In-vitro antimicrobial efficacy of aqueous extract of Areca nut against Enterococcus faecalis

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ABSTRACT

Background and Aim: Successful root canal therapy relies on the combination of proper instrumentation and effective irrigation of the root canal system. The aim of the study was to evaluate out the antibacterial efficacy of areca nut extract and chlorhexidine against E. faecalis. Materials and methods: Areca nut extract was prepared by extraction of 10g dry powdered areca nut with 250ml distilled water for 1 hour. The extract was filtered through a sintered glass funnel, freeze dried and frozen at -70°C. Antimicrobial activity of the areca nut extract was tested by using the agar diffusion and serial dilution method. Results: The areca nut and CHX significantly inhibited the growth of E. faecalis at a dose dependent manner. Conclusion: The areca nut showed potential antibacterial activity against E. faecalis, when compared to CHX.

Key words: Agar Diffusion, Areca nut, Chlorhexidine, E. faecalis.

INTRODUCTION

Primary root canal infection is caused by microorganisms colonizing the necrotic pulp tissue and have been recognized as the primary etiological factors in the development of pulpal and periapical infections. This cleaning of the root canal is carried out by mechanical shaping and chemical disinfection, using a combination of endodontic instruments and chemical agents known as irrigants. These irrigants augment mechanical debridement by flushing out necrotic pulp debris and removing the majority of infected microorganisms from anatomical complexity and inaccessible areas of the root canal system culminating in the long term success of the endodontic therapy (Kandaswamy & Venkateshbabu, 2010). Enterococcus faecalis is the most common and dominant microbial species and sometimes the microorganism isolated from failed endodontic treatment cases and teeth with persistent periodontitis (Stuart, 2006). They are facultative anaerobes and are usually present in asymptomatic cases also. Cleaning of the root canal and the dentinal tubule system as well as proper filling of the canal are essential procedures for the success of the root canal treatment. Chlorhexidine (CHX) has been used as an irrigant in disinfection because of its excellent antimicrobial activity against both gram positive and gram-negative microbes and its adsorption ability to dental hard tissues (Dametto, 2005). CHX has been a promising agent to be used as intracanal medicament (Wang, 2007; Kandaswamy, 2010). Areca nut or betel nut are the seeds of betel palm that are grown in India, Malaysia, Taiwan and many other countries for their economically important seed crop (Nethravathi, 2010). Dried arecanuts are said to strengthen gums, sweeten breath, remove bad taste and produce a stimulant and exhilarant effect. It has also been reported that areca nut exerts a direct antimicrobial effect against oral bacteria including Streptococcus mutans, Streptococcus salivarius, Candida albicans and Fusiform nucleatum (Cyriac, 2012). Tannic acid at concentrations varying from 1.8-18mg/ml inhibited growth of E. corrodens, Porphyromonas gingivalis, C. rectus and Fusiform nucleatum (Hung, 2005).

To our knowledge no study has been done to evaluate the antibacterial efficacy of areca nut against the most common organism found in endodontic infections. Thus the purpose of this study was to find out the antibacterial efficacy of arecanut extract and
chlorhexidine against *E. faecalis* by agar diffusion and serial dilution method.

**MATERIALS AND METHODS**

Two tests were carried out to determine the antibacterial activity of areca nut and CHX. The stock concentrations of areca nut and CHX were 20mg/ml in water +2% DMSO.

**Preparation of the areca nut extract:** Areca nut extract was prepared by extraction of 10g dry powdered areca nut with 250ml of distilled water for 1 hour. The extract was filtered through a sintered glass funnel, freeze dried and frozen at -70°C.

**Serial Dilution Method:** In this test, the minimum inhibitory concentration (MIC) of the test materials against *E. faecalis* were measured by broth micro dilution method (National Committee for Clinical Laboratory Standards, 1999). An overnight culture of *E. faecalis* (MTCC 3159) was harvested in Mueller Hinton broth (MHB) and the concentration was adjusted to optical density of 0.11 at 570 nm. The MIC test was carried out in 96 well microtitre plates containing 100 µl broth. An inoculate of the *E. faecalis* (5 µl/well), containing 10^8 CFU/ml of bacteria, in the presence of different concentration of extract ranging from 2.0 mg/ml to 0.031 mg/ml was used to determine the MIC of the samples by the serial dilution method. The first well (175 µl broth + 5 µl/well bacteria) received 2.0mg/ml concentration of areca nut and CHX (20 µl from stock). Then 100 µl of the solution from the first well was added to the next well (95 µl broth + 5 µl/well bacteria) and mixed completely. This process was performed serially till the last well. Finally, 100 µl from the last well was discarded. Each extract was made a negative control that is without isolate inoculation. Plates were incubated at 37°C for 24 h. MIC was assessed by determining the inhibition of growth of *E. faecalis* at the lowest concentration compared to uninoculated extract well. All the tests OD was done in duplicates and was compared along with the negative control.

**Disc diffusion method:** In the next experiment, zone of inhibition on plates inoculated with *E. faecalis* was investigated to determine the extent of antibacterial activity of areca nut and CHX using disk diffusion method (Jorgensen, 2003). An overnight culture of *E. faecalis* (MTCC 3159; 10^8 CFU/ml of bacteria) was standardized to 0.11 OD measured at 570 nm. *E. faecalis* was spread onto a MHB plate using an L-shaped glass rod and discs were placed on the plates. The different concentration of extract ranging from 2.0 mg/ml to 0.031 mg/ml was added to the discs (20 µl). Sterile saline was used as negative control group. Three replicas were prepared for each sample and the plates were incubated overnight at 37°C for 24 h and the zone of inhibition were measured in millimeters.

**RESULTS**

**Serial Dilution method:** The results of MIC are listed in Figure 1. The MIC values of the tested materials ranged between 2.0mg/ml to 0.031mg/ml. The samples areca nut and CHX significantly inhibited the growth of *E. faecalis* at a dose dependent manner. In the CHX group, 0.031mg/ml concentration inhibited 0.515% of the growth of *E. faecalis*. In the areca nut group, 0.031mg/ml concentration inhibited 8.5% of the growth of microbes after 24h incubation. Thus areca nut showed potential antibacterial activity against *E. faecalis*, when compared to CHX. There were no results obtained on negative control wells.

**Disc diffusion method:** The CHX showed antibacterial activity against *E. faecalis* at four concentrations of 2, 1, 0.5, 0.25mg/disc. The zone of inhibition ranged from 7 to 18 mm in active concentrations of CHX. Whereas in the areca nut group, all the concentrations ranging from 2mg/disc to 0.062 mg/disc showed significant inhibition of the growth of microbes after 24h incubation. Thus areca nut showed potential antibacterial activity against *E. faecalis*, when compared to CHX. There were no results obtained on negative control wells.

![Box plots of the percentage inhibition of different concentrations of areca nut extract and chlorhexidine against *E. faecalis*, which illustrate the mean ± standard deviation of minimum and maximum percentage of inhibitions, as well as the variance in each experimental group](image-url)
DISCUSSION

In this study, antibacterial effect of areca nut extract on E. faecalis was evaluated and compared with that of chlorhexidine. Two standard and routine microbiological tests, agar diffusion and serial dilution were used in this study. In agar diffusion test, the effectiveness of antibacterial material against bacteria is measured in a grown culture. In this method, the diameter of the microbial inhibition zone depends on the solubility and infusibility of the test material. Direct and close contact between the microorganisms and the samples are examined by the direct contact tests, independent of the diffusion properties of the tested material and media, which is an advantage over other tests similar to agar diffusion method (Estrela, 2003). The serial dilution test utilizes serial dilutions of a solution to determine the lowest concentration of the test material that would still show antibacterial properties. E. faecalis was selected because this is the most commonly isolated microorganism in teeth with apical periodontitis that go in for endodontic therapy (Stuart, 2006).

Warnke et al evaluated the antibacterial and antimycotic efficacy of Thyme white oil, Lemon oil, Lemongrass oil, Cinnamon oil, Tea tree oil, Eucalyptus oil, Grapefruit oil, Clove bud oil, Lavender oil, Peppermint oil, Sage oil, Kunzea oil and Sandalwood oil against frequently isolated hospital acquired bacterial strains including MRSA and yeast isolates, including C. krusei by using agar diffusion method. The largest effective zones were measured for Thyme white oil, Lemon oil, Lemongrass oil and Cinnamon oil. These four essential oils showed consistent antibacterial effects against all bacterial strains tested. They proved that essential oils will be alternative for the treatment of localized infections even with severe hospital acquired strains (Warnke, 2009). In our study areca nut showed better antibacterial efficacy against chlorhexidine. The zone of inhibition ranged from 8 to 14 mm in areca nut group and from 7 to 18 in CHX group at concentrations ranging from 2mg/disc to 0.062 mg/disc and 2 to 0.25mg/disc respectively. From the results of our study we prove that areca nut can be used as an antibacterial agent during root canal treatment. The reason for the antibacterial efficacy might be due to its inherent astringent property in addition to the presence of flavonoids and tannins in it.

CONCLUSION

Within the limitations of the study areca nut possess antibacterial efficacy against E. faecalis. Future studies in tooth models are warranted to advocate its use in in-vivo conditions.

REFERENCES


