



HERBAL EXTRACTS TO ERADICATE ENTEROCOCCUS FAECALIS – AN IN-VITRO STUDY

Dental Science

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ABSTRACT

Enterococcus faecalis is one of the most prevalent pathogen isolated from root canals of teeth where previous treatment has failed. Thorough debridement of root canal space is a significant objective for complete reduction of micro-organisms. Intracanal irrigants have been used as an adjunct to intensify antimicrobial effect of cleaning and shaping in endodontics. The antimicrobial efficacy of herbal extracts as endodontic irrigants is evaluated and compared with the standard irrigant sodium hypochlorite and chlorhexidine against clinical strains of enterococcus faecalis. The antimicrobial assay was determined by measuring the mean zone of inhibition (in mm) by agar well diffusion method. Results showed that chlorhexidine and sodium hypochlorite showed the maximum anti-bacterial efficacy. Acacia catechu showed significant anti-microbial activity whereas betel vine showed the least. Thus, it can be used as a probable root canal irrigant for prevention of re infection.

KEYWORDS

acacia catechu, enterococcus faecalis, irrigants.

INTRODUCTION

The utmost objective of root canal treatment is to remove the microorganisms as completely as possible. Chemo mechanical preparation with proper irrigation is essential for achieving a microorganism free root canal space. However, with the materials and methods currently available it is still unachievable, and the remaining microorganisms can lead to reinfection of the root canal space. The most commonly associated pathogen with re-treatment and non-healing cases is Enterococcus faecalis.

E.faecalis is a gram positive, facultative anaerobic cocci, which can survive very harsh environments including extreme alkaline pH(9.6) and salt concentrations. It is found in 4-40% of primary endodontic infections and 24-77% in root filled teeth with periradicular infections. E.Faecalis can adhere to root canal walls and form biofilm which enables the bacteria to become more resistant to phagocytosis and antimicrobials. They can survive starvation by genetic polymorphism and contains various virulent factors.²

In dental practice to eliminate E.faecalis, 5% NaOCl and 2% CHX are commercially used. NaOCl is a standard irrigant of choice widely used for root canal disinfection owing to its tissue dissolving property and antimicrobial activity. But, it is extremely toxic to periapical tissues³, is unable to dissolve smear layer and has detrimental effect on dentin elasticity and flexural strength.

Chlorhexidine is widely accepted intracanal irrigant due to its wide spectrum of antimicrobial activity, substantivity and relatively low toxicity but it causes staining of teeth, alteration in taste and presence of inflammatory exudate and killed microorganisms inhibit the action of CHX in root canal⁴. Due to these disadvantages, the trend of recent medicine attends to use of herbal medicine as efficient alternatives to conventional irrigants.

Herbs have been used by humans since time immemorial for its anti-inflammatory, antibiotic and antimicrobial property suggesting their ability to be used as endodontic irrigant.

Acacia catechu is widely used in Ayurveda, mainly for treating skin diseases. It has powerful antioxidant and astringent activities. The

main chemical constituents are catechin, epicatechin, epigallocatechin, epicatechin gallate, lupenone, procyanidin, kaemferol, afzelchin gum, minerals etc.⁵

Myristica fragrans (Nutmeg) contains myristic acid which is responsible for its antimicrobial activity. Yanti et.al, reported that macelignan isolated from nutmeg has antibiofilm activity against oral bacteria.⁶

Piper betel is used in the Indian traditional system of medicine for its antioxidants and anti-microbial properties. The leaves contain a multitude of biophenolics such as hydroxychancicol, eugenol, charibetol, and piperols.⁴

Hence, we attempted to compare the antibacterial efficacy of Acacia, Myristica and Piper betel extracts against E.faecalis.

Materials and methods

Objective:

The purpose of this study is to assess the susceptibility of E.Faecalis to three traditional indian plants by employing agar well diffusion method.

Collection of plant extracts:

100% aqueous extracts of acacia catechu, piper betel and myristica fragrans were obtained from Greenchem herbal extracts, Bangalore.

Test microorganism:

Enterococcus faecalis was obtained from Department of Microbiology, R.V.Dental College, Bangalore.

E. faecalis was sub-cultured onto blood agar plate and incubated aerobically at 37°C for 24 hrs to obtain a fresh culture which was used at the time of the study.

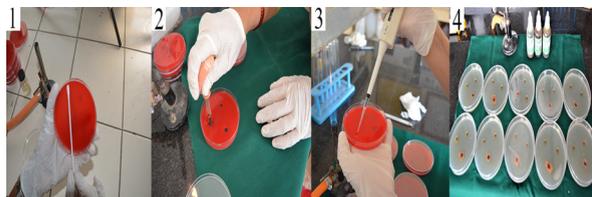
The anti-microbial activity was assayed by Kerby-Bauer agar well diffusion method using Mueller-Hinton and Blood agar plates.

Freshly prepared inoculate with the bacterium in 10ml of sterile peptone water broth was spread evenly on the surface of plates using

sterile cotton swabs.

6 wells (3 plant extracts + 2 positive controls + 1 negative control) of 6 mm diameter were bored into the plates, into which 100ml of the respective test agents were added using a micropipette.

- GROUP 1- 100mg/ml Myristica fragrans
- GROUP 2 – 100mg/ml Acacia catechu
- GROUP 3 – 100mg/ml 2% Chlorhexidine gluconate
- GROUP 4 – 100mg/ml 3% Sodium hypochlorite
- GROUP 5 – 100 mg/ml saline
- GROUP 6 – 100mg/ml Piper Betel

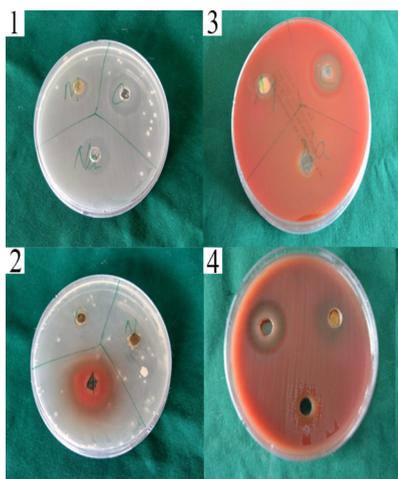


1. Spreading of the bacterial inoculate on the blood agar medium 2. Preparing wells in the agar layer 3. Adding the test materials in the wells 4. agar plates with the extracts

Each test group was assayed ten times under aseptic conditions with the inoculum derived from the initial pure culture.

The plates were incubated at 37°C for 24 hrs, after which the plates were examined for clear zones of bacterial inhibition.

Clear zones within which the bacterial growth was absent were calculated as the zones of inhibition using digital calliper. The size of the zone of inhibition ascertains the effect of extracts in inhibiting the bacteria. The wider the zone, better the effectiveness of the test agent.



Zones of inhibition clearly visible

Results

On Blood Agar plate, mean zone of inhibition of group 2% Chlorhexidine (22.80+2.36) was highest followed closely by group Acacia Catechu (21.06+0.45) with Betel Vine (11.06+0.59) being the least.

On Mueller Hinton Agar plate, mean zone of inhibition of group 3% Hypochlorite (24.86+2.06) was highest amongst all the irrigants followed closely by group 2% Chlorhexidine (23.33+2.99) and group Acacia Catechu (21.33+0.61) with Betel Vine (10.80+0.67) being the least.

The Mean and Standard deviation values of mean zone of inhibition for Enterococcus Faecalis exhibited by the different groups in Blood Agar and Mueller Hinton Agar plate are listed in Table 1 and Table 2, respectively.

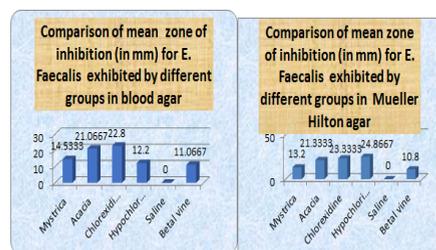
Tukey's post Hoc analysis test was used to assess the level of significance.

Table 1: Comparison of mean zone of Inhibition [in mm] for E.Faecalis exhibited by different groups in Blood Agar using one-way ANOVA test

| Groups | N | Mean | SD | Min | Max | F | P-Value |
|--------------|----|---------|---------|-------|-------|---------|---------|
| Mystrica | 15 | 14.5333 | 1.40746 | 13.00 | 18.00 | 724.390 | <0.001* |
| Acacia | 15 | 21.0667 | .45774 | 20.00 | 22.00 | | |
| Chlorexidine | 15 | 22.8000 | 2.36643 | 18.00 | 26.00 | | |
| Hypochlorite | 15 | 12.2000 | .41404 | 12.00 | 13.00 | | |
| Saline | 15 | .0000 | .00000 | .00 | .00 | | |
| Betel vine | 15 | 11.0667 | .59362 | 10.00 | 12.00 | | |

Table 2 : Comparison of mean zone of Inhibition [in mm] for E.Faecalis exhibited by different groups in Mueller Hinton Agar using one-way ANOVA test

| Groups | N | Mean | SD | Min | Max | F | P-Value |
|--------------|----|---------|---------|-------|-------|---------|---------|
| Mystrica | 15 | 13.2000 | .67612 | 12.00 | 14.00 | 558.946 | <0.001* |
| Acacia | 15 | 21.3333 | .61721 | 20.00 | 22.00 | | |
| Chlorexidine | 15 | 23.3333 | 2.99205 | 13.00 | 26.00 | | |
| Hypochlorite | 15 | 24.8667 | 2.06559 | 19.00 | 27.00 | | |
| Saline | 15 | .0000 | .00000 | .00 | .00 | | |
| Betel vine | 15 | 10.8000 | .67612 | 10.00 | 12.00 | | |



DISCUSSION

The primordial cause of failure of the endodontic treatment is the endurance of microorganisms in the apical portion of root filled teeth. Our challenge as endodontist is to accomplish effective ways of debridement of the diseased pulpal space to prevent the tooth from being a source of infection. It is well documented that instrumentation alone cannot serve the purpose; antibacterial irrigants and interappointment intracanal medicaments are significant inclusions required for root canal disinfection. The irrigants aid in cleaning of the root canal space by dissolving necrotic pulp tissue remnants, lubrication of files, inactivates endotoxin and flushes out debris.

Enterococcus faecalis was chosen in the present study since it is a persistent organism accountable for the presence of periradicular lesion post root canal therapy. Use of good aseptic techniques, increased apical preparation size and 2% chlorhexidine coupled with sodium hypochlorite is presently the most potent method to encounter enterococcus faecalis within root canal system. But the indiscriminate use of antimicrobial drugs has led to development of microbial resistance world-wide. In addition to it, it also causes toxic side effects. Therefore, researchers have made constant efforts to explore new source of anti-microbials of plant origin. Several studies have been undertaken to evaluate the effect of herbal irrigants in eliminating Enterococcus faecalis to improve the success of treatment. Approximately, 25 to 50% of current pharmaceuticals are derived from plants. Botanically derived drugs have gained popularity because of its several advantages: fewer side effects, cheaper alternatives, improved tolerance and better acceptance since time immemorial. During their life cycle, plants face various infectious agents such as bacteria, virus, and fungi. In response to the former, they develop a variety of secondary metabolites capable of destroying them.

This research was undertaken to determine the antibacterial efficacy of plant extracts as root canal irrigants. Aqueous extracts of three traditional plants were chosen to be compared with the standard irrigants, sodium hypochlorite and chlorhexidine. It should be highlighted that very few times in literature have the herbal extracts been assayed as irrigants against enterococcus faecalis.

Blood agar and Mueller hinton agar plates were used in this study to carry out the agar well diffusion method. Blood agar is a differential media used to identify and isolate specific bacteria by studying the haemolytic reaction and enterococcus faecalis exhibits gamma haemolysis. Also, Enterococcus faecalis grows well on Mueller hinton agar plate and gives accurate susceptibility.

Acacia catechu was selected due to its proven antibacterial efficacy against oral bacteria credited to the presence of epigallocatechin, rectin and high tannin content. Acacia catechu wild (family: Fabaceae; subfamily: mimosoidae) is a potent medicinal plant which has found popular use in Ayurveda for multiple diseases (skin diseases). It is commonly used in Kerala as boiled khadira water for drinking purpose. It has also been tried as a topical therapy for sore gums and mouth ulcers. Commonly called as "babul" in India. The extracts exhibit varied pharmacological effects like anti-pyretic, anti-inflammatory, anti-diarrhoeal, hypoglycaemic, antioxidant and antimicrobial. Although it was less potent than chlorhexidine but it has relatively comparable effectiveness in inhibiting the endodontic pathogen in vitro without staining.

The aqueous extracts from myristica and betel vine were very less effective as compared to acacia catechu. Further studies using increased concentrations and the type of extracts may be needed to check their effectiveness.

Results obtained are consistent with the data from literature. A study conducted by yuvraj et al, where acacia catechu was tried against enterococcus faecalis and streptococcus mutans showed potent activity. Similarly, study done by Kumar et al where acacia catechu had significant anti-bacterial influence against acidogenic oral bacteria.

Evaluation of biocompatibility of the chosen extracts was not included as a part of the study. Hence a factual opinion on its toxicity and allergic potential cannot be made. The design criterion did not investigate the ideal properties required to be a root canal irrigant apart from antimicrobial assay. Further research studies are needed to check for the effectiveness of plant extracts against other pulpal pathogenic organisms with respect to varying concentrations and pH.

Thus, trials like these shall result in clinical validation of plant extracts in combating enterococcus faecalis. Clinical evidence of efficacy can then be used in marketing herbal extracts as therapeutic agents.

Conclusion

In conclusion, the results obtained confirm the anti-microbial effectiveness of acacia catechu. The observations form the basis for further phytochemical studies that can check for a range of bacterial strains and can be processed as safer cheaper alternatives for masses.

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Nil

Conflicts of interest

There are no conflicts of interest.

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