Chiang Mai Dental Journal

Original Article

Investigation of Antimicrobial Properties of *Mangifera indica L.* Kernel Extract on Multispecies *Enterococcus faecalis-Candida albicans* Biofilm in an *Ex Vivo* Tooth Model

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Received: February 8, 2021 • Revised: February 25, 2021 • Accepted: March 10, 2021

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Abstract

Objectives: This study aimed to assess the antimicrobial efficacy of kernel extract derived from *Mangifera indica L.* grown in Northern Thailand (Kaew-Moragot cultivar) against multispecies *Entercoccus faecalis-Candida albicans* biofilms in tooth model.

Methods: Agar diffusion and broth microdilution methods were performed to investigate the antibacterial activities of *M. indica* kernel extract against planktonic *E. faecalis* and *C. albicans*. Time-kill assay was conducted to evaluate time-point of the extract which showed bactericidal and fungicidal effects. Antimicrobial activity of *M. indica* kernel extract against *E. faecalis* and *C. albicans* biofilms was investigated through the use of 25 mg/ml extract solution and compared to traditional root canal medicaments. In order to form biofilms in root canal, the microbial suspension of *E. faecalis* and *C. albicans* in 100:1 was placed in the root canal, with the addition of media every other day for 21 days. The tooth samples were divided into 3 groups (n=40): 0.009 mg/ml normal saline solution (as a negative control), 400 mg/ml calcium hydroxide, and 25 mg/ml *M. indica* kernel extract. The root canal medicaments were consequently applied onto the biofilms for 7 days. After 7 days, cylindrical steel burs with a diameter of 1.6 mm and 1.8 mm were used to collect samples from inner dentin and deeper dentin. The remaining tooth samples were collected as residual roots. The samples were cultured for 24 hours, and 200 µl of suspension were place into 96-well microtiter plates for spectrophotometrically testing. The optical density (OD) data were analyzed with two-way ANOVA and Tukey's post hoc tests ($p<0.05$).

Results: *M. indica* kernel extract demonstrated the antimicrobial activity against multispecies *E. faecalis-C. albicans* in root canal. The extract was the most effective in eliminating the biofilms by 30.88% ($p<0.001$) in inner dentin layer. It was comparable to calcium hydroxide. In residual root, the eliminating effects of calcium hydroxide and *M. indica* kernel extract were comparable with percent inhibitions of 30.45% and 26.99%, respectively.

Conclusions: *M. indica* kernel extract had potential to eliminate multispecies *E. faecalis-C. albicans* biofilms up to the residual root. The antimicrobial activity of the extract was comparable to calcium hydroxide, which is a gold standard medicament used in root canal treatment.

Keywords: *M. indica* kernel extract, multispecies biofilm, root canal medicament

Introduction

Endodontic diseases are infection of the root canal system. Microorganisms play essential role in the progression and perpetuation of pulpal and periapical pathologies.[1,2] Oral microbes gain access into the root canal system and form sessile microbial communities called biofilms in the root canals.[3-5] Infections can propagate from the root canals to the periodontal ligament and bone beyond the root apex, ultimately resulting in apical periodontitis. In cases of failed endodontic treatment and canals with persistent infections, *Enterococcus faecalis* and *Candida albicans* have typically been identified.[6] The complex structure of the biofilm allows some degree of co-operation to develop between the species populations.[7,8]

Antimicrobial irrigating solutions and medicaments are used to eradicate microorganisms from root canal system. Broad spectrum and high efficiency antimicrobial effect against microorganisms in biofilms are the ideal properties of a root canal medicament. Moreover, the medicament should be biocompatible and non-caustic to periodontal tissues.[9] Currently, calcium hydroxide ($Ca(OH)_2$) paste is the most widely used inter-appointment medication in endodontics.[10] The mode of action of calcium hydroxide is based on its alkalinity, but there are conflicting results of its antimicrobial activity.[11] For example, gram-positive bacteria, especially *E. faecalis*, have been reported to survive in an alkaline environment.[12] Moreover, potential tissue toxicity and allergic reactions are of concern.[13] Nowadays, there are attempts to find an alternative agent from natural sources which possess both antimicrobial activity and exert minimal tissue-irritating effect.

Mangifera indica L. (M. indica) or mango is a local fruit in Thailand and also in tropical countries.[14,15] This plant belongs to genus *Mangifera*, which exists several species of tropical fruits in the plant family Anacardiaceae. *M. indica* has numerous cultivars, which shown the specific characteristics, depending upon a growth environment.[16] The kernel from mango consumption enveloped by seed shell has been used as a traditional medicine for centuries.[17] Decoction of the kernel has been shown to possess biological activities including antioxidant, anti-inflammatory, and anti-allergy properties.[18-20] Moreover, *M. indica* kernel extract has antimicrobial activity against a wide range of microorganisms.[21,22] Due to these properties, *M. indica* kernel might be a promising source for new root canal medicament. In addition, no previous study of Thai *M. indica* kernel was found on the antimicrobial activity against *E. faecalis, C. albicans* and multispecies biofilms. The aim of the present study was to determine the activity of *M. indica* kernel extract on preformed multispecies biofilms of *C. albicans* and *E. faecalis* in an *ex vivo* tooth model.

Materials and Methods

This study was approved by the Human Experimentation Committee of the institution where the study was conducted (Reference number 6/2019).

Media

RPMI-1640 (R0883, Sigma-Aldrich, Missouri, USA) with 2.0 g/l D-glucose was prepared with morpholinepropanesulfonic acid (MOPS) (Oxoid, Basingstoke, UK) and buffered to pH 7.4. The medium was filtered through a 0.22 μm membrane filter (Sigma-Aldrich, Missouri, USA) and stored at 4°C. The final concentrations of RPMI-1640 used to prepare single species and multi-species biofilms were single strength (1x) and quadruple strength (4x), respectively.

Plant materials and extract preparation

The kernels of raw fruits from *M. indica,* Kaew-Moragot cultivar were derived from a mango processing factory in Lamphun Province, Thailand during the period of May to September, 2020. The samples of tested kernels were then collected and dried at a temperature of 45°C for 24 hours using a hot-air oven. Extraction was carried out following the method of Poomanee *et al.*[22] Briefly, wax compounds were firstly eliminated using hexane extraction for 48 hours, in 3 cycles. The plant residue was then macerated with ethyl acetate using the similar method as hexane extraction. The mixture was then filtrated and the plant residue was fractionally macerated using 95%v/v ethanol in deionized (DI) water. Consequently, ethanol was evaporated using a rotary evaporator to produce the ethanolic *M. indica* kernel extract. The extract was stored at 4°C until usage. Quality control procedure of the extract was performed using high performance liquid chromatography (HPLC) for analyzing fingerprints and gallic acid content.

Culture conditions and formation of multispecies biofilms

The mixed species inoculum was prepared by growing planktonic cultures of *E. faecalis* ATCC 29212 and *C. albicans* ATCC 10231. For *E. faecalis*, one colony was inoculated into 20 ml LB broth (Lennox, Massachusetts, USA) and grown overnight at 37°C in a shaking incubator (at 250 rpm maximum). The cells were harvested by centrifugation (4,500 rpm for 5 minutes), washed twice with sterile phosphate-buffered saline (PBS) (Sigma, Missouri, USA) and then re-suspended in RPMI-1640. The cell density was measured by using a spectrophotometer and adjusted to Optical density; OD_{600} = 0.1, equivalent to $1.0x10^8$ cells/ml. For *C. albicans*, the isolate was cultured on Sabouraud Dextrose (SAB) agar (Oxoid, Basingstoke, UK) and incubated at 37°C for 48 hours before being used to check viability and purity (by culture and microscopy, respectively). One colony was then inoculated into 20 ml yeast peptone dextrose (YPD) broth (Melford, Ipswich, UK) and grown overnight at 37°C in the shaking incubator as detailed above. The cells were harvested by centrifugation (3,000 rpm for 5 minutes), washed twice with sterile PBS and then re-suspended in RPMI-1640. To produce standardized biofilm inoculum, the cell density was measured by using a Neubauer hemocytometer and adjusted to $1.0x10^6$ cells/ml. The culture suspensions of the two microorganisms were mixed in 1:100 of *C. albicans*: *E. faecalis* proportions to create the multispecies biofilms inoculum. The suspensions were incubated in an anaerobic incubator (5% CO_2) at 37°C to develop into biofilms which were then grown for 48 hours before exposure to reagents.

Susceptibility testing

Agar diffusion assay was used to determine the antimicrobial activities of the extracts followed method recommended by the clinical and Laboratory Standards Institute (CLSI).[23,24] Briefly, *C. albicans* and *E. faecalis* suspension was diluted by thioglycollate broth to a final concentration of approximately $1x10^6$ CFU/ml (OD_{600} = 0.1). The diluted bacterial suspension (200 ml) was streaked onto Mueller-Hinton agar. The diluted fungal suspension (200 ml) was streaked onto Mueller-Hinton Agar + 2% Glucose and 0.5 μg/ml Methylene Blue Dye (GMB). The agar was excavated and each sample, dissolved in 25%v/v ethanol in culture broth at a concentration of 50 mg/ml (50 μl), was added into each well (6 mm diameter). The inoculated plates of *C. albicans* and *E. faecalis* were incubated at 37°C, under anaerobic condition for 24 hours. The diameter of the inhibition zone was measured in millimeters using digital vernier caliper (Insize Co., Ltd., USA). Chlorhexidine in 0.02 mg/ml concentration was used as positive controls. Normal saline solution served as a negative control. The experiments were performed in triplicates.

Broth microdilution methods were used to determine the susceptibility of the planktonic *C. albicans* and *E. faecalis* to the extracts. The minimum inhibitory concentration (MIC) was read spectrophotometrically after 24 hours of incubation. Microbial suspensions (20 μl) were added to broth (180 μl) in 96-well cell-culture microtiter plates (F96 MicroWell Plates, NUNC, ThermoFisher Scientific, Leicestershire, UK). Microbial growth was determined by a spectrophotometer Multiscan RC analyser (ThermoFisher Scientific) at 490 nm. The inhibitory concentration of *M. indica* kernel extract that prevented 50% of the bacterial or candidal growth was determined by changes in optical density. In addition, the microbiocidal activity of *M. indica* kernel extract was tested after MICs were determined by streaking 20 μl of the test suspensions from representative wells onto agar and incubating for 48 hours; the results were shown as minimum bactericidal and fungicidal concentration (MBC/MFC). All samples were analyzed in triplicates within separated experiments.

In vitro time-kill assay

Cultures of *C. albicans* and *E. faecalis* grown overnight in brain heart infusion (BHI, Sigma-Aldrich, Dorset, UK) broth at 37°C with shaking at 250 rpm were centrifuged at 2500 rpm for 10 min and washed with phosphate buffer saline (PBS) and finally suspended in BHI medium. The *M. indica* kernel extract (at 1/2MIC, MIC, 2MIC, and 4MIC concentration) and a control (sterile water) were incubated with an adjusted amount of each species equal to 1.5x108 CFU/ml in BHI medium in 96-well plates. Cells were harvested at 0, 4, 8, 12, 24, 48, 72, 96, 120, 144, 168 hours. The cells were also harvested every day until day seven after inoculation. Two-fold serial dilutions were prepared and 10 µl of each dilution was spread on BHI agar plates. After overnight incubation at 37°C, the number of colony forming units (CFUs) was counted. The

experiments were performed in triplicates within separated experiments. The time-kill curve of *M. indica* kernel extract was calculated and expressed as time v.s. - $\log_{10}$ survivor curves.

Tooth collection, storage, and sterilization

One hundred and twenty permanent human single-rooted teeth were collected and were stored in 0.1% (v/v) thymol solution at room temperature. The teeth were extracted as part of orthodontic treatment and the collection of the extracted teeth was approved by the Ethics Committee of the Faculty of Dentistry, Chiang Mai University, Thailand. Written consent for collection was obtained from the patient or their guardian. The inclusion criteria stated that only single root canal teeth with no root curvatures or fractures were collected, and no oval root canal-shaped teeth were selected. All teeth were autoclaved for 20 minutes at 15 psi; this was to ensure that all samples were free from infection and that viable DNA was eliminated. The external root surfaces were debrided of bone, calculus, and soft tissue using a No. 15 Surgical Scalpel Blade (Swann-Morton, Sheffield, UK). Teeth were cut with a diamond disc (Cookson Dental, Birmingham, UK) into six-mm-long and at least four-mm-wide standard root blocks. Root canals were enlarged with Ash Steel Bur No. 4 (Henry Schein Dental, Cardiff, UK) drills under water-cooling to a 1.4 mm diameter to standardize the samples.

Smear layers were removed from the canal walls in an ultrasonic bath with 17% (w/v) EDTA and 0.5% (v/v) NaOCl, 5 min for each. The blocks were autoclaved at 121ºC for 30 min in 3 ml BHI broth. They were then sonicated in fresh broth in an ultrasonic bath for 15 min and finally cultured at 37ºC and 5% CO_2 for 24 hours to confirm sterility. The root canals were dried with sterile paper points (Orbis, Pontault-Combault, France), and the outer surface of the root blocks were covered with nail varnish (Rimmel London Ltd., London, UK) to avoid dehydration during the experiment. The apical-ends of root blocks were sealed with Tenatex pink toughened modelling wax. The blocks were stabilized in a vertical orientation with the root canal opening at the top and embedded firmly into putty-type silicone impression material (Henry Schein Dental, Cardiff, UK) in the wells of sterile 24-well plates (Costar, Corning Incorporated, Corning, NY, USA) in a random order. By embedding in this way no liquid escaped through the bottom of the root canal. The plates were covered with sterile lids to ensure sterility throughout incubation.

Multispecies biofilm formation in a tooth model

Overview of tooth model is shown in Figure 1. The root canals were filled with the multispecies suspensions containing each of $1.5x10^6$ CFU/ml *C. albicans* and $1.5x10^8$ CFU/ml *E. faecalis* in BHI using a 26-gauge needle (Sigma-Aldrich, Dorset, UK). Fresh cell suspension was delivered every second day, and pure broth (BHI) was delivered the days in between. All of the root blocks were cultured for 21 days at 37ºC in order to allow biofilm formation and maturation within the canals.

Medication

The antimicrobial activity of the agents was tested in root canals over a 7-day period. The inoculated root blocks were randomly assigned to three groups (n=40). The root canals were irrigated with 10 ml of sterile saline. Vacuum suction was used during irrigation and root canals were dried with paper points. *M. indica* kernel extract was prepared at 25 mg/ml. 400 mg/ml calcium hydroxide-based paste was used as antimicrobial comparator. 0.009 mg/ml sterile saline was used as an inactive negative control for cell growth. All agents were delivered once into root canals, which were then closed with Cavit (3M Espe; Dental Products, Seefeld, Germany) to seal the top of the root canal to avoid evaporation.

Sampling

The root canal surfaces were irrigated with 10 ml of sterile saline and the canals were dried with paper points. The samples of inner and deeper dentine (100 and 200 µm, respectively) were collected separately with a sterile cylindrical steel bur No. 5 (diameter 1.6 mm) and a cylindrical steel bur No. 6 (diameter 1.8 mm) drills, respectively (Figure 2). The drills were moved gently down and up three times through the root canal with low rotational frequency (<1000 rpm). Dentine powder on the surface of the drill was removed by mixing into 2 ml BHI in Eppendorf tubes. The broth containing dentine was vortexed. The residual roots were placed into separate tubes containing the same amount of broth and vortexed carefully before incubation. All samples were cultured for 24 hours at 37°C.

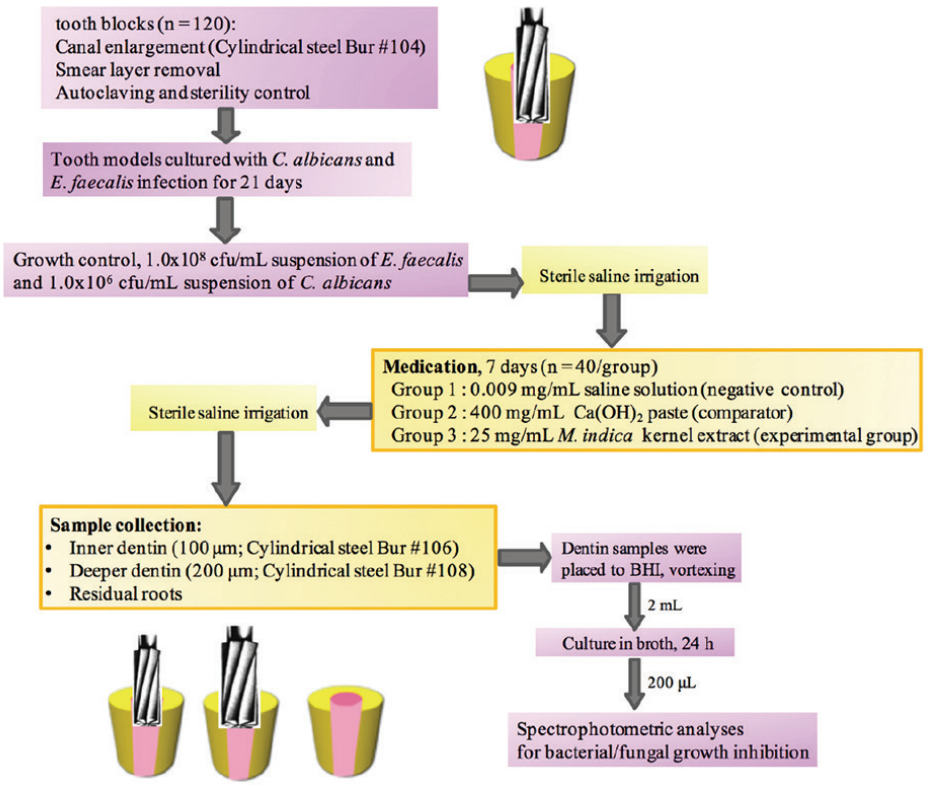

Figure 1: Tooth model; root canals of human dental root blocks were infected with a multispecies biofilm and then exposed to *M. indica* kernel extract, $Ca(OH)_2$, or saline. Spectrophotometric analyses of the resulting cultures in broth were presented as cell growth inhibition.

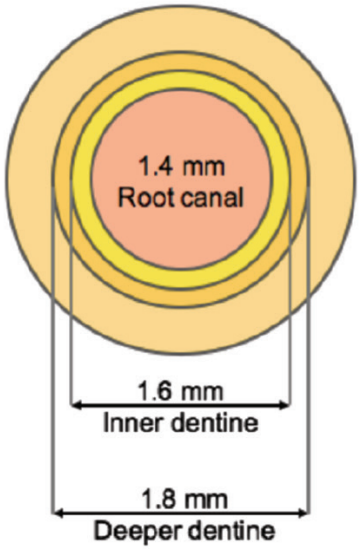

Figure 2: Schematic representation of the circumferential sampling of the dentine. Root canals initially standardized with cylindrical steel bur No. 4 drill were enlarged with cylindrical steel bur No. 5 (inner dentine sample) and cylindrical steel bur No. 6 (deeper dentine sample) drills, and the dentin shavings were collected for analysis of cell growth.

After incubation, 200 μl of each suspension were delivered into 96-well microtiter plates (NUNC; Thermo Fisher Scientific). The changes in optical density (OD_{450}; Multiskan RC, version 6.0; Thermo Fisher Scientific) of the broth, compared to sterile control broth, were detected as cell growth. Based on spectrophotometric analyses, the results were reported as cell growth inhibition (%) with respect to the absence of cell growth of the dentine or residual root suspensions in broth. Culture purity was checked before and after the experiment using a light microscope.

Statistical analysis

The statistical analysis was performed with IBM SPSS Statistics Version 20 (IBM Corp., Armonk, NY, USA). One-way Analysis of Variance (ANOVA) and t-tests were used to investigate significant differences between independent groups of data which followed normal Gaussian distribution. To analyze the multiple comparisons of the data, a Bonferroni correction was applied to the *p* value. The Mann-Whitney U-test or the Kruskal-Wallis test with a Dunn's post-test was used for non-parametric

data to assess differences between independent sample groups. Statistical tests were interpreted at the 5% significance level.

Results

Planktonic susceptibility testing

M. indica kernel extract presented a strong inhibitory effect against *C. albicans* and *E. faecalis* grown planktonically according to their broad inhibition zone (Table 1). However, the antimicrobial activities against all tested microorganisms of the extract were lower than those of chlorhexidine.

Table 1: The activity of *M. indica* kernel extract against *E. faecalis* and *C. albicans* isolates.

Solution	Zone of inhibition (mm ± SD)	
	C. albicans	*E. faecalis*
M. indica kernel extract	14.19±2.45	11.61±1.11
CHX (positive control)	22.19±3.11	15.08±2.36
NSS (negative control)	0	0

The effects of *M. indica* kernel extract on *C. albicans* and *E. faecalis* grown planktonically are summarized in Table 2. The extract shown to have antibacterial and antifungal activities: the MIC for *C. albicans* and *E. faecalis* was 1,250 mg/l and it was bactericidal (MBC) and candicidal (MFC) at 2,500 mg/l.

Table 2: The activity of *M. indica* kernel extract against *E. faecalis* and *C. albicans* isolates.

Organism and phenotype	Destination	kernel extract	
		MIC (mg/ml)	MBC/MFC (mg/ml)
C. albicans	ATCC 10231	1.25	2.50
E. faecalis	ATCC 29212	1.25	2.50

MBC, minimum bactericidal concentration; MFC, minimum fungicidal concentration; MIC, concentration that inhibits 50% of the growth.

Lethal effect against *E. faecalis* and *C. albicans* of the *M. indica* kernel extract by time-kill assays

The *M. indica* kernel extract in concentrations of 1.25, 2.50, 5.00, and 10.00 mg/ml as 1/2MIC, MIC, 2MIC, and 4MIC respectively, were determined for their lethal effects (Figure 3). The viable count of *C. albicans* and *E. faecalis* treated with *M. indica* kernel extract was decreased in dose-dependent manner over time. Generally, 3-log10 reduction in the number of CFU/ml is considered as a bactericidal/fungicidal effect.[25] *M. indica* kernel extract at 4MIC presented stronger bactericidal than fungicidal effect due to its ability to completely kill *E. faecalis* within only 4 hours from log-survivor curves, while the extract at 4MIC reached 3-log10 reduction of planktonic *C. albicans* within 8 hours.

Ex vivo tooth model: spectrophotometric analyses for bacterial/fungal growth inhibition.

M. indica kernel extract significantly inhibited the cell growth in inner dentin and residual roots ($p<0.001$) (Figure 4). In the inner dentin layer, the extract was the most effective in eliminated the biofilms by 30.88% ($p<0.001$) (Figure 4). Also, it was significantly inhibited the cell growth compared to calcium hydroxide ($p<0.001$). The antimicrobial activity of calcium hydroxide and *M. indica* kernel extract was comparable to that of sterile saline at deeper dentine depths. In residual root, calcium hydroxide was the most effective in eliminated biofilm by 30.45% ($p<0.001$) which was comparable to the effect of *M. indica* kernel extract = (26.99%).

Differences in the killing activity of each medicament between dentin depths were significant ($p<0.001$) (Figure 4). The activity of chlorhexidine was better in the deeper dentin than inner dentin or residual roots. However, the activity of calcium hydroxide was better in the residual roots than in the inner and deeper dentin. Whereas the activity of *M. indica* kernel extract was comparable to those medications in inner dentin and residual roots.

Discussion

This is the first study reporting the effectiveness of *M. indica* kernel extract against planktonic *E. faecalis* and *C. albicans*. The selected microorganisms in this study were representative bacterial and fungal in persistent endodontic infection, and have been reported a high prevalence in failed endodontically treated teeth.[26,27] The disc diffusion susceptibility method was used in this study because it is simple and useful for preliminary screening of pure natural substances.[28] The broth dilution method was further used in preliminary screening of potential new antimicrobial agents[29], this method was also used to identify the concentration of the extract that

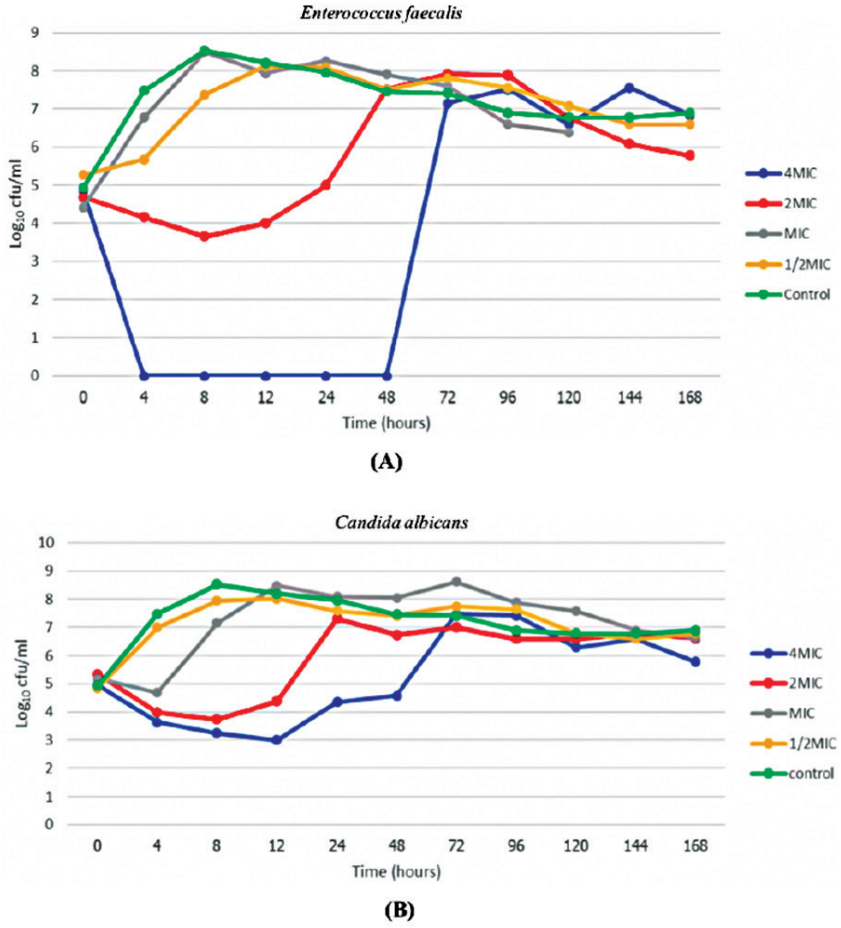

Figure 3: 7 days growth of *C. albicans* (A) and *E. faecalis* (B) in quantitative *in vitro* assays to measure the antimicrobial activity of 1/2MIC, MIC, 2MIC, and 4MIC *M. indica* kernel extract.

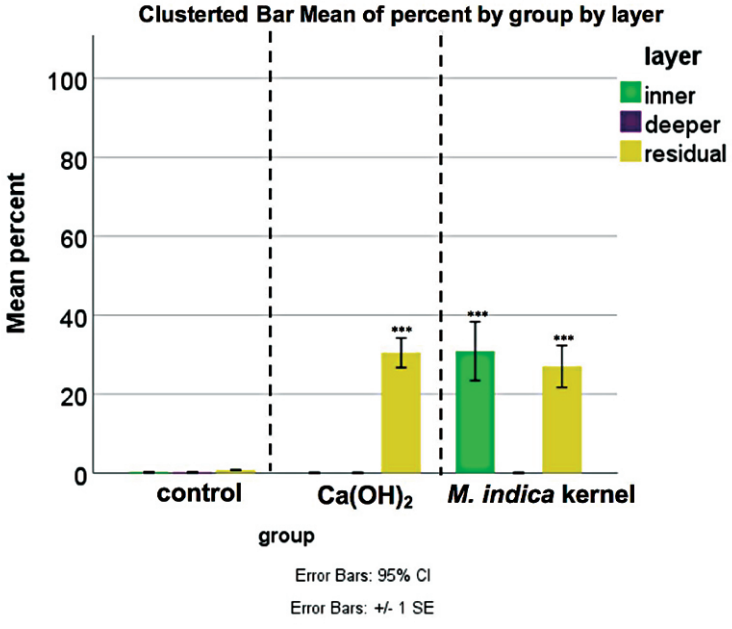

Figure 4: Mean cell growth inhibition (%) compared to negative control in the inner (first 0.1 mm) and deeper (second 0.1 mm) dentine and in the residual roots with standard error of mean (***p<0.001 comparison between the dentine depth).

inhibit the growth of microorganisms.[30] In the present study, *M. indica* kernel extract at the concentration of 25 mg/ml showed bactericidal and fungicidal effect, this concentration was lower than another *in vitro* study tested on *Propionibacterium acnes*.[22] In addition, time-kill assay was used to assess the dynamic interactions between antimicrobial agents and microbial strains and time required for bactericidal or fungicidal effect.[31] *M. indica* kernel extract represented bactericidal and fungicidal effect within 8 hours. However, this study performed the time-kill assay until 168 hours in order to replicate the period of intra-canal medicament in clinical scenario.[32] The development of a multispecies biofilm root canal model provides a platform for understanding the interactions of individual species within a biofilm. Moreover, the evaluation of novel root canal medicaments was accomplished *ex vivo* to mimic the clinical situation. Our study showed that *M. indica* kernel extract was superior to conventional calcium hydroxide in that they reduced the viability of candida and bacteria within biofilms. This study showed that this natural agent has the potential to be used as intra-canal medicaments in endodontic treatment. Moreover, the efficacy of new agent as potential medicaments was shown to mimic the clinical scenario of bacterial and candidal biofilm infections during endodontic treatment. The major consideration was microbial invasion into dentinal tubules; many factors including the nutritional environment, structure of dentine and cell adhesion play an important role.[33,34] Therefore, the inoculation period was set to 21 days to provide sufficient time for bacterial/fungal penetration and biofilm maturation in dentinal tubules.[35-39] In this study, the dentin excavated in the drilled samples showed that *C. albicans* and *E. faecalis* treated were able to migrate into the dentinal tubules. Moreover, *M. indica* kernel extract was able to penetrate and effectively treat these infections compared to calcium hydroxide medicament.

In this study, we additionally showed that saline was a suitable negative control because both *C. albicans* and *E. faecalis* possibly grew after 7 days incubation. Calcium hydroxide, the gold standard of intra-canal medicament, was used as a comparator in this study. The results showed the inferior antimicrobial activity of calcium hydroxide against multispecies biofilms compared to *M. indica* kernel extract in the inner dentine depth. This may be explained by the resistance to calcium hydroxide of *E. faecalis*[40] and *C. albicans.*[41]

The antimicrobial activity of intra-canal medicaments may be naturally lower in the deeper dentinal tubules because of the decreasing in concentration of antimicrobial agents.[42] *Ex vivo* studies have demonstrated that dentine can inactivate the antibacterial activity of calcium hydroxide.[43,44] However, this study showed the better inhibition of cell growth by calcium hydroxide in the residual roots compared to other dentine depths. These findings may be explained by a water-based consistency of calcium hydroxide which can penetrate into the deeper layer of dentine (>200 μm).[45] When the calcium hydroxide particles penetrate into the dentinal tubules they will be in direct contact with microorganisms, and the dissociated calcium hydroxide could continuously dissolve into aqueous form and produce OH^- ions. Therefore, antimicrobial activity will be stronger in the deeper layer. The inhibition of cell growth by *M. indica* kernel extract in residual roots was also comparable to calcium hydroxide and perhaps this is so because it is also water-soluble.

On the other hand, there are some limitations of this study. Firstly, only two isolates of microorganisms were used in the model. In clinical situations, polymicrobial flora with an average of four to seven intra-canal species have been recovered from the necrotic pulpal tissues in primary root canal infections; these microbes were mostly gram-negative obligatory anaerobic bacteria.[46,47] However, this study was focused on the treatment of secondary or persistent infections which Gram-positive facultative anaerobes, especially *E. faecalis,* were predominant.[26] Secondly, the antimicrobial activity of *M. indica* kernel extract was not tested in the presence of potential inhibitors such as hydroxyapatite, dentine, albumin and other serum proteins present in infected root canals. This topic requires further study in order to evaluate the potential of the agent to be used in endodontic treatment. Finally, this study is performed *ex vivo* to mimic the environments in clinical situations. The clinical efficacy and any side effects, if applicable by means of a pre-clinical and clinical evaluation must be evaluated. The antimicrobial activity and efficacy of *M. indica* kernel extract as root canal medicament should be assessed next in a clinical setting in a randomized controlled clinical trial.

Conclusions

Within the limits of the present study, it was concluded that the ethanolic extract of *M. indica* kernel had antibacterial and antifungal effect against planktonic *E. faecalis* and *C. albicans*. Moreover, the extract had potential to eliminate multispecies *E. faecalis*-*C. albicans* biofilms up to the residual root. The antimicrobial activity of *M. indica* kernel extract was comparable to calcium hydroxide, which is a gold standard medicament used in root canal treatment.

Conflicts of interest

No conflict of interest declared.

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