In vitro antimicrobial activity of propolis, BioPure MTAD, sodium hypochlorite, and chlorhexidine on *Enterococcus faecalis* and *Candida albicans*

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ABSTRACT

الأهداف: تقييم تأثير سوائل التروية مثل: العكبر (صمغ النحل)، وبيو بيور متاد (MTAD)، و5% من هيبوكلوريت الصوديوم، و2% من كلورالهكسيدين ضد نمو المكروبات الدقيقة المتمثلة في المبيضة البيضاء، والمكورات المعوية، بالإضافة إلى تحليل التركيز المثبط الأدنى، والتركيز الأدنى القاتل التي تملكه هذه السوائل ضد نمو المكروبات.

الطريقة: أُجريت هذه الدراسة في كلية الأسنان والصيدلة بجامعة إيرسايس، القيصرية، تركيا وذلك خلال الفترة من فبراير إلى إبريل 2010م. لقد تم استخلاص الإيثانول من العكبر الذي تم جمعه من مدينة القيصرية، تركيا. وبعد ذلك تم تحضير مستنبتات المكروبات المناسبة بواسطة الوسط الزرعي المعقم وذلك بهدف الوصول إلى معدلات التركيز النهائية لسوائل التروية والتي كانت نتائجها كالتالي: 2.4-0.002 ملغ/ملتر للعكبر، 0.512-0.000125 ملغ/ملتر لكلورالهكسيدين، فيما تراوح معدل التخفيف لمركبي هيبوكلوريت الصوديوم وبيو بيور متاد ما بين 1:2-1:4096. ولقد تم اللجوء إلى طريقة التخفيف باستخدام الوسط الزرعي من أجل تقييم التركيز المثبط الأدنى، والتركيز الأدنى القاتل التي تملكه سوائل التروية ضد نمو المبيضة البيضاء، والمكورات المعوية.

النتائج: أظهرت نتائج الدراسة فعالية تأثير سوائل التروية ضد نمو المكروبات الدقيقة مثل المبيضة البيضاء، والمكورات المعوية، ولقد كانت فعالية التركيز المنخفض لكل من العكبر، وهيبوكلوريت الصوديوم أكثر من سوائل التروية الأخرى. وبالمقابل فقد كانت فعالية التركيز المنخفض لكلورالهكسيدين وبيو بيور متاد ضد جراثيم المكورات المعوية أعلى من المبيضة البيضاء.

خاتمة: أثبتت الدراسة مدى نشاط العكبر المضاد للأحياء الدقيقة مثل المكورات المعوية والمبيضة البيضاء، حيث كان من سوائل التورية الفعالة التي يمكن استخدامها ضد نمو المكروبات داخل قنوات جذور الأسنان.

Objectives: To evaluate the antimicrobial effect by measuring the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of propolis, BioPure MTAD, 5% sodium hypochlorite (NaOCl), and 2% chlorhexidine (CHX) on *Enterococcus faecalis* (*E. faecalis*) and *Candida albicans* (*C. albicans*) in vitro.

Methods: This study was performed in the Faculty of Dentistry and Pharmacy at Erciyes University, Kayseri, Turkey from February to April 2010. Ethanol extract of propolis (EEP) was prepared from propolis collected from Kayseri, Turkey, and proper media for microorganisms were prepared using sterile broth medium to give final concentrations between 0.002-2.4 mg/ml for propolis, 0.000125-0.512 mg/ml for CHX, and 1:2-1:4096 dilutions for NaOCl and BioPure MTAD. Using the macrobroth dilution method, MIC, and MBC values of irrigants on the growth of *E. faecalis* and *C. albicans* were determined.

Results: Propolis and other irrigants were found to be effective on *C. albicans* and *E. faecalis*. Propolis and NaOCl were more effective in lower concentrations on *C. albicans* than on *E. faecalis*. In contrast, CHX and MTAD were more effective in lower concentrations on *E. faecalis* than on *C. albicans*.

Conclusion: Propolis showed antimicrobial activity against *E. faecalis* and *C. albicans*. It appears that propolis is an effective intracanal irrigant in eradicating *E. faecalis* and *C. albicans*.

Saudi Med J 2011; Vol. 32 (5): 479-483

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Received 11th December 2010. Accepted 14th March 2011.

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Removal of organic debris and microorganisms is an important factor for a good root canal treatment. Residual microorganisms in pulpal spaces and dentin tubules may give rise to persistent infection after endodontic therapy. During mechanical instrumentation, irrigating solutions are used to disinfect the root canal system. Irrigating solutions may arrive at branching canals and pass through the dentinal tubules.[1,2] A variety of antibacterial irrigation solutions may be used in various concentrations to irrigate and disinfect root canals in conjunction with root canal preparation. Sodium hypochlorite (NaOCl), which has been extensively used in endodontics as an irrigating solution, is an effective antimicrobial agent and an excellent organic tissue solvent,[3] but it can elicit severe inflammatory reactions on the periapical tissues,[4] and may also cause allergic reactions.[5] Chlorhexidine gluconate (CHX) is a broad-spectrum antimicrobial agent that has substantive antibacterial activity and relatively low toxic effects.[6] However, it does not dissolve organic tissues.[3] BioPure MTAD (a mixture of a tetracycline isomer [doxycycline], an acid [citric acid], and a detergent [Tween 80]) can safely remove the smear layer, and kill the strain of *Enterococcus faecalis* (*E. faecalis*).[7] Propolis is a resinous substance collected by *Apis mellifera L.* from various tree buds, and is used by bees both for coating hive parts, and for sealing cracks and crevices in the hive. Its antimicrobial potency keeps the growth of microbes under control, which is supported by recent findings regarding its biological properties, such as antibacterial, antifungal, antiviral, anti-inflammatory, local anesthetic, antioxidant, and cytostatic properties.[8] Propolis is non-toxic, and reports of allergic reactions are not uncommon.[9] The *E. faecalis* and *Candida albicans* (*C. albicans*) are known to be important resistant species in infected root canals, and they may cause treatment failures.[2] Several studies have been conducted on alternative intracanal disinfection methods that abate resistant microorganisms more rapidly.[2,3,6,7] The purpose of this study was to evaluate the antimicrobial activity of propolis, BioPure MTAD, NaOCl, and CHX on the viability of some endodontic organisms associated with refractory endodontic infections.

Methods. This study was performed in the Faculty of Dentistry and Pharmacy at Erciyes University, Kayseri, Turkey from February to April 2010. Origin, extraction, and chemical analysis of propolis. A poplar propolis sample was collected from Kayseri (Central Anatolia) in Turkey. The hand-collected propolis sample was kept desiccated in the dark until it was processed. Subsequently, 30 g crude propolis was dissolved in 70% ethanol by shaking 3 times in a day for 3 days. The aqueous ethanol extract was filtered through a Whatman 1 paper, and evaporated at 50°C. The resin obtained was dissolved in 70% ethanol to a final concentration of 9.6 mg/ml. Ethanol extract of propolis (EEP) was employed for the antimicrobial assays. The propolis was analyzed by gas chromatography-mass spectrometry (GC-MS).[10,11] The main compounds of propolis were flavonoids (mainly chrysin), aromatic and fatty acids, alcohol, and ketones. Chrysin (10 %) was the major component of propolis.[10,11]

Susceptibility testing. The strains of *E. faecalis* American Type Culture Collection (ATCC) 29212 and *C. albicans* ATCC 90028 obtained from the ATCC (Manassas, VA, USA) were used in this study. Antimicrobial activity testing was performed using the macrobroth dilution method according to the Clinical Laboratory Standards Institute (CLSI) guidelines. By adjusting density standards (Phoenix Spec Nephelometer, Becton Dickinson, NJ, USA), the microorganism suspensions were prepared to equal the density of a 0.5 McFarland standard (10^8 CFU/ml for bacteria, 1-5 x 10^6 CFU/ml for yeast). Stock solutions were used as follows: Group 1 - EEP (9.6 mg/ml); Group 2 - BioPure MTAD (30 mg/ml doxycycline hyclate) (Dentsply, Tulsa, OK, USA); Group 3 - 2% CHX (Ceraxidin-C, IMICRYL Corporation, Konya, Turkey); Group 4 - 5% NaOCL (WERAX, SDD, Izmir, Turkey). In this study, Mueller-Hinton broth medium for *E. faecalis* and Sabouraud dextrose broth medium for *C. albicans* were used. Proper media for microorganisms were prepared using sterile broth medium to give final concentrations of 2.4-0.002 mg/ml for propolis, 0.512-0.000125 mg/ml for CHX, and 1:2-1:4096 dilutions for NaOCL and BioPure MTAD. Microorganisms were then inoculated as $5x10^5$ colony forming unit (CFU)/ml for *E. faecalis,* and $2.5x10^3$ CFU/ml for *C. albicans* in the final concentrations. The *C. albicans* was incubated at 37°C for 24-48 hours, and *E. faecalis* at 37°C for 24 hours. Control samples included: growth control; only microorganism, negative control; only medium, solvent control; and ethanol (concentrations ranged from 70-0.03%). All determinations were performed twice. The minimum inhibitory concentration (MIC) was defined as the lowest concentration that did not result in any visible growth of the microorganisms compared with the growth in the control tubes. The minimum bactericidal concentration (MBC) was determined by spreading samples from each tube with a concentration

Disclosure. The authors declare that they have no conflicting interest, not supported or funded by any drug company.

equal, or higher than the MIC onto the surface of sheep blood agar plates for *E. faecalis* and Sabouraud dextrose agar plates for *C. albicans*, and then the *C. albicans* was incubated at 37°C for 24-48 hours, and *E. faecalis* was incubated at 37°C for 24 hours. The MBC was determined to be the lowest concentration that precluded bacterial growth on the agar plate.

Results. The results of the MIC and MBC are listed in **Tables 1 & 2**. Propolis and other irrigants were found to be effective on *C. albicans* and *E. faecalis*. Propolis and NaOCl were more effective in lower concentrations on *C. albicans* than on *E. faecalis*. In contrast, CHX and MTAD were more effective in lower concentrations on *E. faecalis* than on *C. albicans*. While propolis was more effective in lower concentrations on *C. albicans* than in CHX, CHX was more effective in lower concentrations on *E. faecalis* than in propolis. The MTAD was more effective in lower concentrations in both *C. albicans* and *E. faecalis* than NaOCL. While the inhibition effect of 70% ethanol was observed on tested microorganisms, ethanol (from 17.5-0.03%) that was used in EEP dilutions had no inhibition effect on the tested microorganisms.

Discussion. In this study, all the tested irrigants were shown to inhibit and eliminate the tested strains. The use of the most effective antimicrobial irrigant has clinical importance for successful endodontic treatment. The NaOCl solution is widely used as a root canal irrigant, but the optimal concentrations of the solution is controversial.[2] Higher concentrations have an increased toxicity, and can irritate periapical and periodontal tissues.[4,12] Its antimicrobial activity is proportional to the drug concentration. The recommended acceptable cytotoxic level of NaOCl is 0.5%, but this concentration is less effective.[2]

The CHX is an agent that has been used in periodontology for more than 20 years due to its antimicrobial properties and low cytotoxicity.[13] It can be used as an endodontic irrigant,[14] and as an intracanal medicament.[15] BioPure MTAD has been described as a universal irrigating solution.[16] It can eliminate bacteria in human root canals that have been infected by whole saliva.[17] The antimicrobial effect of MTAD, Tetraclean, Cloreximid, and NaOCl on *E. faecalis*, *Porphyromonas gingivalis, Prevotella intermedia* was studied by Giardino et al,[18] and 5.25% NaOCl showed the highest antimicrobial activity against anaerobic bacteria. The MTAD and Tetraclean showed a high antimicrobial activity against both strictly anaerobic and facultative anaerobic bacteria. The CHX + Cloreximid showed the lowest antibacterial activity against both the facultative and strictly anaerobic bacteria tested. An investigation was carried out to determine the antimicrobial effect of MTAD as a final irrigant on 8 strains of *E. faecalis*, and the MIC/ minimum lethal concentration (MLC) tests showed that MTAD inhibited the growth of most strains of *E. faecalis* when diluted 1:8192 times, and killed most strains of *E. faecalis* when diluted 1:512 times.[19] In our study, MTAD inhibited *E. faecalis'* growth when diluted 1:2048, and killed *E. faecalis* when diluted 1:1024.

Torabinejad et al[7] showed that MTAD is significantly more effective than NaOCl in killing *E. faecalis* when the solutions are diluted. This superior antimicrobial effect of MTAD was also shown in our study. The superior bactericidal effect of MTAD may have been caused by a carry over effect of doxycycline. Furthermore, the antimicrobial effect of doxycycline against several oral pathogens was shown in previous studies.[20-22] Using the contact test, Sassone et al[23] showed that 0.12% CHX was ineffective against *E. faecalis*, while 0.5% CHX, 1% CHX, 1% NaOCl, and 5% NaOCl were antibacterial against *E. faecalis*. Valera et al[24] reported that 1% NaOCl was effective in considerably reducing *C. albicans* and *E. faecalis* counts immediately after root canal preparation.

In another investigation,[25] the antimicrobial activity of varying concentrations of NaOCL (0.5%, 1.0%, 2.5%, and 5.25% w/v) on the endodontic microorganisms was studied. All concentrations of NaOCl lowered cfu below the limit of detection after 10 seconds in the case of *C. albicans*. The *E. faecalis* proved to be more resistant to NaOCl. Using 0.5%

Table 1 - Minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) values of irrigants.

Irrigants	*Candida albicans*		*Enterococcus faecalis*	
	MIC	MBC	MIC	MBC
	mg/ml			
Propolis (9.6 mg/ml)	0.075	0.150	0.3	0.6
2% chlorhexidine gluconate	0.512	0.512	0.032	0.256

Table 2 - Minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) values of BioPure MTAD and sodium hypochlorite.

Irrigants	*Candida albicans*		*Enterococcus faecalis*	
	MIC	MBC	MIC	MBC
	Dilution			
BioPure MTAD (30 mg/ml doxycycline hyclate)	1:32	1:32	1:2048	1:1024
5% sodium hypochlorite	1:16	1:16	1:8	1:4

NaOCl for 30 minutes reduced cfu to zero for both strains tested. This compares with 10 minutes for 1.0%, 5 minutes for 2.5%, and 2 minutes for 5.25%.[25] Ruff[26] showed that 6% NaOCl and 2% CHX were equally effective and statistically significantly superior to BioPure MTAD, and 17% EDTA in antifungal activity on *C. albicans*. In a dilution test studied by Estrela et al,[27] NaOCl solution showed MIC equal to 0.1% for *E. faecalis* and *C. albicans*, and CHX (2%) presented MIC equal to 0.02% for *E. faecalis* and *C. albicans*. Despite the fact that NaOCL and CHX showed antimicrobial effects against *E. faecalis* and *C. albicans* in our study, the MIC and MBC values were different from those reported in previous studies.[25-27] These differences in antimicrobial susceptibility tests are due to variations in methodology, since several factors (inoculum amount, medium composition, pH, incubation) can influence the interaction between microorganisms and antimicrobial agents, thus affecting the value obtained for MIC.

The resinous hive product has been used as a remedy for treatment of many diseases in folk medicine since ancient times.[28] Many studies showed that EEP was effective on important virulence factors and biofilm formation in *Staphylococcus aureus* and mutant *Streptococci*.[29-31] Furthermore, EEP presents good properties for endodontic use, such as promoting bone regeneration and inducing hard tissue bridge formation in pulpotomies, or pulp capping.[32,33] In addition to its antimicrobial activity, propolis is accepted as safe in low doses.[28]

Organic and inorganic compositions of propolis have been reported to vary greatly depending on the region where bees collect the samples. Because of the changing plant variety and limited bee travel distance from the collected propolis to places of deposit, the composition of propolis may change in the same region.[34] Therefore, before testing propolis in laboratory and clinical studies, chemical analysis of propolis should be performed. Using the microbroth dilution method, Silici and Koc[10] showed that propolis (collected from Kayseri, Turkey) was active against yeast isolated from patients, and that the MIC value of propolis was 3.75 µg/ml. However in our study, the MIC value of propolis was 75 µg/ml against *C. albicans*. The differences of MIC values between these 2 studies could be attributed to different methodologies. Oncag et al[35] observed that propolis had good in vitro antibacterial activity against *E. faecalis* in the root canals of extracted teeth, suggesting that it could be used as an alternative intracanal medicament. In a study of the effectiveness of propolis and calcium hydroxide as a short-term intracanal medicament against *E. faecalis*, the results showed that Jordanian propolis (30% propolis) was significantly more effective than non-setting calcium hydroxide against *E. faecalis*.[36] The propolis extract in our study was 20 times more effective in MIC than it was in Ferreira's study[37] against *E. faecalis*. In a macrobroth dilution test studied by Ferreira et al,[37] Brazilian propolis and other substances were effective against *E. faecalis*. The values of Brazilian propolis was 6425 µg/ml for MIC, and 7640 µg/ml for MBC. In our study, effective propolis concentration against *E. faecalis* was found to be lower. This positive effect in our study was attributed to differences in propolis origins and compositions. The composition of propolis depends upon the vegetation of the area from which it is collected.[34] Propolis from temperate zones (Asia, Europe, North America, and so forth) contains predominantly phenolic compounds, including several flavonoids, aromatic acids, and their esters collected from poplar buds.[38] In the poplar propolis sample used in our study, the major component was chrysin (flavonoid). Flavonoids are the most important pharmacologically active, and they are thought to account for much of the antimicrobial activity in propolis.

In this study, some samples were prepared as dilution and some of them as concentration. Therefore, all results were not comparable to each other. This condition is an important limitation of our study.

In conclusion, propolis showed an antimicrobial effect on *E. faecalis* and *C. albicans* as in other tested irrigants in this study. Additional clinical and laboratory studies should be performed to evaluate the beneficial use of propolis as an intracanal irrigant, or any other endodontic material.

References

1. Menezes AC, Zanet CG, Valera MC. Smear layer removal capacity of disinfectant solutions used with and without EDTA for the irrigation of canals: a SEM study. ***Pesqui Odontol Bras*** 2003; 17: 349-355.
2. Tirali RE, Turan Y, Akal N, Karahan ZC. In vitro antimicrobial activity of several concentrations of NaOCl and Octenisept in elimination of endodontic pathogens. ***Oral Surg Oral Med Oral Pathol Oral Radiol Endod*** 2009; 108: 117-120.
3. Ercan E, Ozekinci T, Atakul F, Gül K. Antibacterial activity of 2 % chlorhexidine and 5.25 % sodium hypochlorite in infected root canal: in vivo study. ***J Endod*** 2004; 30: 84-87.
4. Gomes-Filho JE, Aurelio KG, Costa MM, Bernabe PF. Comparison of the biocompatibility of different root canal irrigants. ***J Appl Oral Sci*** 2008; 16: 137-144.
5. Davdakis C, Lambrianidis T, Boura P. Immunonogic evaluation of dental patient with history of hypersensitivity reaction to sodium hypochlorite. ***Endod Dent Traumatol*** 2000; 16: 184-187.
6. Mohammadi Z, Abbott PV. The properties and applications of chlorhexidine in endodontics. ***Int Endod J*** 2009; 42: 288-302.
7. Torabinejad M, Shabahang S, Aprecio R, Kettering JD. The antimicrobial effect of MTAD: An in vitro investigation. ***J Endod*** 2003; 29: 400-403.
8. Sforcin JM. Propolis and the immune system: a review. ***J Ethnopharmacol*** 2007; 113: 1-14.

9. Castaldo S, Capasso F. Propolis, an old remedy used in modern medicine. ***Fitoterapia*** 2002; 73: 1-6.
10. Silici S, Koc AN. Comparative study of in vitro methods to analyze the antifungal activity of propolis against yeasts isolated from patients with superficial mycoses. ***Lett Appl Microbiol*** 2006; 43: 318-324.
11. Ozbilge H, Gunduz Kaya E, Albayrak S, Silici S. Anti-leishmanial activities of ethanolic extract of Kayseri propolis. ***African Journal of Microbiology Research*** 2010; 4: 556-560.
12. Tanomaru Filho M, Leonardo MR, Silva LA, Anibal FF, Faccioli LH. Inflammatory response to different endodontic irrigating solutions. ***Int Endod J*** 2002; 35: 735-739.
13. Berchier CE, Slot DE, Vander Weijden GA. The efficacy of 0.12 % chlorhexidine mouth rinse compared with 0.2 % on plaque accumulation and periodontal parameters: a systematic review. ***J Clin Periodontol*** 2010; 37: 829-839.
14. Roças IN, Siqueira JF Jr. Comparison of the in vivo antimicrobial effectiveness of sodium hypochlorite and chlorhexidine used as root canal irrigants: a molecular microbiology study. ***J Endod*** 2011; 37: 143-150.
15. Ercan E, Dalli M, Dulgergil CT, Yaman F. Effect of intracanal medication with calcium hydroxide and 1 % chlorhexidine in endodontic retreatment cases with periapical lesions: an in vivo study. ***J Formos Med Assoc*** 2007; 106: 217-224.
16. Torabinejad M, Khademi AA, Babagoli J, Cho Y, Johnson WB, Bozhilov K, et al. A new solution for the removal of the smear layer. ***J Endod*** 2003; 29: 170-175.
17. Shabahang S, Aprecio R, Kettering JD. In vitro antimicrobial efficacy of MTAD and sodium hypochlorite. ***J Endod*** 2003; 29: 400-403.
18. Giardino L, Savoldi E, Ambu E, Rimondini R, Palezone A, Debbia EA. Antimicrobial effect of MTAD, tetraclean, Cloreximid, and sodium hypochlorite on three common endodontic pathogens. ***Indian J Dent Res*** 2009; 20: 12-15.
19. Newberry BM, Shabahang S, Johnson N, Aprecio RM, Torabinejad M. The antimicrobial effect of biopure MTAD on eight strains of E. faecalis: an in vitro investigation. ***J Endod*** 2007; 33: 1352-1354
20. van der Vyver PS, Botha FS, Herbst D, Ismail M. Antimicrobial efficacy and irrigating potential of irrigation solutions using different activation methods. ***SADJ*** 2009; 64: 56, 58-60, 62.
21. Le Corn DW, Vertucci FS, Rojas MF, Progulske-Fox A, Belanger M. In vitro activity of amoxicillin, clindamycin, doxycycline, metronidazole, and moxifloxacin against oral Actinomyces. ***J Endod*** 2007; 33: 557-560.
22. Lakhssassi N, Elhajoui N, Lodter JP, Pineill JL, Sixou M. Antimicrobial susceptibility variation of 50 anaerobic periopathogens in aggressive periodontitis: an interindividual variability study. ***Oral Microbiol Immunol*** 2005; 20: 244-252.
23. Sassone LM, Fidel RAS, Murad CF, Fidel SR, Hirata R. Antimicrobial activity of sodium hypochlorite and chlorhexidine by two different tests. ***Aust Endod J*** 2008; 34: 19-24.
24. Valera MC, Silva KCG, Maekawa LK, Carvalho CAT, Koga-ito CY, Camargo CHR, et al. Antimicrobial activity of sodium hypochloride associated with intracanal medication for *Candida albicans* and *Enterecoccus faecalis* inoculated in root canals. ***J Appl Oral Sci*** 2009; 17: 555-559.
25. Radcliffe CE, Potouridou L, Qureshi R, Habahbeh N, Qualtrough A, Worthington H, et al. Antimicrobial activity of varying concentrations of sodium hypochlorite on the endodontic microorganisms *Actinomyces israelli, A. naeslundii, Candida albicans* and *Enterococcus faecalis*. ***Int Endod J*** 2004; 37: 438-446.
26. Ruff ML. In vitro antifungal efficacy of four irrigants as a final rinse. ***J Endod*** 2006; 32: 331-333.
27. Estrela CRA, Estrela C, Reis C, Bammann LL, Pecora JD. Control of microorganisms in vitro by endodontic irrigants. ***Braz Dent J*** 2003; 14: 187-192.
28. Astaldo S, Capasso F. Propolis, an old remedy used in modern medicine. ***Fitoterapia*** 2002; 73: 1-6.
29. Scazzocchio F, D'Auria FD, Alessandrini D, Pantanella F. Multifactorial aspects of antimicrobial activity of propolis. ***Microbial Res*** 2006; 161: 327-333.
30. Koru O, Toksoy F, Acikel CH, Tunca YM, Baysallar M, Guclu AU, et al. In vitro antimicrobial activity of propolis samples from different geographical origins against certain oral pathogens. ***Anaerobe*** 2007; 13: 140-145.
31. Duarte S, Rosalen PL, Hayacibara MF, Cury JA, Bowen WH, Marquis RE, et al. The influence of a novel propolis on mutans streptococci biofilms and caries development in rats. ***Arch Oral Biol*** 2006; 51: 15-22.
32. Pileggi R, Antony K, Johnson K, Zuo J, Shannon Holiday L. Propolis inhibits osteoclast maturation. ***Dent Traumatol*** 2009; 25: 584-588.
33. Sabir A, Tabbu CR, Agustiono P, Sosroseno W. Histological analysis of dental pulp tissue capped with propolis. ***J Oral Sci*** 2005; 47: 135-138.
34. Seidel V, Peyfoon E, Watson DG, Fearnley J. Comparative study of the antibacterial activity of propolis from different geographical and climatic zones. ***Phytother Res*** 2008; 22: 1256-1263.
35. Oncag O, Cogulu D, Uzel A, Sorkun K. Efficacy of propolis as an intracanal medicament against *Enterococcus faecalis*. ***Gen Dent*** 2006; 54: 319-322.
36. 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. ***Aust Endod J*** 2009; 35: 52-58.
37. Ferreira FB, Torres SA, Rosa OP, Ferreira CM, Garcia RB, Marcucci MC, et al. Antimicrobial effect of propolis and other substances against selected endodontic pathogens. ***Oral Surg Oral Med Oral Pathol Oral Radiol Endod*** 2007; 104: 709-716.
38. Uzel A, Kadriye S, Önçag Ö, Çogulu D, Gençay Ö, Salih B. Chemical compositions and antimicrobial activities of four different Anatolian propolis samples. ***Microbiol Res*** 2005; 160: 189-195.