Comparison of the antimicrobial efficiency of Curcuma longa and 17% EDTA with 3% sodium hypochlorite against E. faecalis, C. albicans: An in vitro study

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Abstract---Aim: To evaluate the antimicrobial efficacy of Curcuma Longa, 17% EDTA, and 3% sodium hypochlorite against E. faecalis, C. Albicans. Materials and Methods: The antimicrobial activity was determined using disc diffusion test. Ethanol extract of turmeric, 3% Sodium hypochlorite, 17% EDTA, absolute ethanol, Enterococcus faecalis cultures, Candida Albican cultures, Mueller Hinton agar. The disc diffusion test was performed in Mueller Hinton agar plates. The plates were incubated at 37°C for 24 hours and zone of inhibition were recorded. Finally, it was statistically analyzed. Results: Ethanol extract of turmeric showed zones of inhibition suggesting that they had anti-microbial properties. Ethanol extract of turmeric showed significantly greater (almost same) zones of inhibition than 2% chlorhexidine. Henceforth research should be directed towards the use as an irrigant in root canal treatment. Conclusion: Under the limitations of this study, it was concluded that Ethanol extract of turmeric has a significant antimicrobial effect against E. faecalis. Microbial inhibition potential of ethanol extract of turmeric observed in this study opens perspectives for its use as an intra-canal irrigant.

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Introduction

In Endodontic therapy, one of the most important procedure is to completely eliminate intracanal microorganisms from the root canals to ensure the root canal [1] dry and micro free in order to promote the healing process and avoiding reinfection to achieve a long-term success. Infections related to endodontics are predominantly associated with anaerobic microorganism which is polymicrobial in nature. In pulpal and periapical lesion, metabolic products of bacteria, such as its enzymes, and toxins have a vital role in persisting the pathogenesis. Many literatures has reported that persisted intraradicular or secondary infection is the major etiology of failures in endodontic therapy. Studies have shown that microflora in failed root canal therapy are different from those found in tooth with pulpitis. Enterococcus faecalis (E. faecalis) and Candida albicans (C. albicans) are proven to be considered as the most resistant species found in infected root canal which are associated with root canal failures. [2]

E. faecalis is a gram positive microorganism, and is anaerobic coccus which is commonly found in persistent root canal infection. It is usually recovered in over one-third of the canal with endodontic therapy with precise periapical lesions. C. albicans, they are the most predominantly and commonly isolated yeast, that induces failure in root canal treatment with periradicular pathosis. As a result of poor oral hygiene and asepsis during the endodontic treatment procedure C. albicans can access into the root canal or as a result of coronal leakage. It is dentinophilic aerobic microorganisms; they are able to survive in harsh ecologic environment of root canal. [3]

Effective disinfection is achieved in complex anatomy of the root canal by unification of mechanical preparation with antimicrobial irrigants. Irrigants help in removal of debris and microorganisms from the canal that cannot be reached by endodontic instruments[4]. An ideal root canal irrigant should have good antimicrobial efficacy, nontoxic to periapical tissues, capable of dissolving necrotic tissue, able to lubricate the canal, and help in smear layer removal.

Sodium hypochlorite (NaOCl) has been widely used as root canal irrigant as it contains effective antimicrobial activity and tissue dissolving ability[5]. But it has several undesirable characteristics such as tissue toxicity, risk of subcutaneous emphysema, allergic potential, and disagreeable smell and taste [6]. Improper use may lead to sodium hypochlorite accident.

17% EDTA is known to be a suggested root canal irrigant because of its excellent antimicrobial efficacy and substantivity characteristic. The mechanism of action of 17% EDTA is associated with intracellular component leakage by the process of adsorbing onto the cell wall of microorganisms. At low concentration, it has bacteriostatic effect. At higher concentration, has bactericidal effect due to precipitation and coagulation of intracellular constituents. [7]
However, herbal alternatives are always been an advantage in endodontics due to its less potential of side effects, safety concerns, and effectiveness over conventional endodontic irrigants. Extracts of plant origin have been found to have therapeutic properties since thousands of years. As per WHO report, 80% of world’s population relies mainly on traditional therapies. There are many advantages of using herbs as antimicrobials, they have minimal side effects, cost effective, have better patient tolerance, and are renewable in nature. Phytochemical extracts such as Vitis Viniferous-grape seed, Curcuma longa - Turmeric, Azadirachta indica - Neem, Morinda citrifolia - Noni, Propolis, and Triphala have been reported to exert antimicrobial properties suggesting their potential to be used as endodontic irrigants. However, there is a lack of sufficient supporting documentation regarding the antibacterial activity of these extracts in endodontics.\[8\]

Research on the bioactive components of grape seeds extracts, i.e. in flavonoids, started in the beginning of the 20th century, when nutritional scientists were dedicated to the isolation and identification of compounds we came to define as “vitamins.” The Hungarian scientist Albert Szent-Györgyi isolated vitamins C and P from citrus fruits. In contrast to vitamin C, the chemical characterisation of vitamin P appeared to be difficult. As a result, it was not possible to attribute the vitamin P effect to a specific compound or preparation. Moreover, the notion of such a vitamin was abandoned since no deficiency disease could be linked to Szent-Györgyi’s citrus extract. However, the biological effect of “vitamin P”, i.e. its influence on vascular permeability was established as well as its enforcing influence on the anti-scurvy effect of vitamin C. Efforts to identify this putative vitamin P were directed to yellow colored plant pigments, the flavonoids (flavus (Latin) means yellow).\[9\]

The purpose of this study was to compare the in vitro effectiveness of ethanol extract of turmeric and 17% EDTA with 3% sodium hypochlorite against E. faecalis and C. albicans by disc diffusion method.

**Methods and Materials**

**Microorganisms used**

Clinical isolates of E.faecalis and C.albicans were used in the present study. These isolates were sub cultured onto Enterococcus conformational media and HI-chrome candida differential media respectively. They were further confirmed by standard biochemical tests and stock culture and stored at -20 degree C for further use.

**Preparation of Turmeric Ethanolic Extract**

50g of turmeric powder measured using an electronic balance and transferred into a test tube, 2ml of ethanol is measured using a 2ml syringe and transferred into the test tube containing turmeric powder. The test tube is sealed with a piece of aluminium sheet. The test tube is left for 3-5 days and is checked on daily, ethanol is added if required. After 5 days, the test tube is centrifuge for 10 minutes, twice. The supernatant is transferred into a sterile test tube. Then it was
kept in hot air oven at 50°C to evaporate the ethanol content to achieve the turmeric ethanolic extract.

**Preparation of Medium**

0.5 ml of distilled water is added into the test and mixed well. The solution is made up to 1ml by adding distilled water. Freshly prepared culture plates of Mueller Hilton agar and Sabourand Chloramphenicol agar were prepared. McFarland standard 0.5 turbidity adjusted suspensions of E.Faecalis and C. Albicans were lawn cultured onto the media plates respectively. After a brief drying, sterile disc were soaked with 10µl ,15µl and 20µl of 3% NaOCl, 17% EDTA, turmeric extract in each one of the disc respectively These plates were incubated at 37°C for 24 hours. Results were recorded by measuring the zone of inhibition of each irrigant E.Faecalis and C. Albican after 24 hours, and was statistically analysed.

**Statistical Analysis**

To compare the mean values between groups one way ANOVA is applied .SPSS version 22.0 is used to analyse the data. Significance level is fixed as 5% (α = 0.05).

**Result**

The mean values of growth inhibition produced by different test groups against the test microorganisms are given. Table1 shows the mean zone of inhibition of 3% sodium hypochlorite, 2% chlorhexidine and 5% grape seed extract and there was statistical significance difference between the groups. 3% sodium hypochlorite performed better than all the test groups, followed by 5% Grape seed extract and, 2% chlorhexidine against all the tested microorganisms after 24 hours incubation.

<table>
<thead>
<tr>
<th>Variation</th>
<th>Group</th>
<th>Mean</th>
<th>Std. Dev</th>
<th>F- Value</th>
<th>P-Value</th>
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<td></td>
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<tr>
<td>E.Faecalis</td>
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<td>0.411</td>
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<tr>
<td></td>
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<td>0.399</td>
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<td>Group 3</td>
<td>28.95</td>
<td>0.401</td>
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</tbody>
</table>

Table 1: Mean zone of inhibition 3% hypochlorite, 17% EDTA and Ethanolic extract of turmeric .where 3% sodium hypochlorite shows highest mean zone of inhibition followed by 17% EDTA and 5% Ethanolic extract of turmeric
Fig 1. Mean zone of inhibition for C. albicans for 3% hypochlorite, 17% EDTA and 5% ethanolic turmeric extract. Where 3% sodium hypochlorite shows the highest mean zone of inhibition followed by ethanolic turmeric extract and 17% EDTA.

Fig 2. Mean zone of inhibition for E. faecalis for 3% hypochlorite, 17% EDTA and 5% ethanolic turmeric extract. Where 3% sodium hypochlorite shows the highest mean zone of inhibition followed by ethanolic turmeric extract and 17% EDTA.

Discussion

A variety of laboratory methods are available to evaluate the in-vitro antimicrobial activity of an extract. The most known and basic methods are the disc-diffusion method. It is commonly used because of providing direct estimation of its antimicrobial activity against a specific microorganism and added advantages of simplicity, low cost, the ability to test enormous numbers of microorganisms and antimicrobial agents, and the ease of results interpretation. Though there are
new technologies in the field of microbiology, disc diffusion is still one of the preliminary tests to assess the antimicrobial activity of a newer material.\textsuperscript{[11]} To further study the antimicrobial effect of a material in depth, time-kill test and flow cytologic methods are recommended, which provide information on the nature of the inhibitory effect.

The importance of root canal treatment is to eliminate the causative agent of pulpal infection (microorganisms) in the root canal. Also, to ensure it prevents their recontamination after a period of time.\textsuperscript{[12]} At the same time it has to remain undisturbed to anatomically challenging areas like fins, lateral or furcal canals, apical deltas, webs and isthmus where the biofilm might disturbs those areas after mechanical debridement. Therefore, in order to ensure complete cleanliness of the canal system it is necessary to use irrigant solutions to complement the action of the mechanical instrumentation.\textsuperscript{[13]}

Root canal infections are multibacterial with anaerobic bacteria being present in more than 70\% of the bacteria isolated. \textit{E. faecalis} had frequently been isolated from root canals of failed endodontic treatment cases. \textit{C. albicans}, the common organism associated with therapy resistant apical periodontitis is more resistant to disinfecting agents used in endodontics.\textsuperscript{[14]} In the present study, 3\% sodium hypochlorite, 2\% chlorhexidine gluconate and turmeric extract were shown to inhibit the \textit{E. faecalis} and \textit{C. albicans} effectively.\textsuperscript{[15]}

Several studies on the antimicrobial activity of irrigation solution in endodontics, such as 0.5\%, 1\%, 2.5\%, 5\% NaOCl are found in literature. On the other hand, lack of studies on phytotherapeutic substances such as chlorophyll, propolis, morinda, citrifolia, neem, grape seed, turmeric extract do not permit more objective conclusion about their use.\textsuperscript{[16]} Several pharmacological activities and medicinal applications of various parts of grape are known.

Interest on this substance is based on its properties like antibacterial, antifungal, antiviral, anti-inflammatory.\textsuperscript{[18]} Inclusion of \textit{E. faecalis} in this study was based on the literature that relates these micro-organisms to pulpal infection, mainly in resistant infection after endodontic treatment.\textsuperscript{[19,20]} Methodology of this study followed the standard established for disc dilution tests. Ideal irrigants should combine antimicrobial action and capacity to dissolve organic and inorganic remnants.\textsuperscript{[21]} NaOCl in full concentration is well known for its bacterial action and cytotoxicity. Moreover, its anti-adherence activity by altering bacterial adhesion and ability of organism to colonize also stimulated the study of this substance.

Since its introduction NaOCl has been considered as the gold standard irrigant for root canal irrigation because of its antimicrobial activity and tissue dissolving capacity.\textsuperscript{[22]} High pH of NaOCl interferes with the cytoplasmic membrane integrity and cause biosynthetic alterations in cellular metabolism attributing to its antimicrobial nature. Tissue dissolving action and dissolution rate of NaOCl is directly proportional to its concentration. But not only its actions like antimicrobial activity, tissue dissolving capacity and smear layer removing ability but also the caustic potential and toxicity of NaOCl also increases with the increase in concentration.\textsuperscript{[23]}
Chlorhexidine gluconate (2%) is a good disinfecting agent with a property of substantivity contributing to its prolonged time of action. On comparison with NaOCl, the irrigant is having less toxicity and foul taste. Chlorhexidine is proposed to be an alternative to NaOCl in open apex cases and NaOCl allergic patients. But the major disadvantage persisting is its inferior tissue dissolving action as a primary endodontic irrigant.\[24\]

In an invitro study conducted in 2015, Ying Liu et al. reported that the antimicrobial efficacy of turmeric extract was comparable to that of EDTA and chlorhexidine.\[25\] The decision to compare 2% chlorhexidine with grape seed extract though the percentage of chlorhexidine being the same for both was because of the fact that herbal product is cost efficient than chlorhexidine and EDTA used separately. As ours is a Government institution catering economically backward patients in particular we were interested in an economically viable alternative to chemical irrigants. \[26\]

Use of turmeric extract as an irrigant might be advantageous because of its biocompatible, antioxidant and thus not likely to cause severe injuries to patients that might occur with NaOCl accidents. 2% CHX is an excellent disinfecting agent with a property of substantivity contributing to its prolonged time of action. On comparison with NaOCl, the irrigant is having less toxicity and foul taste. \[27\] CHX is proposed to be an alternative to NaOCl in open apex cases and NaOCl allergic patients. But the major disadvantage is persisting its inferior tissue dissolving action as an endodontic irrigant. Antimicrobial activity of Ethanol extract is mainly due to presence of polyphenolic compounds (causes loss of cell integrity) and also proanthocyanidins. \[28\] Grape seed extract has moderate activity against E. faecalis. However, present study shows least activity against C. albicans. Antimicrobial activity of turmeric extract is similar to 2% CHX against Enterococcus faecalis.

**Conclusion**

Understanding the consistency, dose–response and biological plausibility of the association between the chemical and herbal (turmeric extract) irrigant is based on the totality of the human intervention studies. These outcomes are substantiated by experimental studies that elucidate the molecular mechanisms of the herbal extract and some of its individual components. In this regard, the knowledge on the mode of action of flavanols in general parallels and supports as irrigant are more refined and prepared specifically insights into the physiological processes has been described in the studies on the herbal product that embody the specific monomeric and oligomeric flavanols extract. In the quest for unravelling the molecular action of herbal extracts that could be developed as an ideal irrigant in the following generation of endodontic therapy. Studies are ongoing to enhance our existing knowledge and understanding in the intricately related fields of pulpal diseases. The prepared pleiotropic effect of turmeric extract explains why and how it can be applied as an antimicrobial remedy as well as a non-toxic material in the field of endodontic.

Within the limitations of the study, it can be concluded that turmeric extract demonstrated antimicrobial activity against E,faecalis, and C.albicans, Since the
study is qualitative analysis further testing needs to be done to final quantitative analysis of the antimicrobial activity of turmeric extract. Henceforth, further dilution studies need to be carried out to find out the better activity of turmeric extract. This study warrants the use of herbal-based non-irritant non-toxic irrigant in place of chemical ones. Our team has extensive knowledge and research experience that has translate into high quality publications [29-48]

References


