

Antibacterial activity of propolis-based toothpastes for endodontic treatment

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This study evaluated the antibacterial activity of propolis-based toothpastes used as intracanal medication in endodontic treatment. The propolis-based toothpastes were prepared using an extract established in previous studies (identified as A70D and D70D). Calcium hydroxide paste was used as a control. The bacteria employed were *Streptococcus mutans* (ATCC 25175), *Staphylococcus aureus* (ATCC 6538), *Staphylococcus aureus* (ATCC 25923), *Kocuria rhizophila* (ATCC 9341), *Escherichia coli* (ATCC 10538), *Pseudomonas aeruginosa* (ATCC 27853), *Enterococcus hirae* (ATCC 10541), *Streptococcus mutans* (ATCC 25175). Five field strains isolated from saliva were used: *Staphylococcus* spp. (23.1 - coagulase positive), *Staphylococcus* spp. (23.5 - coagulase negative), *Staphylococcus* spp. (26.1 - coagulase positive), *Staphylococcus* spp. (26.5 - coagulase negative) and *Staphylococcus epidermidis* (6epi). The diffusion-well method on double-layer agar was used in a culture medium of Tryptic Soy Agar. The plates were kept at room temperature for two hours to allow the diffusion of pastes in the culture medium, and then incubated at 35° C for twenty-four hours in aerobiosis and in microaerophilia (*S. mutans*). After this period, the total diameter of the inhibition halo was measured. The results were analyzed by ANOVA analysis of variance, followed by the Tukey test at $p < 0.05$. The propolis-based toothpastes presented antibacterial activity against 83.3% of the analyzed bacteria. For 66.7% of these bacteria, the propolis-based toothpastes exhibited greater antibacterial activity than calcium hydroxide. The present results allow us to conclude that the experimental pastes A70D and D70D showed good activity against aerobic bacteria, proving more effective than calcium hydroxide.

Uniterms: Propolis/antibacterial activity. Tooth paste/with propolis/antibacterial activity. Endodontics/treatment.

O objetivo deste estudo foi avaliar a atividade antibacteriana de formas farmacêuticas a base de própolis para uso no tratamento endodôntico como medicação intracanal. As formulações de própolis, em forma de pastas, foram preparadas a partir de um extrato pré-estabelecido em estudos anteriores e identificadas como A70D e D70D. Como controle, foi utilizado pasta de hidróxido de cálcio. As bactérias utilizadas foram: *Streptococcus mutans* (ATCC 25175), *Staphylococcus aureus* (ATCC 6538), *Staphylococcus aureus* (ATCC 25923), *Kocuria rhizophila* (ATCC 9341), *Escherichia coli* (ATCC 10538), *Pseudomonas aeruginosa* (ATCC 27853), *Enterococcus hirae* (ATCC 10541), *Streptococcus mutans* (ATCC 25175) e 5 cepas de campo isoladas da saliva: *Staphylococcus* spp. (23.1 - coagulase positiva), *Staphylococcus* spp. (23.5 - coagulase negativa), *Staphylococcus* spp. (26.1 - coagulase positiva), *Staphylococcus* spp. (26.5 - coagulase negativa) e *Staphylococcus epidermidis* (6epi). Foi utilizado o método poço difusão em camada dupla de ágar, em meio de cultura Tryptic Soy Agar. As placas foram mantidas à temperatura ambiente por 2 h para permitir a difusão das pastas no meio de cultura, e então incubadas a 35 °C por 24 h em aerobiose e em microaerofilia (*S. mutans*). Após este período, foi medido o diâmetro total do halo de inibição. Os resultados foram submetidos ao teste de análise de variância ANOVA seguido do teste de Tukey com $p < 0,05$. As pastas a base de própolis apresentaram atividade antibacteriana contra 83,3% das bactérias analisadas. Para 66,7% das bactérias, as pastas de própolis apresentaram maior atividade

antibacteriana que o hidróxido de cálcio, e este foi mais efetivo apenas para *Streptococcus mutans* (ATCC 25175), *Staphylococcus aureus* (ATCC 25923) e *Pseudomonas aeruginosa* (ATCC 27853). De acordo com a metodologia utilizada, pode-se concluir que as pastas experimentais A70D e D70D apresentam boa atividade contra bactérias aeróbias, sendo superior ao hidróxido de cálcio.

Unitermos: Própolis/atividade antibacteriana. Pasta de dente/com própolis/atividade antibacteriana. Endodontia/tratamento.

INTRODUCTION

Intracanal medication helps in the fight against root canal system infection, and can also stimulate the repair process of apical tissues. To achieve this, it must eliminate the bacteria and fungi surviving in biomechanical preparation, act as a physicochemical barrier against reinfection by mouth microorganisms, and decrease peri-radicular inflammation.

Propolis, a substance produced by bees for protection of the hive against microorganisms (Trevisan, 1983), has been widely used in popular medicine. In the scientific community, propolis has demonstrated antibacterial (Wu-yuan, Green, Birch, 1990; Grange, Davey, 1990; Santos *et al.*, 2002; Koru *et al.*, 2007), antifungal (Kujumgiev *et al.*, 1999; Quintero-Mora *et al.*, 2008), anti-inflammatory (Dobrowolski *et al.*, 1991; Hu *et al.*, 2005; Sosa, 2007), cicatrizant (Perri de Carvalho, Tagliavini, Tagliavini, 1991) and immunomodulator (Dimov *et al.*, 1992; Simões *et al.*, 2004) action.

Few studies have associated propolis and its anti-inflammatory and antibacterial effects with endodontic treatment, such as those by Heys (1980), Matos (1989) and Sabir *et al.* (2005). Perhaps this is due to the intrinsic difficulty of introducing propolis and removing it from the internal side of the root canal.

Therefore, the purpose of this study was to evaluate the antibacterial activity of propolis-based pharmaceutical forms for use in endodontic treatment as intracanal medication.

MATERIAL AND METHODS

The propolis-based formulas were prepared from a brown extract (called PPE1) predefined in previous studies (Victorino *et al.*, 2007), which was collected in the Northeastern state of Paraná, Brazil. The solvent used in the formulas was an ethylic alcohol based solution. The formulas, in the form of toothpastes, were labeled A70D and D70D. P.A. calcium hydroxide prepared with propylene glycol was used as a control formula.

Twelve bacteria were used in the study [eleven aerobic and one facultative anaerobic - *Streptococcus mutans*

(ATCC 25175)], seven of which were standard strains: *Staphylococcus aureus* (ATCC 6538), *Staphylococcus aureus* (ATCC 25923), *Kocuria rhizophila* (ATCC 9341), *Escherichia coli* (ATCC 10538), *Pseudomonas aeruginosa* (ATCC 27853), *Enterococcus hirae* (ATCC 10541) and *Streptococcus mutans* (ATCC 25175) and five, community field strains isolated from saliva: *Staphylococcus* spp. (23.1 - coagulase positive), *Staphylococcus* spp. (23.5 - coagulase negative), *Staphylococcus* spp. (26.1 - coagulase positive), *Staphylococcus* spp. (26.5 - coagulase negative) and *Staphylococcus epidermidis* (6epi).

The technique employed to evaluate the toothpastes' antibacterial activity was that of diffusion on double-layer Agar, described by Grove, Randall (1955), which was performed in quadruplicate. The culture medium *Tryptic Soy Agar* (Difco™, USA) was distributed in petri dishes (20x100mm) sterilized to form a base-layer of 12.0 mL. After medium solidification, 8.0ml of *Tryptic Soy Agar* containing 1% of bacterial inoculation (at half-scale concentration proposed by McFarland), were spread over the base-layer to form a *seed* layer (seeding). Subsequently, six 5.0mm-wide wells were made in each Petri dish. The A70D, D70D and calcium hydroxide toothpastes were placed within the wells using sterilized metallic spatulas.

The dishes were pre-incubated at room temperature (25 °C) for two hours to allow diffusion of the toothpastes by the culture medium, and then incubated at 35 °C for twenty-four hours in aerobiosis (*S. aureus*, *Staphylococcus* spp., *K. rhizophila*, *E. coli*, *P. aeruginosa*, *E. hirae*), or in microaerophilia using the candle jar method (*S. mutans*). After this period, the total inhibition zone diameter was measured with a millimeter ruler, without deducting the diameter of the well. The results were submitted to ANOVA analysis of variance, followed by the Tukey test at $p < 0.05$.

RESULTS

The inhibition zone of test and calcium hydroxide toothpastes, as well as the arithmetic mean of respective antibacterial activities (in quadruple), are expressed in Figure 1 and Table I, respectively. The propolis-based toothpastes demonstrated antibacterial activity in ten out

TABLE I - Susceptibility test of pathogenic microorganisms to propolis-based toothpastes for use in endodontics. Mean inhibition zone (mm)*

	A70D	D70D	Ca(OH) ₂
<i>Staphylococcus aureus</i> (ATCC 6538)	19.8	19.8	16.3
<i>Staphylococcus aureus</i> (ATCC 25923)	20.3	20.8	25.5
<i>Staphylococcus</i> spp. (23.1 - coagulase positive)**	20.5	20.5	14.8
<i>Staphylococcus</i> spp. (23.5 - coagulase negative)**	25.8	24.5	20.3
<i>Staphylococcus</i> spp. (26.1 - coagulase positive)**	17.3	16.8	12.0
<i>Staphylococcus</i> spp. (26.5 - coagulase negative)**	20.5	20.5	16.8
<i>Staphylococcus epidermidis</i> (6epi)**	20.0	20.0	13.5
<i>Kocuria rhizophila</i> (ATCC 9341)	28.8	28.0	22.8
<i>Escherichia coli</i> (ATCC 10538)	13.0	13.0	11.5
<i>Streptococcus mutans</i> (ATCC 25175)	6.3	7.0	20.8
<i>Pseudomonas aeruginosa</i> (ATCC 27853)	0	0	11.8
<i>Enterococcus hirae</i> (ATCC 10541)	0	0	0

* tests in quadruple. **community field strain (saliva)

of twelve analyzed bacteria (83.3%), with the exception of *P. aeruginosa* (ATCC 27853) and *E. hirae* (ATCC 10541), which showed resistance even to calcium hydroxide.

For the calcium hydroxide paste, the mean inhibition zone ranged from zero to 25.5 mm, while the propolis-based toothpastes this ranged from zero to 28.0 mm. For eight out of the twelve bacteria (66.7%), the propolis-based toothpastes presented higher antibacterial activity

than calcium hydroxide, while the latter was more effective against *S. mutans* (ATCC 25175), *S. aureus* (ATCC 25923) and *P. aeruginosa* (ATCC 27853). The improved results found for propolis-based toothpastes over calcium hydroxide were statistically significant, according to ANOVA, followed by Tukey test ($p < 0.05$).

DISCUSSION

This study is part of a line of research line pursuing the development of intracanal medication, in which propolis is the main component. Perhaps, this study may serve to explain why previous microbiologic studies have employed propolis in its raw form, or as an extract. Since this extract presents a resinous quality, it does not represent a good option because the medication must be introduced directly into root canals, making its removal extremely difficult. The development of a paste formula with aqueous components, containing the minimal quantity of propolis extract to maintain its biological activities, would render this therapeutic option viable.

Among the substances employed as intracanal medication, calcium hydroxide has been the most widely used and recommended given its biological properties and antimicrobial action (Sathorn, Parashos, Messer, 2007.). Its high pH (around 12.5, resulting from the release of hydroxyl ions) exerts a deleterious effect over the bacterial cells as it damages their respective cellular membranes (Freeman, Crapo, 1982), denatures their proteins (Siqueira, Uzeda, 1997), and alters their DNA (Imlay, Linn, 1988).

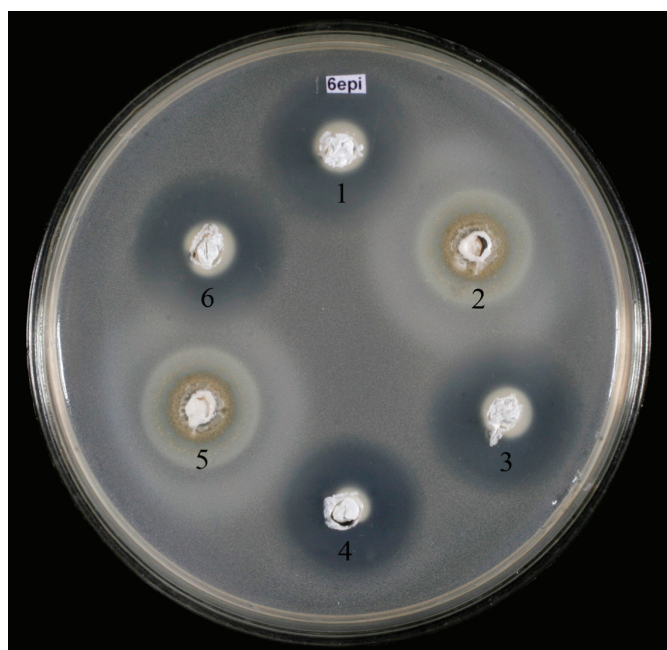


FIGURE 1 - Inhibition zones for *S. epidermidis*. 1: Paste A70D, 2: Ca(OH)₂, 3: Paste D70D, 4: Paste A70D, 5: Ca(OH)₂, 6: Paste D70D.

The antimicrobial property of propolis has been attributed mainly to flavonoids (Greenaway, Scaysbrook, Whatley, 1990; Viuda-Martos *et al.* 2008). However, Kujumgiev *et al.* (1999) have shown that propolis antimicrobial activity results from the synergy between its own components. In this study, the authors have analyzed both the chemical composition and antimicrobial activity from extracts collected in different world regions, including tropical as well as temperate areas. All of these extracts have demonstrated antimicrobial activity, albeit with different chemical characteristics. This result has also been observed by Miorin *et al.* (2003), who analyzed chemical and microbiological extracts from different regions of Brazil.

Many studies have examined propolis antimicrobial activity using serial dilution tests (Sonmez *et al.*, 2005; Victorino *et al.*, 2007) and diffusion tests on agar (Kujumgiev *et al.*, 1999; Somnes *et al.*, 2005; Bruschi *et al.* 2006).

Studies of European propolis employing diffusion test on agar have found diverse results. Metzner *et al.* (1979) found no diffusion zones, probably due to low diffusion of propolis components through agar. Brumfitt *et al.* (1990) achieved inhibition zones of 13.0mm for *S. aureus* and *S. epidermidis*, but no activity against *Streptococcus* spp. Dobrowolsky *et al.* (1991) however, observed inhibition zones of 16.0 mm for both *S. aureus* and for *Streptococcus pyogenes*. Boyanova *et al.* 2006 used the same methodology to evaluate antimicrobial activity of Bulgarian propolis against anaerobic Gram-positive and Gram-negative bacteria, and recorded inhibition zones greater than or equal to 15 mm for the majority of Gram-positive bacteria, at concentrations of 30 µL of propolis per well.

In the present study, growth inhibition zones were observed for the majority of analyzed bacteria, demonstrating that both propolis-based toothpaste and calcium hydroxide toothpaste were able to diffuse and exert their antibacterial activity in the culture medium.

The experimental toothpastes presented similar results, and failed to present activity against *P. aeruginosa* and *E. hirae*. Against the different strains of *S. aureus*, the mean inhibition zone ranged from 16.8 to 25.8 mm, a mean similar or higher than those found by the cited authors. The weak activity against strains of *Streptococcus mutans* also confirms data found in the literature (Brumfitt *et al.*, 1990; Bruschi *et al.*, 2006; Duarte *et al.*, 2006).

Compared to calcium hydroxide, the propolis-based toothpastes showed slightly better results; the latter having inhibited the growth of *P. aeruginosa*, while performing significantly better against *S. mutans*.

It is important to take into account that, not only have standard strains been utilized, but also field strains of *Staphylococcus* spp. from patients' saliva, which dental

professionals routinely encounter in the clinic.

Although the endodontic microbiota is mixed and contains largely anaerobic bacteria, the presence of residual aerobic bacteria within the root canal system, after seventy-two and ninety-six hours after the chemomechanical preparation, was demonstrated by Leonardo (1965) and Assed *et al.* (1996). However, since this was a preliminary study, we used aerobic bacteria and a single facultative anaerobic bacterium (*S. mutans*). Thus, the possible lack of effect in this group of bacteria may be an exclusion or change factor for the formulas.

CONCLUSION

The results obtained using this methodology allow us to conclude that the experimental propolis-based toothpastes showed good antibacterial activity against the analyzed bacteria, proving more effective than calcium hydroxide-based toothpaste. However, subsequent studies should be conducted to examine different specific anaerobic bacteria that are commonly present in cases of endodontic infections.

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Received for publication on 13th November 2008.

Accepted for publication on 07th April 2009.