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Original Article

Laboratory evaluation of the antibacterial and cytotoxic effect of alpha-mangostin when used as a root canal irrigant

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ABSTRACT

Background/objectives: The aim of this study was to investigate the antibacterial activity against *Enterococcus faecalis* of alpha-mangostin and to compare its activity with sodium hypochlorite (NaOCl) and chlorhexidine (CHX) as well as to assess its biocompatibility using human periodontal ligament (PDL) cell.

Methods: Alpha-mangostin was extracted from the pericarps of mangosteen by thin layer chromatography. The inhibitory effect of alpha-mangostin on *E. faecalis* was determined by cultures and an applied broth dilution test. The rate of bactericidal activity was evaluated by time-kill. Human PDL cell culture was used to assess the cytotoxicity of alpha-mangostin.

Results: Alpha-mangostin was effective against *E. faecalis*, the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) were 1.97 and 3.94 µg/ml, respectively. The *E. faecalis* killing activity of alpha-mangostin was higher than that of CHX at 2 × MBC and 4 × MBC, respectively. When compared at the same concentration, there was no significant difference between the alpha-mangostin-treated group and the NaOCl-treated group ($P < 0.05$). From the cytotoxicity test demonstrated that no significant difference of cell viability was observed between the alpha-mangostin-treated group and control group in all of the treatment time intervals.

Conclusion: These findings indicated that alpha-mangostin showed promising antibacterial activity against *E. faecalis* and low toxicity to human PDL cells in vitro, suggesting its potential to be used as a root canal irrigant.

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1. Introduction

Bacteria play a fundamental role in the development of pulp and periapical diseases. Therefore, one of the most important

objectives of endodontic treatment is to eliminate bacteria from the infected root canal.¹ *Enterococcus faecalis*, a gram-positive and facultative anaerobe, has been found to be three times more prevalent in refractory cases than primary

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endodontic diseases.² Moreover, it is the most frequently detected species in the canal of root filled teeth with persistent periapical lesions.³ The ability of *E. faecalis* to tolerate starvation, extremes of pH, biofilm formation, dentin tubular invasion, and emergence of antibiotic resistant strains has made their extermination challenging in endodontically treated teeth.^{4–6}

For many years, root canal irrigants have been used as an adjunct to enhance the antibacterial effect of cleaning and shaping in endodontic treatment. Sodium hypochlorite (NaOCl) is the most frequently used in the treatment of infected root canals because of its dissolving action on pulp tissue and its antibacterial activity. But it also has a cytotoxic effect when injected into the periapical tissues, leaves a bad smell and taste, has a corrosive potential, and may cause allergic reactions.⁷ Chlorhexidine (CHX) has been used as an irrigating solution during root canal treatment. CHX is a cationic bis-bi-guanide with broad-spectrum antibacterial activity. However, the use of CHX as a root canal irrigant is limited because it has no tissue solvent activity and some patients might have allergic reactions to CHX,⁸ moreover, it can discolor teeth.⁹ Previous study has shown that both NaOCl and CHX were highly cytotoxic to human periodontal ligament (PDL) cells by inhibiting mitochondrial activity.¹⁰

An increasing number of reports have determined the activity and possible applications of natural products for root canal disinfection.^{11–13} The pericarps of mangosteen, *Garcinia mangostana* Linn., have been used as a traditional medicine for treatment of abdominal pain, diarrhea, infected wound, and chronic ulcers in Southeast Asia for many years.¹⁴ The major bioactive compounds of *G. mangostana* are xanthone derivatives such as alpha-mangostin, beta-mangostin, and gamma-mangostin.¹⁵ Alpha-mangostin was reported to have a great variety of pharmacological activities including antioxidant,¹⁶ antifungal,¹⁷ antibacterial,^{18,19} anti-HIV,²⁰ anti-inflammation,²¹ antihistamine,²² and anticancer activities.^{23,24} In addition, previous studies have demonstrated low toxicity of alpha-mangostin and the mangosteen pericarp extract.^{25,26}

The literatures have shown that alpha-mangostin has antimicrobial activity, low toxicity, and therapeutic effects, suggesting its potential to be used as a root canal irrigant, but this not been reported previously. The aim of this study was to investigate the antibacterial activity against *E. faecalis* of alpha-mangostin and to compare its activity with NaOCl and CHX as well as to assess its biocompatibility using human PDL cell.

2. Material and methods

2.1. Preparation of alpha-mangostin

Plant materials were identified, collected and confirmed by comparison with specimens available at the Department of Pharmacognosy and Pharmaceutical Botany, the Faculty of Pharmaceutical Sciences, Chulalongkorn University, Thailand. Crude extract and purified alpha-mangostin were prepared based on methods in a previous report.¹⁷ Briefly, dried and ground pericarps were macerated in hexane for 24 h to

remove nonpolar substances. The resulting macerate was subsequently macerated in ethyl acetate for 24 h. The ethyl acetate extract was then recrystallized, and ground into powder. The yield of mangosteen crude extract from the dried pericarp was approximately 3% (w/w). To obtain alpha-mangostin, the crude extract was chromatographed on a silica gel column, and eluted with increasing percentages of ethyl acetate in hexane (0–25%). A hexane–ethyl acetate (4:1) elute was selected based on the thin layer chromatography profile. The selected fraction was further identified as alpha-mangostin (Fig. 1) by using mass spectrometry, nuclear magnetic resonance spectroscopy and a Gallenkamp melting point apparatus. The yield of alpha-mangostin from the dried pericarp was approximately 0.4% (w/w) and the concentrated stock solution was prepared in dimethyl sulfoxide (DMSO).

2.2. Bacterial growth

E. faecalis strain ATCC 29212 used in this study was obtained from the microbiology laboratory of the Department of Medical Sciences, Ministry of Public Health, Thailand. The working stock cultures of *E. faecalis* were maintained on brain heart infusion (BHI) agar slants (Becton, Dickinson and Company, Sparks, MD, USA) at 4 °C until further use. *E. faecalis* to be tested were grown in BHI broth for 24 h at 37 °C.

2.3. Antibacterial susceptibility testing

A broth dilution technique was employed to determine the susceptibility of the *E. faecalis* to alpha-mangostin, NaOCl and CHX. Susceptibility was expressed as minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). NaOCl and CHX were used as positive control. Solvent and media controls were used for reference. The BHI broth containing varying amounts (serially and two-fold diluted) of alpha-mangostin was inoculated with actively dividing *E. faecalis*. The initial density of *E. faecalis* was approximately 1.5×10^8 colony forming units (CFU)/ml. After 24-h incubation, the growth was monitored both visually and colorimetrically (at 600 nm). The MIC was defined as the lowest concentration that arrested the growth of the bacteria at the end of the 24-h incubation. The MBC determined by sub-culturing a 0.01 ml aliquot of the medium drawn from the culture tubes shows no macroscopic growth at the end of 24 h of culture on BHI agar plates to determine the number of vital organisms. The culture was then incubated for appearance of bacterial growth. The MBC was defined as the lowest concentration of the agent that reduced the number of viable organisms by 99.9%.²⁷ Testing was performed in triplicate.

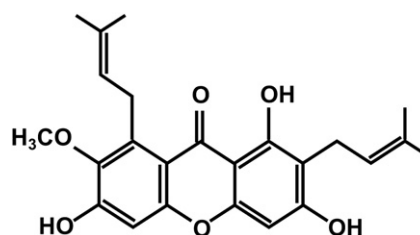


Fig. 1 – Structure of alpha-mangostin.

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