

ORIGINAL ARTICLE

***In vitro* antimicrobial and anti-endotoxin action of *Zingiber Officinale* as auxiliary chemical and medicament combined to calcium hydroxide and chlorhexidine**

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Abstract

Objective. This study was conducted *in vitro* to compare the effectiveness of *Zingiber Officinale* as an auxiliary chemical substance followed by placement of different intra-canal medication in removing endotoxins and cultivable micro-organisms from infected root canals. **Materials and methods.** Seventy-two root canals were contaminated with *Enterococcus faecalis*, *Candida albicans* and *Escherichia coli* for 28 days. After, the teeth were instrumented using *Zingiber Officinale* and divided into six groups according to the intra-canal medication: chlorhexidine gel; calcium hydroxide + chlorhexidine gel; glycolic ginger extract; calcium hydroxide + glycolic ginger extract; calcium hydroxide + saline solution and saline solution (control). Sample collections were performed after root canal contamination (Baseline; S1), after instrumentation (S2), 7 days after instrumentation (S3), after 14 days with intra-canal medication (S4) and 7 days after removal of intra-canal medication (S5). The results were analyzed by the Kruskal-Wallis and Dunn tests. **Results.** It was observed that in S2 and S3 there was significant reduction of the micro-organisms and the quantity of endotoxins after instrumentation. In samples S4 and S5 there was complete elimination of micro-organisms and significant reduction of endotoxins. **Conclusion.** It was concluded that *Zingiber Officinale* as an auxiliary chemical substance was effective on the micro-organisms tested, yet was unable to eliminate the endotoxins. Similarly, the intra-canal medication were effective on micro-organisms, yet did not completely eliminate the endotoxins.

Key Words: endotoxins, micro-organisms, root canal irrigants, root canal medicaments, *Zingiber Officinale*

Introduction

Root canals of teeth with necrotic pulps associated with pulp and periapical lesions are a reservoir of micro-organisms and their byproducts, which induce periapical inflammatory reaction [1]. Endodontic infections are predominantly polymicrobial, involving facultative and strict anaerobes, aerobes and fungi [2–5].

Gram-negative bacteria present an endotoxin (lipopolysaccharide) in their cell wall, which is released during duplication or cell death, causing inflammatory reactions and resorption of periradicular tissues, which may be associated with endodontic signs and

symptoms [6,7]. *Escherichia coli* presents endotoxins in the cell wall with the basic structure of the lipid component, which represents the active center responsible for the toxicity of LPS [8]. For this reason, even though this bacteria is not commonly found in root canals with necrotic pulp, it has been used to evaluate substances that may be able to neutralize endotoxins [9].

Among the micro-organisms frequently found in the root canal system, *Enterococcus faecalis* is the most prevalent species and is associated with persistent periapical lesions, being able to survive even in adverse conditions, indicating its importance in the etiology of endodontic treatment failure [10,11].

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Candida albicans is also frequently isolated in root canals. This fungus may be present in 7–55% of infected root canals [3], being considered another possible cause of endodontic treatment failure [5,11,12].

The world literature demonstrates an increase for natural products in medical specialties, encouraging alternative therapies. *Zingiber officinale*, popularly known as ginger, has been studied because of its antimicrobial, anti-inflammatory and analgesic properties [13,14]. It presents 1–3% of essential oil, 2.5–5% of gingerol and shogaol and 60% of starch, which are able to significantly inhibit the production of E2 prostaglandins induced by the LPS of *E. coli* [13].

Considering that studies conducted on ginger have demonstrated antimicrobial effectiveness, this study evaluated, *in vitro*, the action of *Zingiber Officinale* as an auxiliary chemical substance followed by its use as intra-canal medication associated and compared with other drugs commonly used during the intra-canal medication stage.

Materials and methods

This study was approved by the Ethical Committee of São José dos Campos School of Dentistry–UNESP, protocol n. 07/2008-PH/CEP.

Preparation and contamination of specimens

The study was conducted on 72 single-rooted human teeth whose crowns were sectioned to standardize the length of specimens in 16 ± 0.5 mm. The root canals were instrumented using saline solution throughout their extent up to Kerr file n. 30 (Dentsply Ind. Com. Ltda, Petrópolis, RJ, Brazil) followed by irrigation with 3 ml saline solution after each instrument. Afterwards, the canals were filled with EDTA for 3 min and irrigated with 10 ml of saline solution. Then, the roots were sealed at the apical region with light cured composite resin Z-100 (3M, Saint Paul, MN) and externally sealed with two layers of epoxy adhesive (Brascola, São Paulo, SP, Brazil), except for the cervical opening. The specimens were randomly distributed and fixated with chemically cured acrylic resin (Dencor Artigos Odontológicos, São Paulo, SP, Brazil) on cell culture plates with 24 wells (TPP, Trasadingen, Switzerland), with 12 teeth in each. All cell culture plates and materials were sterilized by Cobalt-60 gamma irradiation (20 KGy for 6 h) to neutralize the pre-existent endotoxins [15].

The micro-organisms used were *Candida albicans* (ATCC 18804), *Enterococcus faecalis* (ATCC 29212) and *Escherichia coli* (ATCC 25922). Separate suspensions were prepared in apyrogenic saline containing 10^6 cells/ml, checked by reading in a spectrophotometer.

The root canals were contaminated with 10 μ l of suspension of *E. coli* and 10 μ l of BHI broth (brain-heart infusion) (Himedia Laboratories, Mumbai, India). Apyrogenic cotton pellets soaked in BHI broth were placed in the root canal openings and the specimens were kept in an oven at $37^\circ\text{C} \pm 1^\circ\text{C}$, in relative humidity, for 7 days. After this period, 5 μ l of suspension of *C. albicans*, 5 μ l of suspension of *E. faecalis* and 10 μ l of BHI broth were added to the root canals. A new apyrogenic cotton pellet soaked in BHI broth was placed in the root canal openings and the specimens were kept in an oven at $37^\circ\text{C} \pm 1^\circ\text{C}$, in relative humidity, for 21 days, with addition of BHI broth to the root canals every 2 days [9,16].

After the contamination period (28 days), the initial sample (baseline, S1) was collected from all specimens to confirm the root canal contamination and quantify the endotoxins.

Division of study groups

After verifying the contamination, the root canals were instrumented into their full length up to Kerr file n. 50, with 20% glycolic ginger extract (Apis Flora, Ribeirão Preto, São Paulo, Brazil) as an auxiliary chemical substance during the instrumentation and using 3 ml of apyrogenic saline solution. Furthermore, at each change of instrument, the 20% glycolic ginger extract solution was renewed. In order to determine the antimicrobial activity of the *Zingiber Officinale* (20% glycolic ginger extract), a second sampling specimen was performed (S2). The teeth were filled with apyrogenic saline solution and maintained for 7 days. Throughout the study period, the specimens were kept in an oven at $37^\circ\text{C} \pm 1^\circ\text{C}$. After 7 days the third sample was collected (S3). The root canals were then filled with EDTA for 3 min, followed by root canal irrigation with 10 ml of apyrogenic saline solution and division of specimens into six experimental groups ($n = 12$), according to the intra-canal medication used (Table I).

Medications of 2% chlorhexidine gel (CHX), 20% glycolic ginger extract (GENG) and saline (SS; control) were applied using 3 ml syringes up to complete root canal filling. The medications were associated in a 1:1 ratio in volume and placed in the root canal using a Kerr file n. 30.

After root canal filling with the intra-canal medication, the teeth were sealed with apyrogenic cotton pellets. The teeth were kept in an oven at 37°C for 14 days. After this period, the medication was removed with a Kerr file n. 50 and 10 ml of apyrogenic saline solution, followed by collection of the fourth sample (S4). The root canals were filled with apyrogenic saline solution and the fifth sample (S5) was collected after 7 days.

Table I. Division of the experimental groups.

Groups	Intra-canal medication	Origin
CHX	2% chlorhexidine gel	Byofórmula, São José dos Campos, Brazil
Ca(OH) ₂ + CHX	Calcium Hydroxide associated with 2% chlorhexidine gel	Biodinâmica, Ibiporã, PR; Byofórmula, São José dos Campos, Brazil
GENG	20% glycolic ginger extract	Apis Flora, Ribeirão Preto, São Paulo, Brazil
Ca(OH) ₂ + GENG	Calcium Hydroxide associated with 20% glycolic ginger extract	Biodinâmica, Ibiporã, PR; Apis Flora, Ribeirão Preto, São Paulo, Brazil
Ca(OH) ₂ + SS	Calcium Hydroxide associated with saline solution	Biodinâmica, Ibiporã, PR, Brazil
SS (Control)	Saline solution	Laboratório Sanobiol, Pouso Alegre, MG, Brazil

Collections of root canal content

All samples (S1, S2, S3, S4 and S5) were carried in the same standard way: the root canals were filled with apyrogenic saline solution and 100 µl of the root canal content was collected and transferred to eppendorf tubes containing 900 µl of apyrogenic saline solution. All samples were submitted to microbiological tests and quantification of endotoxins to verify the presence of micro-organisms and endotoxins in the root canals.

Evaluation of antimicrobial action (culturing procedure)

Aliquots of 100 µl of all samples were plated in duplicate in three different culture media, selective for each micro-organism: agar Sabouraud Dextrose (Himedia Laboratories, Mumbai, India) with chloramphenicol (Vixmicina, União Química Farmacêutica S/A, Embu-Guaçu, SP, Brazil) for *C. albicans*, agar Enterococcosel (Becton, Dickinson and Company, Sparks, MD, USA) for *E. faecalis* and agar MacConkey (Himedia Laboratories, Mumbai, India) for *E. coli*.

The plates were kept in an oven at 37°C for 24 h. In all collections, the antimicrobial activity of treatments and substances was analyzed by counting of colony forming units per milliliter (CFU/ml). The results were statistically analyzed by the *Kruskal-Wallis* and *Dunn* tests, at a significance level of 5%.

Table II. Percentages of reduction of CFU/ml obtained in the second (S2) and third (S3) collections in relation to the baseline (S1).

Reduction	Micro-organisms	Mean	SD	Median	HG
S1 × S2	<i>C. albicans</i>	99.99	0.01	100	A
	<i>E. faecalis</i>	99.87	0.58	99.99	B
	<i>E. coli</i>	99.99	0.04	100	A
S1 × S3	<i>C. albicans</i>	99.98	0.11	100	A
	<i>E. faecalis</i>	99.79	0.61	99.96	B
	<i>E. coli</i>	99.63	3.04	100	A

HG, Homogenous groups; indicate statistical significance.

Quantification of endotoxins (endotoxins procedures)

The neutralization of endotoxins was verified by the kinetic chromogenic method of *Limulus Amebocyte Lysate test* (LAL) (Cambrex, São Paulo, SP, Brazil), using the kinetic reader QCL (Cambrex) connected to a computer with specific software WinkQCL (Cambrex). The concentrations of standard curve (between 0.005–50 EU/ml) were performed according to the manufacturer; calculation of the parameters of standard curves and values of endotoxin samples (EU/ml) were automatically performed by the software. The present statistical analysis was based on the percentage of alteration related to a decrease or increase in the quantity of endotoxin (EU/ml) of each sample in relation to the baseline (S1).

Results were subjected to statistical analysis using *Kruskal-Wallis* and *Dunn* tests (significance level of 5%).

Results

Microbiological analysis

At the baseline (S1), micro-organisms were recovered from 100% of the contaminated-root canals. The results of the *Kruskal-Wallis* test comparing the

Table III. Percentages of reduction of CFU/ml obtained in the fourth collection (S4) in relation to the baseline (S1).

	S1 × S4					
	<i>C. albicans</i>		<i>E. faecalis</i>		<i>E. coli</i>	
	Median	HG	Median	HG	Median	HG
CHX	100	A	100	A	100	A
Ca(OH) ₂ + CHX	100	A	100	A	100	A
GENG	100	A	100	A	100	A
Ca(OH) ₂ + GENG	100	A	100	A	100	A
Ca(OH) ₂ + SS	100	A	100	A	100	A
SS	99.98	B	99.98	B	100	A

n = 12 for each group.

HG, Homogeneous groups; indicate statistical significance.

Table IV. Percentages of reduction of CFU/ml obtained in the fifth collection (S5) in relation to the baseline (S1).

Groups	S1 × S5					
	<i>C. albicans</i>		<i>E. faecalis</i>		<i>E. coli</i>	
	Median	HG	Median	HG	Median	HG
CHX	100	A	100	A	100	A
Ca(OH) ₂ + CHX	100	A	100	A	100	A
GENG	100	A	100	A	100	A
Ca(OH) ₂ + GENG	100	A	100	A	100	A
Ca(OH) ₂ + SS	100	A	100	A	100	A
SS	100	A	99.96	B	99.97	A

n = 12 for each group.

HG, Homogeneous groups; indicate statistical significance.

percentage of reduction of micro-organisms after instrumentation (S2) and 7 days after instrumentation (S3) compared to the baseline (S1) are expressed in Table II. It is possible to observe a reduction of nearly 100% of micro-organisms after preparation using the 20% ginger glycolic extract as an auxiliary chemical substance, demonstrating great efficacy against *C. albicans*, *E. faecalis* and *E. coli*.

The mean, standard deviation, median and homogeneous groups of sample 4 (S4; after placement of intra-canal medication) and sample 5 (S5; 7 days after removal of intra-canal medication), compared to the baseline (S1), are expressed in Tables III and IV. Concerning the SS group (control group), there was a reduction in the number of micro-organisms immediately after instrumentation (S2), re-taking the growth during the study (S4 and S5).

Analysis of endotoxins

The results of quantification of endotoxins revealed a reduction of 92.32% using the ginger glycolic extract as an auxiliary chemical substance during instrumentation (S2). Seven days after instrumentation (S3), there was a reduction of 87.44% (Table V).

The mean and median values obtained in the countings of EU/ml, as well as the homogeneous groups of samples S4 and S5, are presented in Table VI. In sample S4, Ca(OH)₂ + SS and Ca

Table V. Percentages related to the reduction in the quantity of endotoxin (EU/ml) obtained in the second (S2) and third (S3) collections in relation to the baseline (S1).

Reduction	<i>n</i>	Mean	SD	Median	HG
S1 × S2	72	92.32	11.60	96.15	A
S1 × S3	72	87.44	30.68	95.67	A

HG, Homogeneous groups; indicate statistical significance.

Table VI. Percentages related of the reduction in the quantity of endotoxin (EU/ml) obtained in the fourth (S4) and fifth (S5) collections in relation to the baseline (S1).

Groups	S1 × S4			S1 × S5		
	Mean	Median	HG	Mean	Median	HG
CLX	99.65	99.79	BC	99.55	99.88	A
CLX + (CaOH) ₂	99.75	99.97	AB	99.91	99.96	A
Geng	98.28	99.60	BC	92.55	97.16	B
Geng + Ca(OH) ₂	99.74	99.90	B	99.64	99.83	AB
SS + Ca(OH) ₂	99.99	99.99	A	99.98	99.99	A
SS	74.8	90.50	C	85.39	89.62	B

n = 12 for each group.

HG, Homogeneous groups; indicate statistical significance.

(OH)₂ + CHX presented the greatest reduction of endotoxin (99.99% and 99.74%, respectively). In sample S5, the groups Ca(OH)₂ + SS (99.99%), Ca(OH)₂ + CHX (99.96%), CHX (99.88%) and Ca(OH)₂ + GENG (99.83%) exhibited the greatest reduction of endotoxin levels.

Discussion

In the present study, the specimens were contaminated with standardized suspensions of *Enterococcus faecalis*, *Candida albicans* and *Escherichia coli*, already described in previous studies [17–19]. Some microbial species, such as *E. faecalis* and *C. albicans*, may present resistance to calcium hydroxide, a commonly used intra-canal medication [16,20]. This study revealed that calcium hydroxide, pure or associated with chlorhexidine and 20% glycolic ginger extract, was effective on the micro-organisms tested. The antimicrobial action of Ca(OH)₂ has been demonstrated in other studies [9,21]. Even though reports demonstrate that Ca(OH)₂ is not effective on *Enterococcus faecalis* [22], it presented 100% effectiveness in this study.

Recent studies demonstrate the action of glycolic and alcoholic ginger extract on micro-organisms as *Streptococcus mutans*, *Staphylococcus aureus*, *Escherichia coli*, *Porphyromonas endodontalis* and *Prevotella intermedia* [14]. Components of essential oil of rhizomes of *Zingiber officinale* reduce the growth rate of a large variety of bacteria and fungi, including *Staphylococcus* and *Candida* [23]. It was demonstrated that ethanolic extracts of *Z. Officinale* were able to inhibit the growth of Gram-negative and Gram-positive bacteria [24], even though the effect was more pronounced on Gram-positive bacteria [25]. Also, Habsah et al. [26] observed high activity of this rhizome on *Pseudomonas aeruginosa*, a highly resistant Gram-negative bacteria. In this study, after instrumentation using ginger glycolic extract as auxiliary chemical

substance, there was complete elimination of *C. albicans* and *E. coli* and more than 99% of *E. faecalis* could also be eliminated.

Notwithstanding the favorable results in the elimination of micro-organisms, the ginger glycolic extract was unable to completely eliminate the endotoxins present in the root canal system, demonstrating similar results as those obtained with sodium hypochlorite and chlorhexidine, which are auxiliary chemicals commonly employed in the endodontic practice [7,27]. This study used 20% ginger glycolic extract, whose main active principle is gingerol, which probably presents greater antimicrobial effects [14]. Studies indicate that the volatile oils gingerol and shogaol seem to be responsible for the beneficial action of this rhizome [13]. Park et al. [14] suggest that compound isolated from ginger, as gingerol were effective on *Porphyromonas gingivalis*, *Porphyromonas endodontalis* and *Prevotella intermedia*. Although the mechanism of action of ginger extract on micro-organisms is not elucidated in the literature, studies reveal effective antimicrobial action of this extract on the micro-organisms [14]. Other investigations further demonstrated that ginger is biocompatible, being used also in the diet [28,29]. However, there are no studies on the effects of active principles of ginger. The ideal time of action of this product is also not known. The results of this substance immediately after preparation (S1) suggested that the peak action of ginger occurs at nearly 7 days, with a loss of activity after this period. However, further studies should be conducted to evaluate the period of action of this phytotherapeutic substance.

Concerning the use of intra-canal medications, all medications analyzed were able to completely eliminate the micro-organisms, in agreement with other studies [9,30]; however, they were unable to eliminate endotoxins present in the root canals. Among the medications used, associations with calcium hydroxide revealed the best outcomes. The antimicrobial activity of calcium hydroxide is associated with its high pH [31] by the release and diffusion of hydroxyl ions. To be effective on bacteria located in the dentinal tubules, hydroxyl ions should diffuse in the dentine with sufficient concentrations, reaching alkaline pH high enough to destroy the bacteria [32]. Similarly, OH⁻ ions are able to induce the lipid peroxidase, leading to destruction of phospholipids. Moreover, the maintenance of alkaline pH by Ca(OH)₂ causes rupture of ionic bonds and alters the structure of cell membrane proteins, causing interruption of the cell metabolism of bacteria [32].

Therefore, it was concluded that ginger glycolic extract may be a good option as an auxiliary chemical in endodontic practice and calcium hydroxide intra-canal medications are the most indicated, especially to neutralize endotoxins.

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References

- [1] Kakehashi S, Stanley HR, Fitzgerald RJ. The effects of surgical exposures of dental pulps in germ-free and conventional laboratory rats. *Oral Surg Oral Med Oral Pathol* 1965;20:340–9.
- [2] Nair PN, Sjogren U, Krey G, Kahnberg KE, Sundqvist G. Intraradicular bacteria and fungi in root-filled, asymptomatic human teeth with therapy-resistant periapical lesions: a long-term light and electron microscopic follow-up study. *J Endod* 1990;16:580–8.
- [3] Waltimo TM, Siren EK, Torkko HL, Olsen I, Haapasalo MP. Fungi in therapy-resistant apical periodontitis. *Int Endod J* 1997;30:96–101.
- [4] Siqueira JF Jr, Rocas IN, Lopes HP. Patterns of microbial colonization in primary root canal infections. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2002;93:174–8.
- [5] Sunde PT, Olsen I, Debelian GJ, Tronstad L. Microbiota of periapical lesions refractory to endodontic therapy. *J Endod* 2002;28:304–10.
- [6] Jacinto RC, Gomes BP, Shah HN, Ferraz CC, Zaia AA, Souza-Filho FJ. Quantification of endotoxins in necrotic root canals from symptomatic and asymptomatic teeth. *J Med Microbiol* 2005;54:777–83.
- [7] Martinho FC, Gomes BP. Quantification of endotoxins and cultivable bacteria in root canal infection before and after chemomechanical preparation with 2.5% sodium hypochlorite. *J Endod* 2008;34:268–72.
- [8] Oliveira LD, Jorge AO, Carvalho CA, Koga-Ito CY, Valera MC. In vitro effects of endodontic irrigants on endotoxins in root canals. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2007;104:135–42.
- [9] Valera MC, da Rosa JA, Maekawa LE, de Oliveira LD, Carvalho CA, Koga-Ito CY, et al. Action of propolis and medications against *Escherichia coli* and endotoxin in root canals. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2010;110:e70–4.
- [10] Siqueira JF Jr, Rocas IN. Polymerase chain reaction-based analysis of microorganisms associated with failed endodontic treatment. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2004;97:85–94.
- [11] Gomes BP, Pinheiro ET, Jacinto RC, Zaia AA, Ferraz CC, Souza-Filho FJ. Microbial analysis of canals of root-filled teeth with periapical lesions using polymerase chain reaction. *J Endod* 2008;34:537–40.
- [12] Gomes BP, Vianna ME, Sena NT, Zaia AA, Ferraz CC, de Souza Filho FJ. In vitro evaluation of the antimicrobial activity of calcium hydroxide combined with chlorhexidine gel

- used as intracanal medicament. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2006;102:544–50.
- [13] Lantz RC, Chen GJ, Sarihan M, Solyom AM, Jolad SD, Timmermann BN. The effect of extracts from ginger rhizome on inflammatory mediator production. *Phytomedicine* 2007; 14:123–8.
- [14] Park M, Bae J, Lee DS. Antibacterial activity of [10]-gingerol and [12]-gingerol isolated from ginger rhizome against periodontal bacteria. *Phytother Res* 2008;22:1446–9.
- [15] Csako G, Elin RJ, Hochstein HD, Tsai CM. Physical and biological properties of U.S. standard endotoxin EC after exposure to ionizing radiation. *Infect Immun* 1983;41:190–6.
- [16] Valera MC, Silva KC, Maekawa LE, Carvalho CA, Koga-Ito CY, Camargo CH, et al. Antimicrobial activity of sodium hypochlorite associated with intracanal medication for *Candida albicans* and *Enterococcus faecalis* inoculated in root canals. *J Appl Oral Sci* 2009;17:555–9.
- [17] Valera MC, Maekawa LE, de Oliveira LD, Jorge AO, Shygei E, Carvalho CA. In vitro antimicrobial activity of auxiliary chemical substances and natural extracts on *Candida albicans* and *Enterococcus faecalis* in root canals. *J Appl Oral Sci* 2013;21:118–23.
- [18] Maekawa LE, Valera MC, Oliveira LD, Carvalho CA, Camargo CH, Jorge AO. Effect of *Zingiber Officinale* and Propolis on microorganisms and endotoxins in root canals. *J Appl Oral Sci* 2013;21:25–31.
- [19] Valera MC, Maekawa LE, Chung A, Cardoso FGR, Oliveira LD, Oliveira CL, et al. The effect of sodium hypochlorite and ginger extract on microorganisms and endotoxins in endodontic treatment of infected root canals. *Gen Dent* 2014;62:25–9.
- [20] Waltimo TM, Siren EK, Orstavik D, Haapasalo MP. Susceptibility of oral *Candida* species to calcium hydroxide in vitro. *Int Endod J* 1999;32:94–8.
- [21] Gondim JO, Avaca-Crusca JS, Valentini SR, Zanelli CF, Spolidorio DM, Giro EM. Effect of a calcium hydroxide/chlorhexidine paste as intracanal dressing in human primary teeth with necrotic pulp against *Porphyromonas gingivalis* and *Enterococcus faecalis*. *Int J Paediatr Dent* 2012;22:116–24.
- [22] 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.
- [23] Martins AP, Salgueiro L, Goncalves MJ, da Cunha AP, Vila R, Caniguel S, et al. Essential oil composition and antimicrobial activity of three Zingiberaceae from S.Tome e Principe. *Planta Med* 2001;67:580–4.
- [24] Mascolo N, Jain R, Jain SC, Capasso F. Ethnopharmacologic investigation of ginger (*Zingiber officinale*). *J Ethnopharmacol* 1989;27:129–40.
- [25] Alzorekya N, Nakahara K. Antibacterial activity of extracts from some edible plants commonly consumed in Asia. *Int J Food Microbiol* 2003;80:7.
- [26] Habsah M, Amran M, Mackeen MM, Lajis NH, Kikuzaki H, Nakatani N, et al. Screening of Zingiberaceae extracts for antimicrobial and antioxidant activities. *J Ethnopharmacol* 2000;72:403–10.
- [27] Vianna ME, Horz HP, Conrads G, Zaia AA, Souza-Filho FJ, Gomes BP. Effect of root canal procedures on endotoxins and endodontic pathogens. *Oral Microbiol Immunol* 2007;22: 411–18.
- [28] Kim M, Hamilton SE, Guddat LW, Overall CM. Plant collagenase: unique collagenolytic activity of cysteine proteases from ginger. *Biochim Biophys Acta* 2007;1770:1627–35.
- [29] Fouda AM, Berika MY. Evaluation of the effect of hydroalcoholic extract of *Zingiber officinale* rhizomes in rat collagen-induced arthritis. *Basic Clin Pharmacol Toxicol* 2009;104:262–71.
- [30] Silveira CF, Cunha RS, Fontana CE, de Martin AS, Gomes BP, Motta RH, et al. Assessment of the antibacterial activity of calcium hydroxide combined with chlorhexidine paste and other intracanal medications against bacterial pathogens. *Eur J Dent* 2011;5:1–7.
- [31] Gomes BP, Montagner F, Berber VB, Zaia AA, Ferraz CC, de Almeida JF, et al. Antimicrobial action of intracanal medicaments on the external root surface. *J Dent* 2009;37:76–81.
- [32] Siqueira JF Jr, Lopes HP. Mechanisms of antimicrobial activity of calcium hydroxide: a critical review. *Int Endod J* 1999; 32:361–9.