Thai Propolis Extract as a Root Canal Medication against *Enterococcus faecalis* Infection

Sasiwan Pengchum Pattama Chailertvanitkul** Malinee Laopaiboon*** Pinpana Thaweesit***
*PV Abbott**** Suttichai Krisanaprakornkit******

ABSTRACT

The aim was to evaluate the efficacy of Thai propolis extract compared to calcium hydroxide against Enterococcus faecalis after root canal medication. One hundred and seventy two mandibular premolars were infected with E. faecalis. Six roots were filled with normal saline, and four roots were prepared for scanning electron microscopy to check for bacterial penetration. The remaining 162 roots were categorized into six groups and filled with one of three medicaments: Thai propolis extract, Thai propolis extract mixed with calcium hydroxide, and calcium hydroxide, for 2 or 7 days. Root dentine powder was assessed for viable bacteria in colony forming units. Thai propolis extract significantly eliminated more E. faecalis at day 2, but less than Thai propolis extract mixed with calcium hydroxide and calcium hydroxide at day 7 ($p<0.001$). At days 2 and 7, Thai propolis extract mixed with calcium hydroxide had similar efficacy to calcium hydroxide.

Keywords: *Calcium hydroxide/ Enterococcus faecalis/ Propolis; Root canal medication*

Introduction

Enterococcus faecalis is the most prevalent species, isolated from root canals of previously root-filled teeth with apical periodontitis[1]. It has an ability to invade dentinal tubules[2]. Several studies have shown that *E. faecalis* cannot be eliminated effectively by calcium hydroxide, which has been used as a common endodontic medicament for many years[3-5]. Calcium hydroxide was initially recommended for use as an intracanal medicament, based on its antibacterial effect after being placed in the root canal over 7 days[6]. However, long-term use of calcium hydroxide can compromise the integrity of root dentin structure if left in the canal longer than two months owing to its high pH (12.5) that adversely affects the hydroxyapatite structure[7]. The antibacterial effect of calcium hydroxide is due to its alkalinity as the hydroxyl ion possesses a destructive effect on bacteria cell membrane and protein structure[8]. Different vehicles used with calcium hydroxide also play an important role in the antibacterial action of calcium hydroxide that can influence ionic dissociation into calcium and hydroxyl ions[9].

Because calcium hydroxide is not effective in killing *E. faecalis* as aforementioned, there have been several studies testing new synthetic or natural chemicals used as a sole intracanal medicament or in combination with calcium hydroxide for their antibacterial action[10]. Propolis or bee glue is a natural hive product collected by bees and has been used as a remedy in folk medicine for a very long time[11]. Its chemical compositions and biological properties are complex and varied among propolis extracts from different geographical areas and climates. In general, the propolis extract contains various organic compounds, such as phenols, esters and flavonoids which are shown to possess anti-inflammatory, antibacterial, antifungal and antioxidant properties[12].

In dentistry, propolis was reported to exert an antibacterial effect against *Streptococcus mutans, Streptococcus gordonii* and *E. faecalis* [13-15]. Moreover, a combined preparation of calcium hydroxide with Brazilian propolis extract as a drug solvent showed greater inhibition of *E. faecalis* by an agar well diffusion

* Dental Department, Jainad Narendra Hospital, Amphur Muang, Chai Nat.
** Department of Restorative Dentistry, Faculty of Dentistry, Khon Kaen University, Amphur Muang, Khon Kaen.
*** Department of Public Health, Faculty of Biostatistics and Demography, Khon Kaen University, Amphur Muang, Khon Kaen.
**** School of Dentistry, University of Western Australia, Australia.
***** Center of Excellence in Oral and Maxillofacial Biology, Department of Oral Biology and Diagnostic Sciences, Faculty of Dentistry, Chiang Mai University, Amphur Muang, Chiang Mai.

method than calcium hydroxide or the extract alone[10, 16]. Therefore, the aim of this *in vitro* and *ex vivo* study was to evaluate the antibacterial efficacy of Thai propolis extract in comparison with that of calcium hydroxide against *E. faecalis* infection for 2 and 7 days when used alone or combined with calcium hydroxide as a root canal medicament.

Materials and methods

- **Preparation of propolis extract**

The extract of propolis collected from beehives in Nong Khai province, Thailand, was prepared as previously described[17]. Briefly, propolis was ground into fine particles and dissolved in 95% ethanol (1 g/5 mL). The mixture was stirred at 25°C in an orbital shaker at 200 rpm for 5 days. The propolis extract was filtered with Whatman No. 1 filter paper. Ethanol was removed in a rotary evaporator at 40°C and the semi-solid brownish extract with a concentration of 908.77 mg/mL was obtained by lyophilization in a freeze dryer (CHRIST, ALPHA 2-4 LD plus, Martin Christ, Germany). The extract was kept in a bottle wrapped with aluminum foil and stored at 4°C for further uses.

- ***In vitro* antibacterial assays**

E. faecalis strain DMST 4736, purchased from the Department of Medical Sciences, Ministry of Public Health, Thailand, was incubated overnight at 37°C, 5% CO_2 for 24 h and suspended in sterile tryptic soy broth. The optical density (OD) of *E. faecalis* suspension was adjusted spectrophotometrically to 0.1, which was approximately equivalent to 1.5×10^8 colony forming units (CFU)/mL. The *in vitro* antibacterial effect of Thai propolis extract against *E. faecalis* was first determined by an agar well diffusion method according to Bauer *et al.*[18]. In brief, sterile tryptic soy broth, mixed with the suspension of *E. faecalis* (OD = 0.1), was poured into the petri dish. Each agar plate was punched out using a cork border into three wells, each of which was for adding the propolis extract at 100 mg/mL, non-setting calcium hydroxide paste (Odontex, Bangkok, Thailand) or sterile normal saline. The agar plate was then incubated at 37°C for 48 h. The zones of inhibition were measured in mm.

In addition, the minimum bactericidal concentration (MBC) of the extract was determined by a broth microdilution method according to the Clinical & Laboratory Standards Institute guidelines. The MBC value is defined as the lowest concentration that completely inhibits bacterial growth. The propolis extract was dissolved in phosphate-buffered saline (PBS) and diluted with tryptic soy broth to a concentration of 200 mg/mL. Further two-fold serial dilutions were performed by the addition of tryptic soy broth to reach the concentrations of 100, 50 and 25 mg/mL. Then, a 100-µL quantity of each dilution was pipetted into 96-well plates. Each test or control well was inoculated with 100 µL of the bacterial suspension. The well plates were incubated at 37°C for 24 h. Then, a 0.1-mL quantity withdrawn from both test and control wells was streaked on agar plates and incubated at 37°C for 24 h, and any visible colony of *E. faecalis* on agar plates was recorded.

The combination of calcium hydroxide in mg and Thai propolis extract in mL was prepared at three different ratios, 300:1, 400:1 and 500:1, based on the viscosity of mixture that could be easily carried into root canals using a spiral root filler. The antibacterial activity was determined by an agar well diffusion test as aforementioned. Each agar plate was punched out using a cork border into four wells. Calcium hydroxide alone and calcium hydroxide mixed with Thai propolis extract at each of the three ratios were placed in the plates and incubated at 37°C for 48 h. The zones of inhibition were measured in mm. All *in vitro* antibacterial experiments were performed in triplicate.

● **Preparation of tooth root specimens**

The research protocol (#HE 582121) of this study was approved by the office of the Khon Kaen University Ethics Committee in Human Research. One hundred and seventy-two single-rooted human mandibular premolars without carious exposure, root canal treatment and periodontal disease that were extracted for orthodontic purposes were collected from 100 participants (18-24 years old) with their informed consent. All teeth were stored in 0.1% thymol after being decoronated to 12-mm root length using a diamond disk with water coolant (Isomet 1000, Buehler, IL, USA). The root canals were instrumented 1 mm short of the root apex up to a size of 20 K-files (Dentsply, Maillefer, Switzerland) and then prepared using the Wave One 40/08 rotary file (Dentsply) according to the manufacturer's instructions. Irrigation was performed with 2 mL of 5.25% NaOCl during root canal preparation. The root specimens were placed in an ultrasonic bath of 17% EDTA for five minutes followed by 5.25% NaOCl for five minutes to remove smear layer. The roots were then placed in brain heart infusion (BHI) broth, autoclaved (WACS-1045 autoclave, Taipei, Taiwan) for 15 minutes at 121°C and cultured to check the efficacy of sterilization.

● ***Ex vivo* antibacterial activity of Thai propolis extract**

The study procedures were performed in a microbiological safety cabinet (Napco NapFLOW 1200 Type A/B3, Boston, MA, USA) using sterilized instruments and materials. All 172 root specimens were placed in test tubes, containing 2 mL of BHI broth inoculated with 0.5 mL of the *E. faecalis* suspension (1.5 x10^8 CFU/mL), and kept at 37°C for 21 days. The broth was replaced every three days and randomly checked to confirm free of contamination with bile esculin agar and 6.5% sodium chloride. The infected roots were divided into a scanning electron microscope (SEM) group (n = 4), a positive control group (n = 6) and six experimental groups (n =27) as shown in Figure 1. The conditions of eight groups were as follows:

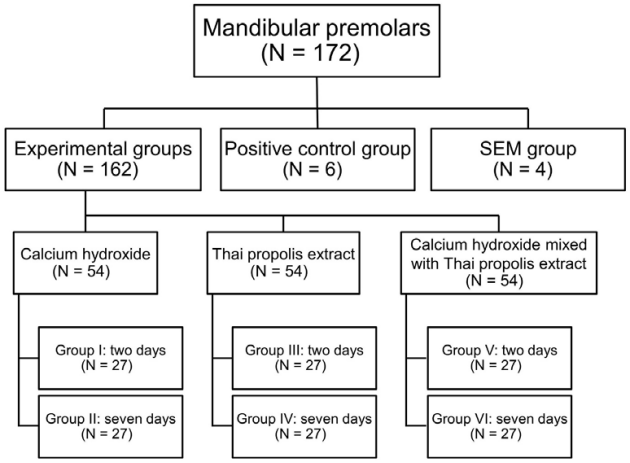

Figure 1. A flow chart summarizing a research design for this ex vivo study to assess the antibacterial activity of Thai propolis extract, using 172 human single-rooted premolars from 100 participants. See more details in the text. SEM = scanning electron microscope.

SEM group - four roots were sectioned longitudinally into two halves and fixed in 2.5% glutaraldehyde for two h. Each specimen was washed in 0.1 M PBS three times for five min each. Dehydration was performed using ascending concentrations of ethyl alcohol (30%, 50%, 70%, 90% and 100%) for ten min each. After dehydration, the samples were dried, gold coated using K500X Manual Sputter Coater (Quorum Technologies Ltd., Brighton, UK), and examined by SEM under a magnification power of 5,000x (S-3000N Hitachi, Tokyo, Japan).

Positive control group - each root canal was filled with sterile normal saline, sealed with Cavit G (3M ESPE, Seefeld, Germany) and incubated at 37°C for 7 days.

Groups I and II - each root canal was filled with non-setting calcium hydroxide paste using a spiral root filler, sealed with Cavit G and incubated at 37°C for 2 and 7 days, respectively.

Groups III and IV – each root canal was filled with Thai propolis extract soaked in a sterile paper point at 100 mg/mL, the MBC against *E. faecalis* based on the broth microdilution test, sealed with Cavit G and incubated at 37°C for 2 and 7 days, respectively.

Groups V and VI – each root canal was filled with calcium hydroxide paste mixed with Thai propolis extract at the most effective ratio of 300:1 (mg:mL) as determined by the agar diffusion test using a spiral root filler, sealed with Cavit G and incubated at 37°C for 2 and 7 days, respectively.

- **Microbiological sampling**

After the end of the medication periods for 2 and 7 days, a size-4 Gates Glidden drill (Kerr Corporation, Orange, CA, USA) was passed through the root canal to a depth of 11 mm to remove dentin powder. The powder was collected in a test tube, containing 1 mL of PBS, and the tube was shaken for 60 sec. A 10-fold dilution was made, and a 0.1-mL quantity of the dentin suspension was streaked on agar plates and incubated at 37°C for 24 h, and the number of *E. faecalis* was counted as CFU/mL.

- **Statistical analysis**

The inhibition zone was reported as mean, and the significance differences between groups were determined by the independent sample *t* test or the Mann-Whitney *U* test. The CFU data were transformed to $\log_{10}$ values, and the normality test showed that the CFU data of each group were not normally distributed. Therefore, the median differences of CFU data among different medicaments in each medication period or each medicament between two different periods were compared by the Kruskal-Wallis test, followed by the Mann-Whitney *U* test. The statistical significance was set to 5%. The Statistical Package for the Social Science software version 19.0 (Chicago, IL, USA) was used for statistical analysis.

Results

The *in vitro* antibacterial effect of Thai propolis extract was first assessed by the agar diffusion test, and the extract exhibited a larger growth inhibition zone than did calcium hydroxide paste or sterile normal saline, as a negative control (Fig. 2A). Statistically, the extract demonstrated a significant greater mean inhibition zone than calcium hydroxide ($p < 0.001$; Table 1). In addition, the MBC of Thai propolis extract against *E. faecalis* was found at 100 mg/mL, and calcium hydroxide mixed with Thai propolis extract at the ratio of 300:1 (mg:mL) showed the largest growth inhibition zone when compared with that of calcium hydroxide or of calcium hydroxide mixed with Thai propolis extract at the two ratios, including 400:1 and 500:1 (Fig. 2B). Statistically, calcium hydroxide mixed with Thai propolis extract at 300:1 exhibited a significant greater mean inhibition zone than the other groups ($p < 0.05$; Table 2).

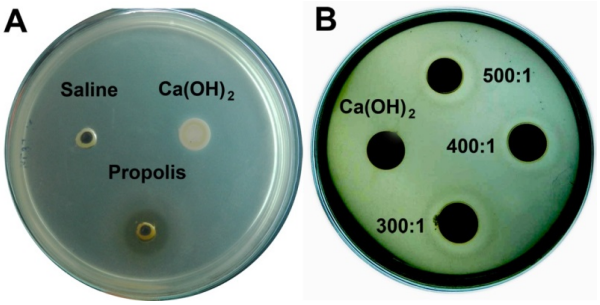

Figure 2 Assessment of antibacterial activity against *Enterococcus faecalis* of Thai propolis extract by an agar diffusion test. A) A representative image of inhibition zones on an agar diffusion plate with normal saline, calcium hydroxide [$Ca(OH)_2$], and 100 mg/mL of Thai propolis extract. B) A representative image of inhibition zones on an agar diffusion plate with calcium hydroxide [$Ca(OH)_2$] and three different ratios of calcium hydroxide mixed with Thai propolis extract, including 300:1, 400:1 and 500:1.

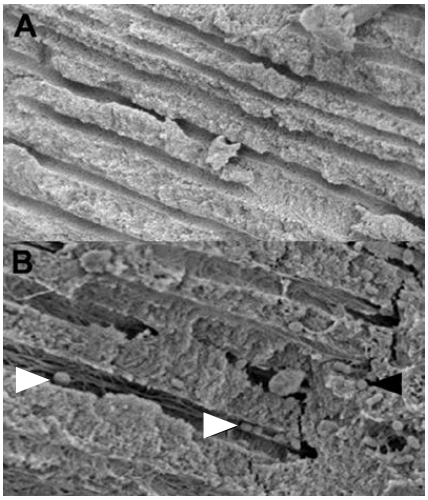

Figure 3 Infected root dentin with *Enterococcus faecalis* evaluated by scanning electron microscopy. A representative image of dentinal tubules from sterile dentin (A), as a negative control, and infected dentin for 21 days (B) at a magnification power of 5,000x. Arrowheads in B indicate entombed *E. faecalis* with a spherical shape in dentinal tubules.

The presence of *E. faecalis* entrapped in dentinal tubules of infected roots after inoculation for 21 days (arrowheads in Fig. 3B) was first revealed by scanning electron microscopy as compared to its absence in sterile root dentin (Fig. 3A). The medians of colony forming units in CFU/mL for infected root canals, treated with calcium hydroxide, Thai propolis extract at 100 mg/mL, and calcium hydroxide mixed with Thai propolis extract at the ratio of 300:1, at 2 and 7 days of medication periods are shown in Table 3. It was revealed that Thai propolis extract had significantly eliminated more *E. faecalis* than calcium hydroxide and calcium hydroxide mixed with Thai propolis extract after 2 days of intracanal medication ($p < 0.001$). On the contrary, it had significantly less antibacterial efficacy than calcium hydroxide mixed with Thai propolis extract and calcium hydroxide after 7 days ($p < 0.001$). At both 2 and 7 days of medication periods, calcium hydroxide mixed with Thai propolis extract had similar antibacterial efficacy to calcium hydroxide used alone (Table 3). When comparing the antibacterial efficacy of each medicament between 2 and 7 days of intracanal medication, it was found that there were significant decreases in median colony forming units by calcium hydroxide and calcium hydroxide mixed with Thai propolis extract at day 7, whereas there was a significant increase by Thai propolis extract alone ($p<0.001$; Table 3).

Table 1 Antibacterial activity against *Enterococcus faecalis* of Thai propolis extract, assessed by an agar diffusion method and shown as a mean growth inhibition zone in mm.

Medication	Inhibition zone (mm)	Confidence interval (95%CI)
Thai propolis extract (100 mg:ml)	18.51 [a]	18.30,18.72
Calcium hydroxide (control)	7.66 [a]	7.23,8.08

[a] Statistically significant difference at $p<0.001$ by the Independent sample *t* test

Table 2 Antibacterial activity against *Enterococcus faecalis* of calcium hydroxide mixed with Thai propolis extract at three different ratios, assessed by an agar diffusion method and shown as a mean growth inhibition zone in mm.

Medication	Inhibition zone (mm)	Confidence interval (95%CI)
Calcium hydroxide mixed with Thai propolis extract 300:1 (mg:ml)	18.19 [b c d]	18.17,18.22
Calcium hydroxide mixed with Thai propolis extract 400:1 (mg:ml)	17.62 [b]	17.42,17.82
Calcium hydroxide mixed with Thai propolis extract 500:1 (mg:ml)	17.48 [c]	17.44,17.53
Calcium hydroxide (control)	7.57 [d]	7.40,7.74

[b,c,d] Statistically significant differences at $p<0.05$ by the Mann–Whitney U test

Table 3 Comparsions of the median colony forming units in CFU/mL of *Enterococcus faecalis*, recovered after intracanal medication by three different medicaments for two or seven days. The quantity of E. faecalis inoculated at the beginning was 1.5 x 10^8 CFU/mL.

Intracanal medication	Median colony forming units (x 10^4 CFU/ml)	
	For two days	For seven days
Calcium hydroxide	46.10 [a †]	4.44 [c †]
Thai propolis extract (100 mg:ml)	13.40 [a b ‡]	81.10 [c d ‡]
Calcium hydroxide mixed with Thai propolis extract 300:1 (mg:ml)	72.40 [b ζ]	6.97 [d ζ]

[a,b,c,d,†,‡,ζ] Statistically significant differences at $p<0.001$ by the Mann–Whitney *U* test

Discussion

This study used a broth microdilution method to obtain accurate minimum bactericidal concentrations of Thai propolis extract. Higher values of MBC of Thai propolis extract were observed as compared to the findings from Ferraira *et al.*[19] and Jafarzadeh Kashi *et al.*[20]. This may be a result of lower flavonoids (7.33 ±0.18 mg quercetin) and phenolic compounds (29.50±0.27 mg gallic acid) in the Thai propolis extract[21] than in the propolis extract from other regions of the world[22]. Any vehicle added to calcium hydroxide gives the chemical characteristics influencing the rate of ionic dissociation and diffusion [9]. This study used Thai propolis extract as the vehicle at the ratio of 300:1 (mg:ml) because it had the greater mean of the growth inhibition zone than other groups (Table 2) and could be easily handled and inserted into the root canal by a spiral root filler. Sahamanta *et al.*[10] showed that a mixture between propolis and calcium hydroxide at the ratio of 1:3 had the largest zone of growth inhibition at 48 and 72 h, which is similar to the current study.

The results of this study suggest that Thai propolis extract may be used as a short-term root canal medicament. Thai propolis extract after 2 days of medication showed higher antimicrobial effect compared to calcium hydroxide (Table 3). This may be attributed to the qualities of the active compounds in Thai propolis extract whilst the time of application was not sufficient for the calcium hydroxide to act against *E. faecalis*. According to Sjögren *et al.*[6], calcium hydroxide must be applied at least 7 days to act as an effective antimicrobial agent. Awawdeh *et al.*[13] and Madhubala *et al.* [23] have shown that propolis was significantly more effective than calcium hydroxide against *E. faecalis* with short-term application, which is in agreement with this study.

After 7 days of medication, calcium hydroxide had similar antibacterial efficacy to calcium hydroxide mixed with Thai propolis extract while the Thai propolis extract had the lowest antibacterial efficacy (Table 3). This may be a result of flavonoid and polyphenol reduction at high temperature.[24]

Calcium hydroxide mixed with Thai propolis extract had antibacterial efficacy similar to calcium hydroxide alone after 2 and 7 days of medication. This is possibly because the pH of calcium hydroxide mixed with Thai propolis extract was still high (pH 12.4). The antibacterial effect of this mixture may be the result of high pH of calcium hydroxide rather than the flavonoid in the Thai propolis extract. However, some *E. faecalis* still remained in all root canals after medication, which confirms that chemo-mechanical instrumentation is essential during root canal treatment.

The SEM finding in the infected root specimens indicated that the microorganisms penetrated into dentin in a similar manner to that reported by Love in 1996,[25] who showed that *E. faecalis* penetrated into the dentinal tubules for up to 200 μm. Since the diameter of the dentinal tubules may affect the results, this study controlled the diameter of the dentinal tubules by limiting the age of the patients, from which the extracted teeth were collected, and by standardizing the root length to 12 mm.

A single examiner, who was blinded to the different medicaments, performed the colony counting and that examiner was self-calibrated with a substantially high value of intra-class correlation coefficient to minimize the measurement bias (Kappa =0.935). Microbiological sampling was accomplished using a Gates Glidden drill at the same length to abrade the dentin powder, and the bur was also submerged in the broth. According to Awawdeh *et al.*,[13] this method is the most effective microbiological sampling technique. Further laboratory and clinical investigations should be carried out to validate the findings of some beneficial use of Thai propolis extract as an intracanal medicament.

In conclusion, Thai propolis extract at the concentration of 100 mg/mL was effective in eliminating *E. faecalis* at 2 days of medication. However, the Thai propolis extract had the lowest antibacterial efficacy at 7 days of medication whilst calcium hydroxide mixed with Thai propolis extract at the ratio of 300:1 and calcium hydroxide has similar efficacy to at both 2 and 7 days.

Acknowledgements

Financial support from Khon Kaen University to P.C. and from the Thailand Research Fund (#BRG6080001) to S.K. was gratefully acknowledged.

Conflict of Interests

None declared. All authors have read, edited and approved this manuscript.

References

1. Sundqvist G, Figdor D, Persson S, Sjögren U. Microbiologic analysis of teeth with failed endodontic treatment and the outcome of conservative re-treatment. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1998;85:86-93.
2. Figdor D, Davies JK, Sundqvist G. Starvation survival, growth and recovery of Enterococcus faecalis in human serum. Oral Microbiol Immunol 2003;18:234-9.
3. Han GY, Park SH, Yoon TC. Antimicrobial activity of $Ca(OH)_2$ containing pastes with *Enterococcus faecalis in vitro.* J Endod 2001;27:328-32.
4. Evans M, Davies JK, Sundqvist G, Figdor D. Mechanisms involved in the resistance of *Enterococcus faecalis* to calcium hydroxide. Int Endod J 2002;35:221-8.
5. Sukawat C, Srisuwan T. A comparison of the antimicrobial efficacy of three calcium hydroxide formulations on human dentin infected with *Enterococcus faecalis*. J Endod 2002; 28:102-4.
6. Sjögren U, Figdor D, Spangberg L, Sundqvist G. The antimicrobial effect of calcium hydroxide as a short-term intracanal dressing. Int Endod J 1991; 24:119-25.
7. Andreasen JO, Farik B, Munksgaard EC. Long-term calcium hydroxide as a root canal dressing may increase risk of root fracture. Dent Traumatol 2002;18:134-7.
8. Siqueira JF Jr, Loped HP. Mechanisms of antimicrobial activity of calcium hydroxide: a critical review. Int Endod J 1999;32:361-9.
9. Estrela C, Pécora J, Souza-Neto MD, Estrela C, Bammann LL. Effect of vehicle on antimicrobial properties of calcium hydroxide paste. Braz Dent J 1999;10:63-72.
10. Sahamanta CG, Gabriel AS, Isela FC, Laura VFG, Hugo MZ. Antimicrobial activity with mixture of calcium hydroxide and propolis. Int J Pharm Bio Sci 2011;2:203-10.
11. Ghisalberti E. Propolis:a review. Bee World 1979;60:59-84.
12. Uzel A, Sorkun K, Öncag Ö, Cogulu D, Gencay Ö, Salih B. Chemical compositions and antimicrobial activities of four different Anatolian propolis samples. Microbiol Res 2005;160:189-95.
13. Awawdeh L, Al-Beitawi M, Hammad M. Effectiveness of propolis and calcium hydroxide as a short-term intracanal medicament against *Enterococcus faecalis*: a laboratory study. Aus Endod J 2009;35:52-8.
14. Kayaoglu G, Ömurlu H, Akca G, Gurel M, Gencay Ö, Sorkun K, Salih B. Antibacterial activity of propolis versus conventional endodontic disinfectants against *Enterococcus faecalis* in infected dentinal tubules. J Endod 2011;37: 376-81.
15. Samaksamarn T, Chailertvanitkul P, Laopaiboon M, Kaentip P, Yim-art P, Sheewakumnuan N. Antibacterial properties of propolis extracted by agar well diffusion method. Khon Kaen Dent J 2012;15:21-8.
16. de Rezende GP, da Costa LR, Pimenta FC, Baroni DA. *In vitro* antimicrobial activity of endodontic pastes with propolis extracts and calcium hydroxide: a preliminary study. Braz Dent J 2008;19:301-5.
17. Prueksakorn A, Puasiri S, Ruangsri S, Makeudom A, Sastraruji T, Krisanapakornkit S, chailertvanitkul P. The preservative effect of Thai propolis extract on the viability of human periodontal ligament cells. Dent Traumatol 2016; 32:495-501.
18. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol 1966;45:493-6.
19. Ferreira FB, Torres SA, Rosa OP, Ferreira CM, Garcia RB, Marcucci MC, Gomes BP. Antimicrobial effect of propolis and other substances against selected endodontic pathogens. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2007; 104:709-16.
20. Jafarzadeh Kashi TS , Kermanshahi RK, Erfand M, Dastjerdie EV, Rezaeia Y, Tabatabaei FS. Evaluating the *in-vitro* antibacterial effect of Iranian propolis on oral microorganisms. Iran J Pharm Res 2011;10:363-8.
21 Chailertvanitkul P, Namsirikul T, Damrongrungruang T, Peerapattana J. Phenolic and flavonoids contents and antibacterial activity of ethanolic extract of propolis. Isan J Pharm Sci 2017;13:59-67
22. Kumazawa S, Hamasaka T, Nakayama T. Antioxidant activity of propolis of various geographic origins. Food Chem 2004;84:329-39.
23. Madhubala MM, Srinivasan N, Ahamed S. Comparative evaluation of propolis and triantibiotic mixture as an intracanal medicament against *Enterococcus faecalis*. J Endod 2011;37:1287-9.
24. Schieber A, Keller P, Carle R. Determination of phenolic acids and flavonoids of apple and pear by high-performance liquid chromatography. J Chromatogr A 2001;910:265-73.
25. Love RM. Regional variation in root dentinal tubule infection by *Streptococcus gordonii.* J Endod 1996;22:290-3.

Corresponding author:

Pattama Chailertvanitkul
Department of Restorative Dentistry,
Faculty of Dentistry, Khon Kaen University,
Amphur Muang, Khon Kaen, 40002.
Tel.: +66 43 202405 #45143-4
Fax.: +66 43 202862
E-mail: patchai@kku.ac.th

สารสกัดพรอพอลิสไทยเมื่อใช้เป็นยาใส่ในคลองรากฟันต่อการต้านการติดเชื้อเอ็นเตอโรคอคคัสฟีคาลิส

ศศิวรรณ เพ็งชุ่ม ปัทมา ชัยเลิศวณิชกุล** มาลินี เหล่าไพบูลย์*** ปิ่นพนา ทวีสิทธิ์***
*PV Abbott**** สุทธิชัย กฤษณะประกรกิจ******

บทคัดย่อ

การศึกษานี้มีวัตถุประสงค์เพื่อประเมินประสิทธิภาพของสารสกัดพรอพอลิสไทยเปรียบเทียบกับแคลเซียมไฮดรอกไซด์ต่อการต้านการติดเชื้อเอ็นเทอโรคอคคัสฟีคาลิสเมื่อใช้เป็นยาใส่ในคลองรากฟัน ฟันกรามน้อยล่างจำนวนเจ็ดสิบสองซี่ถูกทำให้ติดเชื้อเอ็นเทอโรคอคคัสฟีคาลิส ฟันหกซี่ใช้น้ำเกลือเป็นยาใส่ในคลองรากฟัน ฟันสี่ซี่ตรวจการแทรกซึมของแบคทีเรียด้วยกล้องอิเล็กตรอนไมโครสโคปชนิดส่องกราด ฟันอีก 162 ซี่แบ่งเป็นกลุ่มทดลองหกกลุ่ม กลุ่มละ 18 ซี่ ใส่ยาสามชนิด คือ สารสกัดพรอพอลิสไทย แคลเซียมไฮดรอกไซด์และแคลเซียมไฮดรอกไซด์ผสมสารสกัดพรอพอลิสไทย โดยใส่ยาเป็นเวลา 2 หรือ 7 วัน นำผงรากฟันที่กรอได้ไปประเมินปริมาณแบคทีเรียมีชีวิตที่หลงเหลือ ผลการศึกษาพบว่าสารสกัดพรอพอลิสไทยกำจัดเชื้อเอ็นเทอโรคอคคัสฟีคาลิสได้มากที่สุดในวันที่ 2 หลังจากใส่ยา ($p<0.001$) แต่กำจัดเชื้อได้น้อยกว่าสารสกัดพรอพอลิสไทยผสมแคลเซียมไฮดรอกไซด์ และแคลเซียมไฮดรอกไซด์ในวันที่ 7 ($p<0.001$) โดยทั้งวันที่ 2 และ 7 สารสกัดพรอพอลิสไทยผสมแคลเซียมไฮดรอกไซด์ให้ผลกำจัดเชื้อไม่แตกต่างกับแคลเซียมไฮดรอกไซด์

คำไขรหัส: *แคลเซียมไฮดรอกไซด์/ เอ็นเทอโรคอคคัสฟีคาลิส/ พรอพอลิส/ ยาใส่ในคลองรากฟัน*

ผู้รับผิดชอบบทความ

ปัทมา ชัยเลิศวณิชกุล
ภาควิชาทันตกรรมบูรณะ
คณะทันตแพทยศาสตร์ มหาวิทยาลัยขอนแก่น
อำเภอเมือง จังหวัดขอนแก่น 40002
โทรศัพท์: 0 4320 2405 #45143-4
โทรสาร: 0 4320 2862
จดหมายอีเล็กทรอนิกส์: patchai@kku.ac.th

** แผนกทันตกรรม โรงพยาบาลชัยนาทนเรนทร อำเภอเมือง จังหวัดชัยนาท*
*** ภาควิชาทันตกรรมบูรณะ คณะทันตแพทยศาสตร์ มหาวิทยาลัยขอนแก่น อำเภอเมือง จังหวัดขอนแก่น*
**** ภาควิชาชีวสถิติและประชากรศาสตร์ คณะสาธารณสุขศาสตร์ มหาวิทยาลัยขอนแก่น อำเภอเมือง จังหวัดขอนแก่น*
***** School of Dentistry, University of Western Australia, Australia.*
****** ภาควิชาชีววิทยาช่องปากและวิทยาการวินิจฉัยโรคช่องปาก คณะทันตแพทยศาสตร์ มหาวิทยาลัยเชียงใหม่ อำเภอเมือง จังหวัดเชียงใหม่*