

Artigo Original de Pesquisa

Antimicrobial activity of chlorophyll-based solution on *Candida albicans* and *Enterococcus faecalis*

Atividade antimicrobiana da solução de clorofila sobre *Candida albicans* e *Enterococcus faecalis*

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chlorophyll; endodontics;

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Abstract

Chlorophyll is a phytotherapeutic substance that presents curing properties, however it is rarely used in Dentistry. **Objective:** to analyze the antimicrobial activity of a chlorophyll-based solution on isolates of *Candida albicans* and *Enterococcus faecalis* by agar dilution method. **Material and Methods:** oral isolates and *Candida albicans* reference strain (ATCC 18804) and *Enterococcus faecalis* (ATCC 29212) were included in the study. Chlorophyll extract was diluted in Sabouraud dextrose or brain heart infusion (BHI) agar, according to the testing microorganism and poured in Petri plates, obtaining the final concentrations of 50%, 25%, 12.5%, 6%, 3% and 1.5%. *C. albicans* and *E. faecalis* strains were plated with the aid of a Steers' inoculator. Plates were incubated at 37°C for 48 h for *C. albicans* and at 5% CO₂ for *E. faecalis*. The experiments were performed in duplicate. **Results:**

the results showed that the concentrations 50% and 25% chlorophyll extract were effective against *C. albicans*. *E. faecalis* grew in the presence of all the concentrations. **Conclusion:** chlorophyll-based solution presents effective antimicrobial activity of *C. albicans* but did not present any activity on *E. faecalis*.

Palavras-chave:
clorofila; endodontia;
Candida albicans;
Enterococcus faecalis.

Resumo

A clorofila é uma substância fitoterápica que possui propriedades curativas, apesar de ainda ser pouco utilizada na odontologia. O presente trabalho objetivou analisar a atividade antimicrobiana do extrato de clorofila sobre amostras de *Candida albicans* e *Enterococcus faecalis*, utilizando o método de diluição em ágar. Foram incluídos no estudo isolados bucais e amostra-padrão de *Candida albicans* (ATTC 18804) e *Enterococcus faecalis* (ATCC 29212). O extrato de clorofila foi diluído em ágar Sabouraud ou Tryptic soy, de acordo com o microrganismo a ser testado, e vertido em placas de Petri, obtendo concentrações finais de 50%, 25%, 12,5%, 6%, 3% e 1,5%. As amostras de *Candida albicans* e *Enterococcus faecalis* foram semeadas nas placas com auxílio de replicador de Steers. Em seguida, as placas de Petri com *C. albicans* foram incubadas a 37°C por 48 horas; para as de *E. faecalis* procedeu-se do mesmo modo, em 5% de CO₂. Os experimentos foram realizados em duplicata. Os resultados mostraram que as concentrações de 50% e 25% do extrato de clorofila foram efetivas nas amostras de *Candida albicans*, entretanto nas concentrações de 12,5%, 6%, 3% e 1,5% observou-se crescimento. *Enterococcus faecalis* desenvolveu-se em todas as concentrações. Foi possível concluir que o extrato de clorofila, diluído em até 25%, tem efetiva ação antimicrobiana sobre *Candida albicans*, não apresentando atividade sobre *Enterococcus faecalis* nas concentrações testadas.

Introduction

The aim of chemical and surgical preparation is to clean and to disinfect the root canal, aiming to maintain the conditions obtained by the use of irrigation solutions. Also, intracanal medicaments and obturing pastes are important to difficult the metabolic changes between the oral milieu, root canal and support tissue, blocking the recontamination by the microorganisms presents in the oral microbiota, that is related to treatment failures.

Endodontic infections are considered polimicrobial and more than 150 bacterial species are usually found in combinations of 3 to 6 species in each canal [19]. Also, microorganisms such as yeasts may be commonly found in root canals with pulp necrosis [6, 12].

Najzar-Fleger *et al.* (1992) [13], studying the prevalence of *Candida* genus in different sites of the oral cavity, verified that 55% of the root canals presented these microorganisms. Maekawa *et al.*

(2006) [9] analyzed the microbiota from the root canals of teeth with pulp necrosis and showed that in 15.3% of the cases *Candida albicans* was identified. *Enterococcus faecalis* is also frequently isolated from root canals in cases of pulp infection and also recalcitrant infections after endodontic treatment [22, 8].

Waltimo *et al.* (2000) [24] studied the penetration of *Candida albicans* in dentine compared with *Enterococcus faecalis* through an *in vitro* model in human dentine. An effective and deep penetration was observed in the specimens infected with *E. faecalis*. The authors concluded that, although *C. albicans* penetration is possible, that is less significant in relation to the penetration ability of *E. faecalis*.

Rodrigues (2001) [15] related that enteric bacteria, mainly *E. faecalis*, and yeasts, in particular of the *Candida* genus, are commonly isolated from recalcitrant endodontic infections. This microbiota may characterize monoinfection, with the predominance of Gram-positive microorganisms

particularly facultative anaerobes. These are more resistant to antimicrobial procedures performed during the endodontic treatment and can establish themselves without other microorganisms.

Several studies have been developed about the antimicrobial activity of irrigation solutions in Endodontics. Due to several properties, such as ability to solve organic material and neutralize toxic content, sodium hypochlorite is the most frequently employed substance during the instrumentation and irrigation of root canals. It is commercially available at the concentrations of 0.5%, 1%, 2.5% and 5.25% [23, 11, 10, 2, 14, 4, 21, 20]. Cardoso (2003) [1], studying the dentinal permeability of the cervical, medium and apical thirds of the root canals after the rotatory instrumentation with different auxiliary chemical substances, concluded that the use of Endo-PTC associated to Dakin's solution increased the dentinal permeability when compared with EDTA 17% and chlorhexidine 2%. Despite of its advantages, the use of high sodium hypochlorite concentrations may cause injuries in periapical tissues [7, 21], and alternative solutions have been proposed aiming the association between antimicrobial effectiveness and biocompatibility [14].

Chlorhexidine 2% is also largely employed. Many studies proved its effectiveness on several microorganisms such as *Fusobacterium nucleatum*, *Prevotella nigrescens*, *Staphylococcus aureus*, and *E. faecalis* [23, 10, 3].

Chlorophyll is a phytotherapeutic substance that presents curing properties but is scarcely known and studied in Dentistry. Santiago *et al.* (2002) [17], testing chlorophyll solutions, concluded that it might increase the dentinal permeability, an important characteristics in the endodontic therapy. Guillén-Burgos *et al.* (2004) [5] evaluated the cytotoxicity of chlorophyll extract and verified that it can be well tolerated in cell culture by fibroblasts of periodontal ligament, in short and long experimental periods.

Saeki *et al.* (1993) [16] evaluated the antimicrobial effects of the green tea, chlorophyll and sodium copper chlorophyll on oral bacteria and concluded that all the tested substances were effective but each of them presented different specificities. Chlorophyll inhibited the growth of the tested oral bacteria, particularly *Porphyromonas gingivalis* and *Fusobacterium nucleatum*. Copper chlorophyll did not present inhibitory activity on those bacteria.

Perin (2001) [14] tested a lyophilized phytotherapeutic solution obtained from the extract of *Arctium lappa* as an auxiliary substance for the canal preparation and concluded that the tested substances were effective against *C. albicans* but did not present any effect on *E. faecalis*, *Pseudomonas*

aeruginosa and *Staphylococcus aureus*. The phytotherapy substance presented antifungal activity against *C. albicans*, while that other cepas had remained viable.

In Endodontics, chlorophyll can be indicated as an auxiliary antimicrobial solution to assist in the chemical and surgical preparation and to increase the permeability of the dental tubules. Knowing the importance of the removal of the smear layer, originated during the instrumentation due to the action of the instruments cut and being able still to be contaminated, it seems interesting to use a solution provided with antimicrobial effect in the removal of smear layer.

Based on the findings from the literature and the lack of data regarding the chlorophyll and its uses in Dentistry, the present study aimed to evaluate *in vitro* the antimicrobial activity of chlorophyll-based solution on *Candida albicans* and *Enterococcus faecalis*.

Material and methods

The solution tested in this study was composed by sodium copper chlorophyllin (Ervas Vida, Vitória-ES, Brazil). Thirty-two oral isolates and reference strain (ATCC 18804) of *C. albicans* and *E. faecalis* ATCC 29212 were included in the study.

Firstly, the isolates were plated on Sabouraud dextrose agar (Difco, Detroit, USA) or brain heart infusion agar (BHI, Difco, Detroit, USA) and incubated at 37°C for 24 hours, and 37°C/48h/5% CO₂ for *E. faecalis*. Then, standardized cell suspension containing 10⁶ cells per milliliter was turbidimetrically obtained in sterile saline solution (NaCl 0.85%).

Plates containing serial dilutions (50%-1.5%) of the testing solution in Sabouraud dextrose agar (for *C. albicans* tests) and BHI agar (for *E. faecalis*) were obtained.

Isolates were plated on each plate with the aid of a Steers' inoculator (Figure 1). Then, plates were incubated at 37°C for 48 hours (*C. albicans*) and 37°C/48h/5% CO₂ for *E. faecalis*. All the experiments were performed in duplicate. After the incubation period, readings were based on the growth of the isolates in the presence of the dilutions tested.

Aiming at characterizing the chlorophyll-based solution tested, it was submitted to pH measurement (CG710, Schott Mainz, Switzerland).

Results

The concentrations of 50 and 25% of the tested chlorophyll-based solution tested inhibited the growth of *C. albicans* isolates. The concentrations

of 1.5% to 12.5% did not show any antifungal activity. No activity was observed on *E. faecalis*. The pH of the tested solution was of 5.4.

Discussion

Several studies on the antimicrobial activity of irrigation solutions in Endodontics, such as 0.5%, 1%, 2.5% and 5% sodium hypochlorite and 2% chlorhexidine are found in the literature [23, 14, 4, 21]. On the other hand, the lack of studies on phytotherapeutic substances such as chlorophyll, propolis and castor bean detergent do not permit more objective conclusions about their use [14, 3].

The inclusion of *C. albicans* and *E. faecalis* in this study was based on the literature that relates these microorganisms to pulp infections, mainly in recalcitrant infections after endodontic treatment [13, 22, 6, 24, 15, 9, 8].

Chlorophyll is cited as a phytotherapeutic substance provided of several benefits to our organism such as the prevention of anemia, detoxifying effect and improvement of blood circulation (Ervas Vida, Vitória-ES, Brazil). Considering these benefits already studied in the medical area, the interest on chlorophyll is now spreading to Dentistry. However, few studies are still found in the literature.

The interest on this substance is based on these already described properties. Moreover, relates on an increased dentinal permeability stimulated the study of this substance. The increase in the permeability of the dentinal canals may be considered a very interesting characteristic and it is related to better cleaning activity and adaptation of the obturing material.

Santiago *et al.* (2002) [17] observed *in vitro* an increase in the permeability of dentinal canals when compared chlorophyll with Clorox and Dakin' solution and it was indicated to be used during the chemical-surgical preparation of root canal.

Also, Guillén-Burgos *et al.* (2004) [5], in experiments of biocompatibility with chlorophyll, concluded that this extract is biocompatible in human periodontal ligament fibroblast culture at short and long periods.

The methodology of this study followed the standard established for agar dilution tests [18]. This method permits more objective growth control and the use of Steer's replicator makes the testing of several samples possible at the same time.

The results obtained in this study showed that the chlorophyll-based tested solution was effective against *C. albicans* at the concentration of 25%, however no activity was observed against *E. faecalis*.

Considering that *C. albicans* is a common microorganism in pulp infections, this study suggests that in certain concentrations, chlorophyll might be indicated as a good choice as irrigation solution. On the other hand, the results obtained for *Enterococcus faecalis* were not so promising. Bacterial growth was observed in all the concentrations, however more studies on this subject are necessary.

Another fact to be considered is the value of pH, since it is related in the literature that an alkaline permits a better infection control interfering on microbial growth and survival [7]. The pH observed for chlorophyll was 5.6 that is contrary to this affirmative. On the other hand, this result corroborates the study of Guillén-Burgos *et al.* (2004) [5], which states that this pH value shows that chlorophyll has low damage potential for periapical tissues when compared to 0.5% sodium hypochlorite (pH 11.8), 24% EDTA (pH 7.2) and 15% EDTA (pH 7.4).

Considering the results obtained and the characteristics cited before, the data suggest that chlorophyll extract may have a clinical applicability as irrigation solution of the root canal if used in concentrations that might compensate its low antimicrobial potential and might preserve the good cell tolerance and capacity of permeate the dentinal canals. More studies for searching this formulation are necessary.

Conclusions

The chlorophyll-based tested solution presented antifungal activity against *C. albicans* at the concentration of 25%. No activity on *E. faecalis* was observed.

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