COMPARATIVE ANTIBACTERIAL AND ANTIFUNGAL EFFICACY OF 15% ALOE BARBADENSIS MILLER, 0.2% CHLORHEXIDINE (CHX), 18% ETIDRONATE AND 5% SODIUM HYPOCHLORITE AGAINST ENTEROCoccus FAECALIS AND Candida Albicans

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ABSTRACT
The ultimate goal of endodontic treatment is to either prevent the development of apical periodontitis or to create adequate conditions for periapical healing in case the disease is already present. As there is no study to our knowledge comparing the antimicrobial efficacy of 15% Aloe barbadensis miller, 0.2% chlorhexidine (CHX), 18% etidronate and 5% sodium hypochlorite, the purpose of this in vitro study was to evaluate the antimicrobial efficacy of herbal irrigants against Enterococcus faecalis and Candida albicans. The testing of the antibacterial and antifungal efficacy of selected medicaments against Enterococcus faecalis and Candida albicans was done by the agar disc-diffusion method. Pre sterilized Whatman paper disc, 6mm in diameter were soaked with the test solution and placed onto the previously seeded agar petri plates. Each plate was incubated at 37°C for 48 hours. Zones of inhibition were recorded for each plate and the results were statistically analyzed. Saline was used as negative control in this study. Chlorhexidine exhibited maximum antibacterial efficacy followed by sodium hypochlorite and Aloe barbadensis miller. Etidronate showed some amount of antibacterial activity. In case of Candida albicans, Aloe barbadensis miller exhibited maximum antifungal efficacy followed by sodium hypochlorite and Chlorhexidine. Etidronate exhibited minimal efficacy while dimethyl sulphoxide and saline showed no antifungal efficacy.

KEYWORDS: Candida albicans, Enterococcus faecalis, Root canal irrigants.

INTRODUCTION
To achieve successful endodontic treatment it is essential to remove all the causative microorganisms and their by-products from the root canal system. Though chemomechanical preparation exists, the debris is often left behind in the root canal system.19 Endodontic infections are polymicrobial and more than 150 bacterial species are usually found in combination of 3 to 6 species in each canal.19 Researches have shown that Enterococcus faecalis is the most prominent bacteria present in 22-77% of endodontic failure cases.21 It has the ability to survive harsh environmental conditions.21 Evidence exists showing the presence of fungi in the root canal system. Specifically Candida albicans, have been isolated in the pulp spaces and periapical areas through light and electron microscopy and culture techniques.29 Aloe barbadensis miller, is a short succulent herb resembling a cactus, with green fleshy, spiny and well margined leaves filled with a clear viscous gel. Aloe barbadensis miller has potent antibacterial, antifungal and antiviral properties.4,35 Aloe barbadensis miller has been used to relieve thermal burn, sunburn and promote wound healing22 and has antimicrobial activity and can help stimulate the body’s immune system.6 The leaf extracts contain anthraquinones.23 Chlorhexidine (CHX) is a cationic bis-guanide that has a broad spectrum of antimicrobial activity. It is effective against both Gram-positive and Gram-negative bacteria as well as fungi.27

Etidronate also known as Hydroxyethylidene bisphosphonate (HEBP) has been introduced in Endodontics. It is a substance which prevents bone
resorption and is used in patients suffering from osteoporosis or Paget’s disease. It has been used as a substitute for other chelators earlier and it has few effects on dentin. It has the ability to mix with sodium hypochlorite without interfering with the antimicrobial property of the substance.

Sodium hypochlorite (NaOCl) is considered as a gold standard irrigating solution because of its antimicrobial potential and ability to dissolve organic matter. Though it causes irritation to the periapical tissues and possesses various disadvantages like staining of instruments, burning of surrounding tissues, unpleasant taste, high toxicity, is corrosive to instruments, inability to remove smear layer, reduction in elastic modulus and flexural strength of dentin. To overcome these disadvantages of NaOCl studies are being conducted to obtain other alternatives for root canal irrigation.

As there is no study till date comparing the antimicrobial efficacy of 15% Aloe barbadensis miller, 0.2% Chlorhexidine (CHX), 18% Etidronateand 5% Sodium hypochlorite, the purpose of this in vitro study was to evaluate the antimicrobial efficacy of herbal irrigants against Enterococcus faecalis and Candida albicans.

**MATERIALS AND METHODS**

Microorganisms taken for this study were one anaerobic facultative bacteria Enterococcus faecalis and one yeast Candida albicans. Agar disc diffusion test was conducted to evaluate and compare the antimicrobial efficacy of medicaments. The Enterococcus faecalis (ATCC 29212) bacterial strains were reactivated in a brain heart infusion broth (Hi Media, Mumbai, India). The Candida albicans (ATCC 10231) were reactivated in Sabouraud’s dextrose agar broth (Sigma Aldrich, Bengaluru, India) in 48 hours.

The inoculums for each bacterial strain were prepared by picking up four to five colonies (with the help of a circular, previously sterilised loop) and dissolving them into the respective test tube containing 5ml of 0.85% saline solution to produce a turbidity of 0.5 on the McFarland scale. A sterile cotton swab was then rolled in the suspension to streak the plate surface of a Mueller- Hinton agar plate (Sigma Aldrich, Bengaluru, India) for Enterococcus faecalis and a Sabouraud’s dextrose agar plate (Sigma Aldrich, Bengaluru, India) for Candida albicans, in a form that lawn growth was observed.

For Aloe barbadensis miller preparation 150 mg of Aloe powder (Bioprex Labs, Pune, Maharashtra, India) was dissolved in 10ml of Dimethylsulphoxide (DMSO) and made into 15% concentration. Etidronate (Zschimmer & Schwarz Mohsdorf GmbH & Co KG, Burgstadt, Germany) solution was obtained by mixing pure chemicals with distilled water. 5% Sodium hypochlorite (Sigma Aldrich, Bengaluru, India) was also taken as a test group irrigant. 0.2% Chlorhexidine was prepared by dilution of a 20% stock Chlorhexidine (Sigma Aldrich, Bengaluru, India).

Discs of 6-mm diameter were prepared from Whatman paper No. 1 (Sigma Aldrich, Bengaluru, India), which then were sterilised by a hot air oven. These discs were then saturated with 50µl of each medicament and aseptically transferred to the agar plate previously incubated with bacteria.

Then the plates were incubated at 37°C under the appropriate gaseous conditions for 48 hours in a CO₂ incubator, in an atmosphere of 10% CO₂. All assays were repeated four times to ensure reproducibility. Microbial Zones of inhibition were measured in millimetres.

After 48 hours, zones of inhibition were calculated using Hi zone antibiotic scale (Hi Media, Mumbai, India) (Fig 1 and 2).

The values were subjected to statistical analysis by using one way Anova analysis & intergroup comparison with Bonferroni multiple comparison test. Values of p<0.05 were considered statistically significant. Statistical analysis was performed with SPSS (PC. Version 10 software) (IBM, NY, USA)

**RESULTS**

The results showed that there was significant difference between the different groups of irrigants used against Enterococcusfaecalis. The obtained results were condensed in the following order Chlorhexidine > Sodium hypochlorite = Aloe barbadensismiller > Etidronate > Saline = Dimethyl sulphoxide. The above order was based on the p values where p < 0.05 was considered to be significantly different.

The results showed that there was significant difference between the different groups of irrigants used against Candida albicans. The obtained results were condensed in the following order Aloe barbadensis miller > Sodium hypochlorite > Chlorhexidine > Etidronate > Saline = Dimethyl sulphoxide. The above order is based on the p values where p < 0.05 was considered to be significantly different (Tables 1 and 2).
Fig 2: Zones of inhibition against Candida albicans

Table 1 Comparison of the antimicrobial effect of endodontic irrigants against Enterococcus faecalis. [Analysis of variance of zones of inhibition for different irrigants, P < 0.05.]

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Minimum</th>
<th>Maximum</th>
<th>p Value compared with Saline</th>
</tr>
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<tbody>
<tr>
<td>CHX</td>
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<td>14.37</td>
<td>0.47</td>
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<td>0</td>
</tr>
<tr>
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<td>10.5</td>
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<td>9.5</td>
<td>0.57</td>
<td>9</td>
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<td>0</td>
</tr>
<tr>
<td>ETIDRONATE</td>
<td>4</td>
<td>6.75</td>
<td>0.64</td>
<td>6</td>
<td>7.5</td>
<td>0</td>
</tr>
<tr>
<td>SALINE</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Dimethylsulphoxide</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>1</td>
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</table>

Table 2 Comparison of the antimicrobial effect of endodontic irrigants against Candida albicans. [Analysis of variance of zones of inhibition for different irrigants, P < 0.05.]

<table>
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<th></th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Minimum</th>
<th>Maximum</th>
<th>P Value compared with Saline</th>
</tr>
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<td>CHX</td>
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<td>10</td>
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<td>10</td>
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<tr>
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<td>0</td>
<td>0</td>
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<td>0</td>
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<tr>
<td>Dimethylsulphoxide</td>
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<td>0</td>
<td>0</td>
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</table>

DISCUSSION
The tested Endo pathogens were arbitrarily selected and these samples were not from necrotic root canals. Even though an attempt was made from our side to select the most commonly isolated bacteria and fungus from necrotic canals which are mainly responsible for the failed endodontic cases and recurrent infections.

The agar diffusion method has been widely used to test the antimicrobial activity of dental materials, irrigants and medicaments.\(^\text{15,16}\) The advantage of this method is that it allows direct comparison of the materials against the organisms, indicating the potential of the test materials to eliminate microorganisms in the local microenvironment of the root canal system. However, there are certain disadvantages of using this method, the most important being that the results may be variable depending upon the ability of the material to diffuse across the medium and not only on the toxicity of the material for the particular organism.\(^\text{4}\)

Enterococcus faecalis is the most frequently isolated bacterial species in tooth with persistent infection after root canal treatment with incidence of 22-77% in failure cases.\(^\text{34}\) Candida albicans is the most common fungus seen in the root canals, 21% in primary infections and 18% in cases of retreatments.\(^\text{32}\) It can survive harsh conditions and the physicochemical properties of the microorganisms help them to modify according to the prevailing environmental and nutritional conditions.

Aloe barbadensis miller has shown to have anti-inflammatory, immunomodulatory and cell growth stimulatory activities.\(^\text{25}\) It is also shown to be antiviral and anti fungal.\(^\text{19}\) Aloe barbadensis miller has 75 potentially active constituents such as vitamins, enzymes, minerals, sugars, lignins, saponins, salicylic acid, amino acids and anthraquinones which are phenolic derivatives.\(^\text{7}\) Results from the present study showed that Aloe barbadensis miller was effective against both Enterococcus faecalis and Candida albicans. The antibacterial action of Aloe barbadensis miller had been proved in study by Pandey R and Mishra A in 2010.\(^\text{24}\) Even study by Ehsaniet al. in 2013, showed significant antibacterial efficacy of Aloe Vera against Enterococcus faecalis.\(^\text{10}\) It was most effective in eliminating Candida
albicans in comparison to other groups taken in our study. These results are in support of the study by Alemdar S[1] but in contrast to the study by Vinothkumar et al which stated that Aloe barbadensis miller loses its antibacterial and antifungal properties once it is exposed to the environment.[40] Our results were also in contrast to the study conducted by Valera et al. in 2013. This may be due to the different (glycolic) extract of Aloe Vera taken in their study.[38]

Chlorhexidine is used because of its antimicrobial properties, substantivity in the root canal and low cytotoxicity. It has wide range of antimicrobial action.[26] In the present study Chlorhexidine exhibited good antimicrobial activity against Enterococcus faecalis and Candida albicans which is in support of the study published by Ertugrul Ercan.[11] It was most effective in eliminating Enterococcus faecalis when compared to other irrigants used in our study.

Sodium hypochlorite is widely used as root canal irrigant because of its wide range of anti microbial action. It was used in our study as it is considered to be the gold standard for irrigation. Results of the study showed that it had good antimicrobial action against both Enterococcus faecalis and Candida albicans which is in support of the study by Soley Arslan.[3]

HEBP (1-hydroxyethylidene- 1, 1-bisphosphonate), also known as etidronic acid or etidronate, has been proposed as a potential alternative to EDTA or citric acid because this agent shows no short-term reactivity with NaOCl. HEBP is nontoxic and has been systematically applied to treat bone diseases.[18] It also has good antimicrobial action.[23] The results of the present study showed that HEBP exhibited limited antimicrobial efficacy against both tested microorganisms. This is in the support of study Maria Teresa Arias-Molizet al.[3]

Dimethylsulfoxide was used as a test group as Aloe barbadensis miller solution was prepared using it as a solvent.[41] The result of the present study showed that it did not exhibit significant antimicrobial efficacy against both the tested microorganisms. Its antimicrobial activity was similar to that of saline which was taken as the negative control.

CONCLUSION
Aloe barbadensis miller was effective against Candida albicans and Enterococcus faecalis in this study. Also, Chlorhexidine exhibited highest antibacterial efficacy against Enterococcus faecalis and good antifungal efficacy against Candida albicans. Sodium hypochlorite exhibited adequate antibacterial and antifungal efficacy. Before making any claims regarding Aloe barbadensis miller’s use as an endodontic irrigating agent, further in vivo and studies involving dentinal tubule disinfection are warranted.

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