapeutic effects including antibacterial, antifungal, antiviral, antitumor, antihelminthic, analgesic, hypotensive, anti‑inflammatory, and immune enhancing effects (Murray, 2008). *Propolis* also known as bee glue or bee Propolis is a potent antimicrobial, anti-inflammatory and antioxidant agent. It is used to reinforce the combs and to keep the hive environment aseptic. It is a potent antimicrobial, antioxidant, and anti-inflammatory agent. The main chemical elements present in propolis are flavonoids, phenolics, and various aromatic compounds. Flavonoids are well known plant compounds that have antioxidant, antibacterial, antifungal, antiviral, and anti-inflammatory properties (Kandaswamy, 2010). *Green tea polyphenols (GTP)* is known to have range of properties like antibacterial, antioxidant, anti-inflammatory, antifungal etc. Cathechins and the flavins are present in GTPs, and they are considered as microbiologically active ingredients (Pujar, 2011). The aim of this study was to evaluate the antimicrobial effectiveness of various herbal irrigants namely, Morinda Citrifolia, Propolis and Green tea and to compare these herbal irrigants with most commonly used irrigants like sodium Hypochlorite and 2 % Chlorhexidine on Enterococcus Faecalis. Null Hypothesis of this study is that all herbal irrigants have same antimicrobial efficacy with that of sodium hypochlorite and chlorhexidine.

MATERIALS AND METHODS

Preparation of Irrigants

Commercially available extract of Morinda citrifolia juice (Galaxy) and Propolis (Platinum) were obtained and were preserved in refrigerator (Fig. 1) Extract of green tea (Gaia) was prepared by boiling commercially available green tea for 5 minutes. 25 grams of green tea leaves were boiled in 100 ml of water for 5 minutes. The extract was filtered using filter paper and was refrigerated until use.

Preparation of Plates with irrigants

Pure strains of Enterococcus faecalis (ATCC 29212) were isolated and active cultures for experiments were prepared by transferring loopfull of cells to the petri dish of nutrient agar (Hi-media) for bacteria that were incubated for 24 h at 37°C (Fig. 2). 5 Muller hinton agar plates were prepared. To prepare muller hinton agar (Hi-media), 19 grams of muller hinton (Hi-media) was dissolved in 500ml of water in a beaker, autoclaved at 121°C for 20 minutes at 15lbs pressure and was poured in sterile glass petri dishes under aseptic condition to get a minimum thickness of around 6mm. The petri dishes were then incubated for 24 hours at 37°C. 5 Holes of 6mm diameter were punched out on muller hinton agar plate followed of streaking with strain of Enterococcus faecalis (ATCC 29212) on the plate (Fig. 3).

Irrigants were divided into 5 groups as:

Group 1 : Morinda citrifolia juice
Group 2 : Propolis 3%
Group 3 : Green tea
Group 4 : 3%Sodium Hypochlorite
Group 5 : 2% Chlorhexidine

100 µl of each irrigant was pipetted in its respective well and incubated at 37°C for 24 hours. The plates were examined for the presence and size of zone of inhibition. The zone of inhibition for each irrigant was measured with the help of Hi-media scale. All measurements were made with unaided eye while viewing the back of the petri dish with reflected light against a black non-reflecting background.

Fig. 1. Herbal irrigants used in the study Morinda Citrifolia juice, propolis and green tea respectively

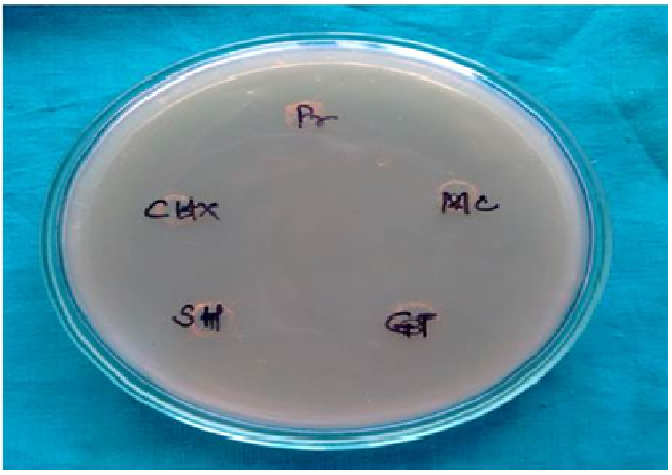

Fig. 2. Wells were prepared and labelled for respective group

Fig. 3. Irrigants were placed in respective well through micropipette

Data Analysis

The data were analysed [Tables 1 & 2] and Figure 1 using SPSS 15.0 software. The readings were subjected to stastical Analysis using one way ANOVA. Differences between the five groups were assessed using Tukey's Test.

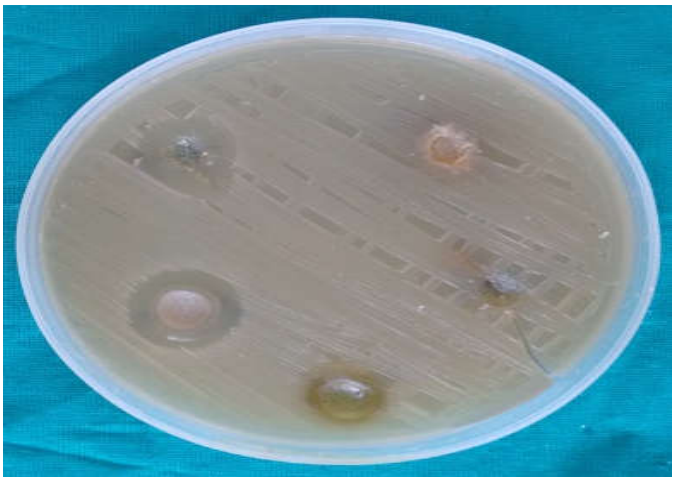

Fig. 4. Zone of inhibition for respective irrigants

The zone of inhibition were tabulated and subjected to statistical analysis.

Zone of inhibition in millimeter

Groups	Plate 1	Plate 2	Plate 3	Plate 4	Plate 5
Group 1	9mm	10mm	9mm	11mm	10mm
Group 2	10mm	11mm	12mm	10mm	11mm
Group 3	7mm	8mm	7mm	7mm	8mm
Group 4	12mm	13mm	12mm	11mm	13mm
Group 5	20mm	18mm	20mm	19mm	20mm

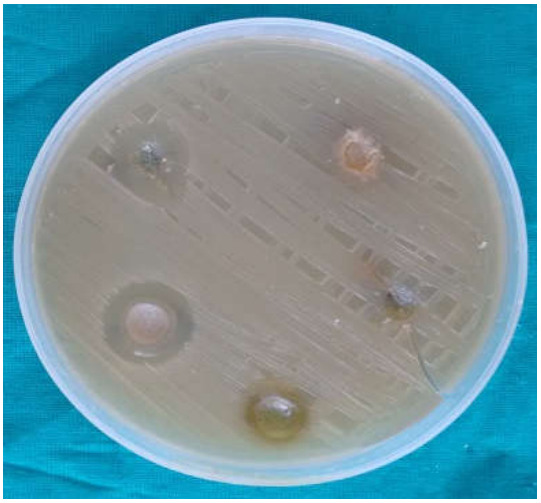

Table 1. Descriptive statistics for zone of inhibition (mm) as per different treatments

	Morinda citrifolia	Propolis	Green tea	Sodium hypochlorite	Chlorhexidine
Mean	9.80	10.80	7.40	12.20	19.40
SD	0.837	0.837	0.548	1.304	1.140
Median	10	11	7	12	19
P-value*	< 0.0001 (HS)				

*Obtained by using *one way ANOVA;* HS: Highly-Significant

Highly significant difference in the mean zone of inhibition across treatment groups.

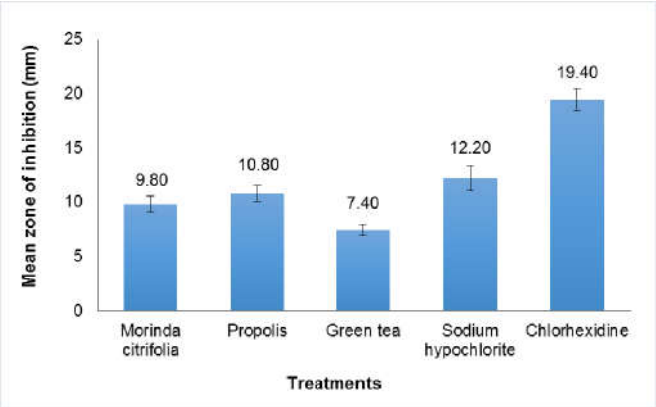

Figure 1. Bar chart showing mean zone of inhibition according to different treatments

Table 2. Pair wise comparison between different treatments across zones of inhibition

Comparison	*P*-value*
Morinda citrifolia - Propolis	0.4961 (NS)
Morinda citrifolia - Green tea	0.0068 (S)
Morinda citrifolia - Sodium hypochlorite	0.0068 (S)
Morinda citrifolia – Chlorhexidine	< 0.0001 (HS)
Propolis - Green tea	0.0001 (S)
Propolis - Sodium hypochlorite	0.1913 (NS)
Propolis – Chlorhexidine	< 0.0001 (HS)
Green tea - Sodium hypochlorite	< 0.0001 (HS)
Green tea – Chlorhexidine	< 0.0001 (HS)
Sodium hypochlorite – Chlorhexidine	< 0.0001 (HS)

*Obtained by using *Tukey's test;* HS: Highly-Significant; S: Significant; NS: Non-Significant
Level of significance: 5%

RESULTS

Chlorhexidine showed the maximum antimicrobial activity, followed by Sodium Hypochlorite, Propolis, Morinda Citrifolia and Green Tea [Figure 1]. There was no statistical difference between NaOCl & Propolis and Propolis & Morinda Citrifolia. There was statistical difference between the efficacy of Chlorhexidine and Morinda Citrifolia, Propolis and green tea. The efficacy of Propolis and Morinda Citrifolia was also significantly more than that of green tea.

DISCUSSION

This study was conducted on *E. Faecalis* because these microorganisms are commonly encountered in endodontic infections. Enterococci are gram positive coccci that occur singly, in pairs ,or as short chains. They are facultative anaerobes, possessing the ability to grow in the presence of or absence of oxygen. These are commonly detected in asymptomatic, persistant endodontic infection. Its prevalence in such infection ranges from 24%to 77%.Use of good aseptic technique ,increased apical preparation sizes, inclusion of 2 % chlorhexidine in combination with sodium hypochlorite are currently most effective methods to combact E Faecalis within root canal system of teeth, Sodium hypochlorite is the most commonly used irrigating solution in clinical practice. Sodium hypochlorite has excellent antimicrobial potency and it has unique tissue dissolving property. But it has unpleasant taste ,toxicity ,and inability to remove smear layer ,it also has detrimental effect on dentin elasticity and flexural strength. So Researchers are constantly in search of Herbal alternatives as a root canal irrigants (Mohammadi, 2008).

Herbal products have been used in dental practice and have become more common now due to their high antibacterial activity, biocompatibility, and antioxidant properties. Herbal and alternative medicine are gaining popularity among the Researchers (Penumudi). In this study, all the tested irrigants were shown to inhibit and eliminate the tested strains. Chlorhexidine showed the best antimicrobial efficacy followed by Sodium Hypochlorite, Propolis, Morinda Citrifolia and Green Tea. Chlorhexidine was best among all the groups. It exhibited excellent antibacterial activity on the bacteria. Chlorhexidine digluconate is widely used in disinfection because of its excellent antimicrobial activity.CHX is a positively charged hydrophobic and lipophilic molecule that interacts with phospholipids and lipopolysaccharides on the cell membrane of bacteria and enters the cell through some type of active or passive transport mechanism. Its efficacy is because of the interaction of the positive charge of the molecule with the negatively charged phosphate groups on microbial cell walls, which alters the cells' osmotic equilibrium.

This increases the permeability of the cell wall, allowing the CHX molecule to penetrate into the bacteria. Damage to this delicate membrane is followed by leakage of intracellular constituents, particularly phosphate entities such as adenosine triphosphate and nucleic acids. As a consequence, the cytoplasm becomes congealed, with resultant reduction in leakage; thus, there is a biphasic effect on membrane permeability (Mohammadi, 2008). Propolis exhibits antimicrobial, anti-inflammatory, healing, anaesthetic and cariostatic properties. In this study, the antimicrobial efficacy of Propolis was found to be similar to Sodium hypochlorite. (Grange, 1990). The antimicrobial property of propolis as observed in the present study was in concurrence of previous reported studies (Murray, 2008). The possible reason for the antimicrobial action of propolis might be attributed to its flavanoid content (Grange & Davey 1990). Grange & Davey (1990) assessed the bacteriocidal ability of propolis against a wide range of gram-positive and gram-negative organisms. They reported complete inhibition of cultures of Staphylococcus aureus, including MRSA strains. The results of the present study was similar to the study of Kandasamy et al. who compared Dentinal tubule disinfection with 2% Chlorhexidine gel, propolis, morinda citrifolia juice, 2% povidone iodine, and calcium hydroxide (Grange, 1990). Propolis has excellent antimicrobialn,anti-inflammatory properties due to faavonids ,phenolics .and various aromatic compounds (Kandaswamy, 2015).

M. citrifolia juice (MCJ) is used as an endodontic irrigant as it is a biocompatible antioxidant and not likely to cause injuries like sodium hypochlorite accidents. MCJ contains the antibacterial compounds L-asperuloside and alizarin and is more effective than 2% CHX against *E. faecalis*. Murray et al compared the effectiveness of MC with sodium hypochlorite and concluded that MCJ could be used as endodontic irrigant in combination with EDTA followed by final flush with M Citrifolia juice (Bhargava, 2015 and Murray, 2008). Green tea extracts (GTEs) contain catechin, which is one of the polyphenols from green tea. It has been reported that GTEs have remarkable anti‐inflammatory, antioxidant, and anti‐carcinogenic effects in a number of animal tumors, cell culture systems, and epidemiological studies (Pujar, 2011). In this study it showed least antimicrobial activity as compared to other groups. In this study, commercially available herbal products were used to check their antimicrobial efficacy as they are easily available, inexpensive and can be prepared in clinical setup itself.

Conclusion

Under the limitations of this study, it was concluded that:

- Chorhexidine performed best among all irrigants on E faecalis followed by sodium hypochlorite .
- Propolis performed equally well as sodium hypochlorite against *E. faecalis* biofilm formed on Muller Hinton agar plate
- Among experimental herbal irrigants, propolis and Morinda citrifolia showed better antimicrobial activity on E faecalis as compared to green tea.

Herbal alternatives used for root canal irrigation will prove to be preferable considering the various unenviable properties of conventional irrigants. Further research is required to recommend the use of herbal alternatives as a root canal irrigant.

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