Evaluation of antimicrobial efficacy of cetrимide and *Glycyrrhiza glabra* L. extract against *Enterococcus faecalis* biofilm grown on dentin discs in comparison with NaOCl

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This study aimed to determine the antimicrobial efficacy of NaOCl, cetrимide, and *Glycyrrhiza glabra* L. extract against *Enterococcus faecalis* biofilms on dentine discs. Broth microdilution method was used to determine minimal bactericidal concentrations (MBCs) of the agents. A biofilm susceptibility assay was performed using *E. faecalis* biofilms grown on dentine discs. Minimal bactericidal concentrations (MBCs) of NaOCl (0.5%), cetrимide (0.015%), and *G. glabra* L. extract (0.25%) were applied for 1, 3, and 5 min, and the mean viable cell counts were recorded and statistically analyzed. There was no significant difference between cetrимide and NaOCl at 1 min ($p>0.05$). NaOCl was the most effective agent at 3 and 5 min ($p<0.05$) while *G. glabra* L. extract was the least ($p<0.05$). The MBCs of NaOCl, cetrимide, and *G. glabra* that eliminated the planktonic *E. faecalis* did not eradicate the biofilms grown on dentin discs.

Keywords: Biofilm, Cetrимide, *Enterococcus faecalis*, *Glycyrrhiza glabra* L., Sodium hypochlorite

INTRODUCTION

Planktonic bacterial cells form complex structurally and dynamically organized biological systems *via* binding to host proteins and coaggregating with other bacteria, which is called “biofilm”. Biofilms in dentinal tubules, apical canal ramifications, isthmuses, and irregularities can resist to disinfection protocols during root canal therapy¹. *Enterococcus faecalis* is the most commonly recovered species from the teeth of failed root canal treatments and persistent root canal infections, with a prevalence of more than 70%².

Sodium hypochlorite (NaOCl) has excellent tissue dissolution properties and antimicrobial activity, however its cytotoxicity and allergic potential have compelled researchers to develop new irrigation materials and protocols³.

Cetrимide is a cationic surface-active agent (surfactant), and in endodontics, it is typically used in combination with other irrigants, as it increases the effectiveness of endodontic irrigants by reducing the surface tension^{4,5}. Recently, the antimicrobial and biofilm dissolving abilities of cetrимide were demonstrated⁶⁻⁸. When used as a single agent, 0.1% cetrимide showed antibacterial activity that was comparable to that of 2% NaOCl and 2% chlorhexidine⁸. Natural products, especially herbal extracts, have been used in medicine and shown to be good alternatives to synthetic chemicals⁹. Antimicrobial activity of *G. glabra* L. extract is associated with its content Glycyrrhizin^{9,10}. Glycyrrhizin is a tripartenic saponin and comprises the

major component of *Glycyrrhiza glabra* L. It demonstrated anti-inflammatory, antimicrobial, anticarcinogenic and hepatoprotective effects⁹. In dental literature, *G. glabra* L. showed a markedly inhibition of bacterial growth and adherence of cariogenic *Streptococcus mutans* and *E. Faecalis*¹¹⁻¹³.

The aim of this study was to determine the antimicrobial efficacy of cetrимide and *G. glabra* L. extract against *E. faecalis* biofilms grown on dentine discs by using the agents at their respective minimal bactericidal concentrations (MBCs) for three different exposure durations in comparison with NaOCl.

MATERIALS AND METHODS

Preparation of the antimicrobial agents

G. glabra L. extract was obtained using *G. glabra* roots and rhizomes. The concentrated extract was standardized to contain 2% Glycyrrhizin by analyzing high performance liquid chromatography (HPLC)^{14,15}. Cetrимide (Alkyltriethylammoniumbromide) powder (Sigma Chemical, Steinheim, Germany) was used to obtain 2% solution. NaOCl 2% (Wizad, Rehber Chemicals, Istanbul, Turkey) and sterile saline (Polifarma, Tekirdag, Turkey) solutions were used.

Determination of MBCs of antimicrobial agents

The disc diffusion method was used to test the antimicrobial activity of the solutions against planktonic *E. faecalis* (ATCC 29212). Six millimeter paper discs were saturated with 10 µL of each solution (NaOCl, cetrимide, *G. glabra* extract and sterile saline) and placed onto Muller-Hinton Broth (MHB) plates that

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were pre-adsorbed with bacterial cells. Tests were performed in duplicate, incubated at 37°C for 24 h, and the growth of inhibition zones were measured. The broth microdilution method was used to determine the minimal inhibitory concentrations (MICs) and minimal bactericidal concentrations (MBCs) of the test agents according to the CLSI Standards¹⁶.

Biofilm model

Under a protocol approved by the Ethics Committee of the Suleyman Demirel University (27.12.2011-no:27/8.), 228 freshly extracted human molars without caries or fillings were collected and embedded in polyester resin. Standard dentin discs ($n=228$) with 6 mm diameter and 2 mm thickness were obtained from the corona of the

tooth and stored in phosphate buffered saline (PBS) at 4°C until use. Stainless steel clamps were attached to the lid of the 24 well polystyrene microtiter plates (Costar, Corning, USA), and the dentin discs were fixed to the clamps to modify Calgary biofilm model¹⁷. The dentin discs were vertically positioned in the center of the wells (Fig. 1). They were sterilized with ethylene oxide following removal of the smear layer with EDTA.

The experimental groups ($n=12$) are shown in Table 1. In each group ($n=19$), 1 sample was used to assess the sterilization of the dentin discs, 1 sample to demonstrate biofilm formation by field emission scanning electron microscopy (FE-SEM, Supra 55, Zeiss, Jena, Germany), 4 samples to determine the initial bacterial counts in the biofilm on the dentin discs, 12 samples to evaluate

Table 1 Experimental groups formed after determination of the MBC's of antimicrobial agents

Antimicrobial agent	Concentration(%)	Duration (min)	<i>n</i>
Cetrimide	0.015	1	19
Cetrimide	0.015	3	19
Cetrimide	0.015	5	19
<i>G.glabra</i> extract	0.25	1	19
<i>G.glabra</i> extract	0.25	3	19
<i>G.glabra</i> extract	0.25	5	19
NaOCl	0.5	1	19
NaOCl	0.5	3	19
NaOCl	0.5	5	19
Sterile saline (negative control)	0.9	1	19
Sterile saline (negative control)	0.9	3	19
Sterile saline (negative control)	0.9	5	19

Fig. 1 Stainless steel clamps attached to the lid of the 24 well polystyrene plates, dentin discs fixed to the clamps for the biofilm formation.

biofilm elimination, and 1 sample to determine the antimicrobial efficacy of each agent by FE-SEM (Fig. 1). For the biofilm formation, *E. faecalis* (ATCC 29212) suspensions in MHB were adjusted to 0.5 McFarland, and then diluted 1:10 in MHB. All the wells of the plate, except the sterilization control well, were filled with 1 mL of this dilution. The sterilization control well was filled with 1 mL of MHB. The lid was closed, and the plate was placed on a 3D rotary shaker for 24 h at 37°C. To test the sterilization, 10 μ L aliquots from the sterilization well were plated on blood agar. The remaining dentin discs were placed into wells filled with 1 mL of 0.9% saline, and the plate was placed on a rotary shaker for 1 min to remove the loosely adherent planktonic bacteria. To assess biofilm formation, 1 sample was removed from the lid, and it was examined by FE-SEM after fixation and dehydration. To count the initial bacteria on the dentin discs, 4 samples were removed from the lid, placed in 1 mL of 0.9% saline and sonicated in an ultrasonic water bath for 5 min. Then, the disrupted biofilms were serially diluted in 0.9% saline, and 10 μ L aliquots were plated onto MHA. The number of colony forming units were counted and recorded after incubation for 24 h at 37°C.

Elimination of the biofilm

Biofilm susceptibility assay was performed in another plate. The wells were filled with 1 mL of a test antimicrobial agent, the lid with the dentin discs was submerged into the wells for 1, 3, or 5 min, and then the lid was transferred to two additional plates. First, it was transferred to a neutralizing plate, containing 1 mL of 0.9% saline per well. Then, it was transferred

to a recovery plate containing 1 mL of 0.9% saline per well and sonicated for 5 min to disrupt the biofilm. The viability of the bacteria was determined by inoculating 10 μ L aliquots from each recovery plate well onto MHA and incubating the agar plates for 24 h at 37°C. Mean viable cell counts were recorded and were log10 transformed to prepare the data for statistical analysis.

FE-SEM evaluation

FE-SEM, which has higher spatial resolution than conventional SEM was used to assess the formation and disruption of biofilms and micrographs were obtained at different magnifications (from 500 $\times$ to 20,000 $\times$).

Statistical analysis

Analysis of Variance (ANOVA) was used to evaluate the differences between the mean bacterial counts (log10) of the biofilm grown on dentin discs before and after exposure to antimicrobial agents. Differences between the bacterial counts in each exposure duration were compared with Tukey's test at a significance level of $p < 0.05$.

RESULTS

Cetrimide showed the largest inhibition zone (26 mm) with disc diffusion method, followed by *G. glabra* L. (9 mm) and NaOCl (9 mm) (Fig. 2). The MICs and MBCs of cetrimide, *G. glabra* L., and NaOCl were determined as 0.00195%, 0.015%, 0.062% and 0.25%, 0.125%, 0.5%, respectively (Table 2). The negative control sterile saline solution showed no inhibitory effect on *E. Faecalis*. The

Fig. 2 Inhibition zones of the tested agents with disc diffusion method.

Table 2 Minimum inhibition (MIC) and minimum bactericidal concentration (MBC) percentages of tested agents over *Enterococcus faecalis* (ATCC 29212)

Antimicrobial agent	MIC (%)	MBC (%)
Cetrimide	0.00195	0.015
<i>G. glabra</i> extract	0.062	0.25
NaOCl	0.125	0.5
Sterile Saline (Negative Control)	Ø	Ø

FE-SEM images of the control samples showed biofilm formation on the dentin discs (Fig. 3). Within 24 h, *E. faecalis* was able to form biofilms on the dentin discs,

Fig. 3 Representative FE-SEM image of dentine disks inoculated with *E. faecalis* for 24 h. Dense bacterial aggregates were observed covering the surface of the dentin. The structures that connected bacteria to the surface and each other could be seen at 5,000× magnification.

with an average of $6.6 \pm 0.2 \log_{10}$ CFU/dentin disc. There was no statistically significant difference in the initial bacterial counts among the groups ($p > 0.05$).

Biofilm disruption after exposure to the antimicrobial agents was observed by FE-SEM (Fig. 4). Change of the mean surviving bacterial count ($\log_{10}$) by time after dentin discs were exposed to antimicrobial agents is shown in Tables 3 and 4. Significant differences were found among the groups in terms of the antimicrobial agents used and the exposure durations ($p < 0.01$). The negative control group did not show any significant effect against biofilms in 1, 3, and 5 min ($p < 0.05$). While there was no significant difference between cetrimide and NaOCl ($p > 0.05$) in 1 min, NaOCl was the most effective agent in 3 and 5 min applications. NaOCl achieved a mean reduction of approximately 4 $\log_{10}$ units, which was highly significant ($p < 0.001$), and complete eradication was observed in 25% of the samples in 5 min application. Five min exposure to cetrimide resulted in a mean reduction of approximately 3.5 $\log_{10}$ units ($p < 0.001$), and complete eradication was observed in 8.3% of the samples. *G. glabra* L. was the least effective agent in all exposure durations ($p < 0.05$), and a 5 min exposure to *G. glabra* L. yielded a reduction of 1.6 $\log_{10}$ units ($p < 0.05$).

Fig. 4 Representant images of dentin disk after exposure to cetrimide (a, b, c), *G. glabra* extract (d, e, f) and NaOCl (g, h, i) for 1 (a, d, g), 3 (b, e, h) and 5 (c, f, i) min.

Table 3 Change of the mean surviving bacterial count (log10) by time, after dentin disks were exposed to antimicrobial agents (CFU/Dentin Disk)

	Non-treated	1 min	3 min	5 min
0.015% Cetrimide	6.7±0.1 ^{Aa}	5.4±0.3 ^{Cb}	4.2±0.2 ^{Cc}	3.2±0.2 ^{Cd}
0.25% <i>G. glabra</i> extract	6.6±0.3 ^{Aa}	6.1±0.1 ^{Bb}	5.5±0.2 ^{Bc}	5.0±0.1 ^{Bd}
0.5% NaOCl	6.6±0.2 ^{Aa}	5.4±0.2 ^{Cb}	4.0±0.2 ^{Dc}	2.6±0.3 ^{Dd}
Sterile saline	6.6±0.2 ^{Aa}	6.6±0.2 ^{Aa}	6.6±0.2 ^{Aa}	6.6±0.2 ^{Aa}

* Superscript different uppercase letters indicate significances among the antimicrobial agents for each exposure duration, different lowercase letters indicate significances among the exposure durations for each antimicrobial agent ($p<0.05$).

Table 4 Mean numbers of recovered bacteria in control and treatment groups

Duration	Antimicrobial agent	<i>n</i>	Mean log CFU ±SD	Mean log red.	NnG (%)
0	0.015% Cetrimide	12	6.7±0.1 ^a	—	—
	0.25% <i>G. glabra</i> extract	12	6.6±0.3 ^a	—	—
	0.5% NaOCl	12	6.6±0.2 ^a	—	—
	Sterile saline	12	6.6±0.2 ^a	—	—
1	0.015% Cetrimide	12	5.4±0.3 ^z	1.3	0 (0)
	0.25% <i>G. glabra</i> extract	12	6.1±0.1 ^y	0.5	0 (0)
	0.5% NaOCl	12	5.4±0.2 ^z	1.2	0 (0)
	Sterile saline	12	6.6±0.2 ^{ax}	0.1	0 (0)
3	0.015% Cetrimide	12	4.2±0.2 ^γ	2.5	0 (0)
	0.25% <i>G. glabra</i> extract	12	5.5±0.2 ^β	1.1	0 (0)
	0.5% NaOCl	12	4.0±0.2 ^δ	2.6	0 (0)
	Sterile saline	12	6.6±0.2 ^{aa}	0.0	0 (0)
5	0.015% Cetrimide	12	3.2±0.2 ^v	3.5	1 (8,3)
	0.25% <i>G. glabra</i> extract	12	5.0±0.1 ^μ	1.6	0 (0)
	0.5% NaOCl	12	2.6±0.3 ^ε	4.0	3 (25)
	Sterile saline	12	6.6±0.2 ^{al}	0.0	0 (0)

n, number of samples per group; Mean log CFU, mean logarithmic number of colony-forming units; SD, standard deviation; mean log red., mean logarithmic reduction; NnG, number of dentin disks with no growth. Superscript different letters indicate significances between the groups ($p<0.05$).

DISCUSSION

In the present study, a biofilm model was designed to evaluate the effectiveness of antimicrobial agents, since in persistent endodontic infections; bacteria are mostly organized as biofilms which can be 100–1,000 fold more resistant to antimicrobial agents compared to their planktonic counterparts¹⁷. Calgary biofilm model, main disadvantage of which is that biofilms are formed on the polished surface of the polystyrene surfaces was modified using dentin discs as substrates for biofilm formation to simulate the roughness and dentin tubules in root canal wall. It is a quick method, provides statistically standardized biofilms and eliminates potential contamination¹⁷. This was proved by confirming no significant difference among the initial bacterial counts of the groups ($p>0.05$). However, a weakness of this

model is that the biofilms on dentin discs are directly exposed to the antimicrobial agents, which differs from their exposure during root canal irrigation. The test microorganism was chosen as *E. faecalis*, since it is known to be associated with failed root canal treatments, and its ATCC 29212 strain was used due to its ability to form biofilms^{6,7,13,18}.

Antimicrobial agents have to be able to penetrate into the extracellular matrix or to break of the bonds in the biofilm to eliminate the microorganisms. The following features are taken into account in the selection of the antimicrobial agents for this study. NaOCl is an agent antimicrobial activity and organic tissue dissolubility of which are proven. It can diffuse into the biofilm by disrupting nucleic acids and proteins of extracellular matrix.

Positively charged hydrophilic portion of cetrimide

interact with negatively charged phospholipids in the membrane structure of bacteria cells and impair the selective permeability of the bacterial cell membrane¹⁹. When the cetrимide enters the cell the enzymes within the cell and its organelles denature²⁰. It was reported that cetrимide is an effective agent on biofilms^{8,18}. It reduces the structural resistance of biofilms by affecting electrostatic bonds in the extracellular matrix. The reasons for choosing this agent for the present study were the residual activity for 40 days at 0.02% concentration and the less cytotoxicity than NaOCl^{21,22}.

Triterpenoid saponins Glycyrrhizin interact with membrane lipids and disrupt the cell membrane integrity causing the cells to leave the intracellular organelles²³. Flavonoids, the other components of *G. glabra* extract are also responsible for the antimicrobial activity by inhibiting the cell membrane electron transport chain²⁴. As well as the antimicrobial activity of saponins, lowering the surface tension was also reported^{13,23}. *Glycyrrhiza glabra* extract containing 7–7.5% glycyrrhizin was found advantageous in terms of cytotoxicity because 87% of periodontal ligament fibroblasts were alive after 48 h¹³.

The effective concentrations of NaOCl (0.05–6%) and cetrимide (0.2–7.5%) in endodontic irrigation are well known, whereas the concentration of the *G. glabra* L. extract is not reported^{7,18,25,26}. For endodontic irrigation, it is important to select the lowest effective concentration of the antibacterial agents to result in the less cytotoxic effect. Therefore, MBCs of the agents used should be known. Thus, this study aimed to compare the chosen agents in the level of their minimum effective concentrations. Efficacy of the MBCs of Cetrимide (0.25%), *G. glabra* L. (0.125%) and NaOCl (0.5%) were examined on biofilm elimination. However, it is predicted that their efficacy may increase at higher concentrations.

In this study, although NaOCl and cetrимide showed similar antimicrobial effects on biofilms in 1 min ($p>0.05$), NaOCl was the most effective agent in 3 and 5 min. Three and 5 min applications of cetrимide remarkably reduced the viable counts; however, it did not reach the efficacy of NaOCl. The reduction of bacterial count was considered to be dependent on the antimicrobial agent used and the exposure duration ($p<0.01$). It is well known, that NaOCl is able to disrupt and dissolve biofilms^{18,27–29}. Meire *et al.*²⁹ reported that treatment with 2.5% NaOCl solution for 5 min or longer completely eliminated *E. faecalis* biofilms on dentin discs; however, a 30 min treatment was required if 0.5% NaOCl was used. In the present study, 5 min exposure to 0.5% NaOCl achieved a mean reduction of approximately 4 log₁₀ units, which was highly significant ($p<0.001$), and complete eradication was seen in 25% of the samples. Arias-Moliz *et al.*³⁰ reported that, 1 min of contact with 0.00625% NaOCl eradicated *E. faecalis* biofilms that were grown on polystyrene pegs. It has been reported that dentine has a buffering ability that reduces the antibacterial effects of agents^{20,21,31,32}. Therefore, testing the efficacy of antimicrobial agents on dentine seems to be more realistic than testing with other non-dentine

models. In the present study, no complete eradication of the biofilms in some samples treated with 0.5% NaOCl, might be related to the buffering ability of dentine.

As a contradiction to the biofilm assay, the disc diffusion assay results showed that, cetrимide had the strongest antibacterial activity against *E. faecalis*. This might be attributed to its ability to spread out wider on the agar plate owing to its lower surface tension. On the other hand, the lower antibacterial activity of NaOCl might be related to OCl ions interact with the agar. In addition, the results of the disc diffusion assay in the present study showed that *G. glabra* L. and NaOCl had similar antibacterial effects. However, in the biofilm model used in this study, *G. glabra* L. was less effective than the other tested agents in all exposure durations ($p<0.05$). This might be attributed to the low saponin amount in 0.25% concentration of glycyrrhizin which can not disturb the extracellular matrix. While 5 min exposure to 0.25% *G. glabra* L. yielded a 1.6 log₁₀ unit reduction ($p<0.05$), biofilm eradication was not provided. This finding was in contrast to the results of Badr *et al.*¹³, who reported that *G. glabra* L. containing 7.5% glycyrrhizin and a mixture of Ca(OH)₂ were able to eliminate *E. faecalis* biofilms grown on cellulose nitrate filters after 48 h. The difference between these results can be explained by the different concentrations of the extracts and substrates chosen for the growing of biofilms.

In addition to its antimicrobial activity, cetrимide has also been reported to have the capacity to decrease the mechanical stability of biofilms by weakening the cohesive forces and disrupting extracellular polymeric substances³³. Wang *et al.*⁸ infected dentin specimens with an *E. faecalis* clinical strain (VP3-181) for 24 h, and they indicated that 1 min exposure to 0.1% cetrимide had comparable antibacterial activity to 2% NaOCl and 2% chlorhexidine, and 3 min exposure to cetrимide showed higher antimicrobial activity than NaOCl and chlorhexidine. Baca *et al.*⁷ reported that 0.2% cetrимide eliminated *E. faecalis* biofilms after 1 min, as did 2.5% NaOCl. Arias Moliz *et al.*⁶ reported that 1 min exposure to low concentration cetrимide (0.0312%) eradicated *E. faecalis* biofilms on polystyrene pegs. In contrast, Guerreiro-Tanomaru *et al.*¹⁸ reported that 2.5% NaOCl was more effective against *E. faecalis* biofilms than 0.2% cetrимide. In the present study, 0.015% cetrимide for 5 min exposure resulted in a reduction of 3.5 log₁₀ units, which was highly significant ($p<0.001$), and complete eradication was detected in 8.3% of samples in this group. It is found that biofilm dissolving activity of NaOCl is more effective than cetrимide at the MBC, and dentin reduced the antimicrobial activity of cetrимide more than that of NaOCl. As a cationic surfactant, cetrимide has acceptable cytotoxicity²¹ and residual antimicrobial effect, similar to that of chlorhexidine⁷. However, unlike chlorhexidine, cetrимide does not form a precipitate with NaOCl, and this is an important superiority. Due to these advantages, it can be a promising agent for use of root canal irrigation.

CONCLUSION

The MBCs of tested agents that eliminated the planktonic *E. faecalis* did not eradicate the biofilms grown on dentin discs. Although NaOCl was the most effective agent in 3 and 5 min, cetrimide showed remarkable antimicrobial effect on biofilms in 1 min similar to NaOCl. *G. glabra* extract had also displayed remarkable reductions of viable counts, whereas its efficacy against biofilms was lower than those of NaOCl and cetrimide. Further studies are needed to evaluate the appropriate concentrations and exposure durations of these new irrigation alternatives in clinical use.

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REFERENCES

- Nair PN. On the causes of persistent apical periodontitis: a review. *Int Endod J* 2006; 39: 249-281.
- Baumgartner JC, Siqueira JFJ, Sedgley CM, Kishen A. Microbiology of endodontic disease. In: Ingle IJ, Bakland LK, Baumgartner JC, editors. *Ingle's Endodontics*. USA: PMPH; 2007. p. 221-308.
- Basrani B, Haapasalo H. Update on endodontic irrigating solutions. *Endod Topics* 2012; 27: 74-102.
- Guerisoli DM, Marchesan MA, Walmsley AD, Lumley PJ, Pecora JD. Evaluation of smear layer removal by EDTAC and sodium hypochlorite with ultrasonic agitation. *Int Endod J* 2002; 35: 418-421.
- Lui JN, Kuah HG, Chen NN. Effect of EDTA with and without surfactants or ultrasonics on removal of smear layer. *J Endod* 2007; 33: 472-475.
- Arias-Moliz MT, Ferrer-Luque CM, Gonzalez-Rodriguez MP, Valderrama MJ, Baca P. Eradication of *Enterococcus faecalis* biofilms by cetrimide and chlorhexidine. *J Endod* 2010; 36: 87-90.
- Baca P, Junco P, Arias-Moliz MT, Gonzalez-Rodriguez MP, Ferrer-Luque CM. Residual and antimicrobial activity of final irrigation protocols on *Enterococcus faecalis* biofilm in dentin. *J Endod* 2011; 37: 363-366.
- Wang Z, Shen Y, Ma J, Haapasalo M. The effect of detergents on the antibacterial activity of disinfecting solutions in dentin. *J Endod* 2012; 38: 948-953.
- Asl MN, Hosseinzadeh H. Review of pharmacological effects of *Glycyrrhiza* sp. and its bioactive compounds. *Phytother Res* 2008; 22: 709-724.
- Fenwick GR, Lutowski J, Nieman C. *Liquorice, Glycyrrhiza glabra* L. —Composition, uses and analysis. *Food Chem* 1990; 38: 119-143.
- Segal R, Pisanty S, Wormser R, Azaz E, Sela MN. Anticariogenic activity of licorice and glycyrrhizine I: Inhibition of in vitro plaque formation by *Streptococcus mutans*. *J Pharm Sci* 1985; 74: 79-81.
- Badria FA, Zidan OA. Natural products for dental caries prevention. *J Med Food* 2004; 7: 381-384.
- Badr AE, Omar N, Badria FA. A laboratory evaluation of the antibacterial and cytotoxic effect of *Liquorice* when used as root canal medicament. *Int Endod J* 2011; 44: 51-58.
- More G, Tshikalange TE, Lall N, Botha F, Meyer JJ. Antimicrobial activity of medicinal plants against oral microorganisms. *J Ethnopharmacol* 2008; 119: 473-477.
- Ozkan G, Baydar H, Erbas S. The influence of harvest time on essential oil composition, phenolic constituents and antioxidant properties of Turkish oregano (*Origanum onites* L.). *J Sci Food Agric* 2010; 90: 205-209.
- Institute CaLS. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; Approved standard-eighth edition. Wayne, PA, USA 2009.
- Ceri H, Olson ME, Stremick C, Read RR, Morck D, Buret A. The Calgary Biofilm Device: new technology for rapid determination of antibiotic susceptibilities of bacterial biofilms. *J Clin Microbiol* 1999; 37: 1771-1776.
- Guerreiro-Tanomaru JM, Nascimento CA, Faria-Junior NB, Graeff MS, Watanabe E, Tanomaru-Filho M. Antibiofilm activity of irrigating solutions associated with cetrimide. Confocal laser scanning microscopy. *Int Endod J* 2014; 47: 1058-1063.
- Gomes BP, Lilley JD, Drucker DB. Variations in the susceptibilities of components of the endodontic microflora to biomechanical procedures. *Int Endod J* 1996; 29: 235-241.
- McDonnell G, Russell AD. Antiseptics and disinfectants: activity, action, and resistance. *Clin Microbiol Rev* 1999; 12: 147-179.
- Oncag O, Hosgor M, Hilmioglu S, Zekioglu O, Eronat C, Burhanoglu D. Comparison of antibacterial and toxic effects of various root canal irrigants. *Int Endod J* 2003; 36: 423-432.
- Baca P, Junco P, Arias-Moliz MT, Castillo F, Rodriguez-Archilla A, Ferrer-Luque CM. Antimicrobial substantivity over time of chlorhexidine and cetrimide. *J Endod* 2012; 38: 927-930.
- Killeen GF, Madigan CA, Connolly CR, Walsh GA, Clark C, Hynes MJ, Timmins BF, James P, Headon DR, Power RF. Antimicrobial saponins of *Yucca schidigera* and the implications of their in vitro properties for their in vivo impact. *J Agric Food Chem* 1998; 46: 3178-3186.
- Haraguchi H, Tanimoto K, Tamura Y, Mizutani K, Kinoshita T. Mode of antibacterial action of retrochalcones from *Glycyrrhiza inflata*. *Phytochemistry* 1998; 48: 125-129.
- Zehnder M. Root canal irrigants. *J Endod* 2006; 32: 389-398.
- Mohammadi Z. Sodium hypochlorite in endodontics: an update review. *Int Dent J* 2008; 58: 329-341.
- Clegg MS, Vertucci FJ, Walker C, Belanger M, Britto LR. The effect of exposure to irrigant solutions on apical dentin biofilms in vitro. *J Endod* 2006; 32: 434-437.
- Del Carpio-Perochena AE, Bramante CM, Duarte MA, Cavenago BC, Villas-Boas MH, Graeff MS, Bernardineli N, de Andrade FB, Ordinola-Zapata R. Biofilm dissolution and cleaning ability of different irrigant solutions on intraorally infected dentin. *J Endod* 2011; 37: 1134-1138.
- Meire MA, Coenye T, Nelis HJ, De Moor RJ. Evaluation of Nd:YAG and Er:YAG irradiation, antibacterial photodynamic therapy and sodium hypochlorite treatment on *Enterococcus faecalis* biofilms. *Int Endod J* 2012; 45: 482-491.
- Arias-Moliz MT, Ferrer-Luque CM, Espigares-Garcia M, Baca P. *Enterococcus faecalis* biofilms eradication by root canal irrigants. *J Endod* 2009; 35: 711-714.
- Deng DM, Buijs MJ, ten Cate JM. The effects of substratum on the pH response of *Streptococcus mutans* biofilms and on the susceptibility to 0.2% chlorhexidine. *Eur J Oral Sci* 2004; 112: 42-47.
- Portenier I, Waltimo T, Orstavik D, Haapasalo M. Killing of *Enterococcus faecalis* by MTAD and chlorhexidine digluconate with or without cetrimide in the presence or absence of dentine powder or BSA. *J Endod* 2006; 32: 138-141.
- Simoes M, Pereira MO, Vieira MJ. Effect of mechanical stress on biofilms challenged by different chemicals. *Water Res* 2005; 39: 5142-5152.