ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF ENDODONTIC INTRACANAL MEDICATIONS

ANDRADA TONEA1, MANDRA BADEA2, LIVIU OANA3, SORINA SAVA1, DAN VODNAR4

1Department of Prosthodontics and Dental Materials, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania
2Department of Prevention in Dentistry, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania
3Department of Anesthesiology and Surgical Propedeutics, University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Romania
4Department of Food Science and Technology, University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Romania

Abstract

Background and aims. The sterilization of the entire root canal system represents the main goal of every endodontist, given the fact that the control of the microbial flora is the key point of every root canal treatment. The diversity of microorganisms found inside the root canal and also the resistance of some bacterial species to intracanal medications led to a continuous development of new endodontic products. The present study focuses on the comparison of the antibacterial and antifungal properties of different endodontic products, two commercially available, one experimental plant based extract, and two control substances.

Methods. The disc diffusion assay was used to determine the antibacterial and antifungal properties of chlorhexidine, calcium hydroxide, a mix extract between Arctium lappa root powder and Aloe barbadensis Miller gel, Amoxicillin with clavulanic acid and Fluconazole (as control substances). Two of the most common microorganisms found in endodontic infections were chosen: Enterococcus faecalis (ATCC 29212) and Candida albicans ATCC(10231).

Results. All tested substances showed inhibition zones around the discs, for Enterococcus faecalis and Candida albicans, including the experimental mix extract of Arctium lappa root powder with Aloe vera gel.

Conclusion. The experimental mix extract of Arctium lappa root powder and Aloe vera gel is able to inhibit very resistant microorganisms, like Enterococcus faecalis and Candida albicans.

Keywords: disc diffusion, antimicrobial, antifungal, endodontic, chlorhexidine, calcium hydroxide, Arctium lappa, Aloe vera
reinfections [3,4]. Although the main preoccupation of researchers was the study of endodontic bacteria, yeasts play a very important role in the occurrence of root canal infection and reinfection. The genus Candida and in particular Candida albicans is the most often recovered yeast from the root canals of teeth with primary endodontic infections [5].

Candida albicans is exceptional isolated alone, in pure cultures, but can be commonly found together with Gram positive and Gram negative microorganisms. It has the capacity to adhere and invade dentinal tubules and to survive to severe environmental conditions [6].

The interest of researchers for intracanal irrigants and antiseptic substances has increased over the years, as they became more aware of the importance of the removal of microorganisms from the endodontic system. Different antiseptic and antibacterial substances were developed, including plant based products, with various actions and therapeutic properties [7].

The aim of this study is to compare the antibacterial and antifungal properties of some intracanal medications and therewith to introduce an experimental antiseptic substance, based on a mix extract between Arctium lappa root powder and Aloe vera gel.

Materials and method

Bacterial strains, culture conditions and tested substances

For the testing of the antibacterial and antifungal activity the following microorganisms and culture conditions were used: two bacterial strains, a facultative aerobic bacteria, Enterococcus faecalis (ATCC 29212) and one yeast, Candida albicans (ATCC 10231), purchased from Microbiologics, Saint Cloud, Minnesota, USA. The tested microorganisms were obtained from the American Type Culture Collection. The bacteria were cultured on Columbia agar for Enterococcus faecalis and on Sabouraud agar for Candida albicans and cultures were stored at 4°C and subcultured once a month. Culture media were purchased from Biomerieux, Lyon, France.

The following substances were tested: chlorhexidine gel 2% (Cerkamed), calcium hydroxide (Calxyd-Spofa) and a plant extract. The plant extract was obtained as follows: 100 mg of burdock powder was suspended in 1 ml distilled water, mixed for 8 hours and filtered by 0.45 µm Millipore filter. The obtained burdock root extract was mixed with 100 µl Aloe vera gel. As control substances, specific antibiotics and antifungals were used as follows: Amoxicillin with clavulanic acid for Enterococcus faecalis and Fluconazole for Candida albicans.

Disc diffusion assay

The primary screening test was carried out by disc diffusion, using 100 µl of suspension containing 10⁶ CFU/mL of Enterococcus faecalis and 10⁶ CFU/mL of Candida albicans, spread evenly on the surface of the Columbia agar and Sabouraud agar. Sterile 6 mm discs were used and processed, in triplicates, to contain 20 µl chlorhexidine 2%, 20 µl calcium hydroxide and 20 µl of the plant based extract. These discs were then placed on the inoculated agar. The control substances were also placed on the inoculated plates.

For Enterococcus faecalis, the inoculated plates were incubated for 48 h at 37°C in gas bags (GenBag Co2, Biomerieux, Lyon, France) and for Candida albicans the inoculated plates were incubated for 2-3 days at 30°C. Inhibition zones around discs indicated the presence of antimicrobial and antifungal activity. For optimal fidelity of results, each assay was repeated three times.

Data analysis

Data analysis was performed by applying the ANOVA one way test, to investigate the statistical significance of the data between test groups.

Results

Antimicrobial efficacy test

Enterococcus faecalis and Candida albicans were efficiently inactivated by some of the tested substances (Figure 1). The average inhibition zone of Enterococcus faecalis for chlorhexidine 2% was 14.15 mm, for calcium hydroxide 0.04 mm, while the plant mix extract showed an average inhibition diameter of 5.69 mm. The control substances (Amoxicillin and clavulamic acid) exhibited an average inhibition diameter of 13.66 mm (Table I).

![Figure 1. Antimicrobial efficacy test for Enterococcus faecalis showing inhibition zones around chlorhexidine 2%, calcium hydroxide, Amoxicillin and clavulamic acid, experimental mix extract. Antimicrobial efficacy test for Candida albicans showing inhibition zones around chlorhexidine 2%, calcium hydroxide, Fluconazole and experimental mix extract.](image)

| Group         | Count | Inhibition zone (mm) |
|---------------|-------|----------------------|
|               |       | Mean   | Standard deviation (+/-) |
| Control       | 3     | 13.66  | 0.030                  |
| CHX 2%        | 3     | 14.15  | 0.020                  |
| Ca(OH)₂       | 3     | 0.04   | 0.020                  |
| Extract       | 3     | 5.69   | 0.010                  |
The average inhibition diameter of Candida albicans for chlorhexidine 2% was 12.18 mm, followed by the average inhibition diameter of the plant mix extract, 11.62 mm. The average inhibition diameter for calcium hydroxide was 0.09 mm. The control substance (Fluconazole) exhibited an average inhibition diameter of 12.68 mm (Table II).

All the test groups showed a statistically significant difference when compared to the control group (p<0.05).

| Group       | Count | Inhibition zone (mm) |
|-------------|-------|----------------------|
|             |       | Mean    | Standard deviation (+/-) |
| Control     | 3     | 12.68   | 0.026                |
| CHX 2%      | 3     | 12.18   | 0.017                |
| Ca(OH)₂     | 3     | 0.09    | 0.026                |
| Extract     | 3     | 11.62   | 0.020                |

**Table II** - Antimicrobial efficacy test: diameters of inhibition zones (mean and standard deviation) for Candida albicans ATCC-10231 around the tested substances.

**Discussion**

Previous research evaluated the effectiveness of different antiseptic products, as root canal therapy agents. Several studies proved the antimicrobial effect of chlorhexidine. From its use as an irrigant solution [8] to the use in form of gel, directly inside the root canal [9], chlorhexidine is known as a reliable antimicrobial agent.

Calcium hydroxide is mostly used for its antiseptic properties. However, the mechanisms of this substance are still being studied. Regarding its role against endodontic biofilms, studies showed that calcium hydroxide is not as effective as other substances used as intracanal medications [10].

In the continuous pursuit of developing the antimicrobial endodontic treatment, a number of plants were studied. Propolis, Morinda citrifolia (noni fruit), Marticaria recutitia (chamomile), Melaleuca alternifolia (tea tree), Aloe vera and Arctium lappa (burdock) are some examples, whose pharmaceutical characteristics have been recognized along the time [11].

Arctium lappa, also known as burdock, is a plant commonly found in Europe [12] and acknowledged for its healing effects. In the traditional medicine, the root of burdock was known for its antioxidant and antidiabetic properties. Antimicrobial effect of Arctium lappa in dentistry, against oral microorganisms and also against microorganisms associated with endodontic infections, were investigated and proven during several studies [13,14].

Antimicrobial, antifungal and anti-inflammatory properties of Aloe vera are highly recognized all over the world. The plant is used in different areas of general medicine, but also of dental medicine. From the use as an oral dentifrice [15] or as a local drug in periodontal pockets [16] in periodontology, to the use of Aloe vera gel against microbial strains in endodontic infections, a great variety of dentistry domains were covered by the properties of this plant. Regarding the endodontic treatment, the antimicrobial properties of Aloe vera were previously investigated and compared with other plants and antiseptic substances [17]. The efficiency of Aloe vera was also studied in case of the root canals of primary teeth, exhibiting a higher antimicrobial activity compared to medications based on eugenoil [18].

The huge diversity of endodontic microorganisms that populate the root canal system and also their different metabolisms influence the response to the intracanal dressings applied during the treatment.

Candida albicans is the predominant yeast identified directly from the infected root canals [19]. It is able to diminish the host defense system with the help of various characteristics such as adhesion to different types of surfaces, production of enzymes, morphologic transition or formation of the biofilm [20]. Candida albicans is present in a high percentage in the previously treated root canals [21].

Enterococcus faecalis is known for its resistance to antiseptic root canal medications. It is frequently present in primary endodontic infections but also in the case of reinfection of previously filled root canals. Enterococcus faecalis can be found in biofilms, together with other microorganisms and rarely alone, as a single bacteria [22,23,24].

**Conclusions**

Based on this study, chlorhexidine 2% exhibits the most efficient antimicrobial activity against Enterococcus faecalis and Candida albicans.

Calcium hydroxide demonstrates a very low efficiency against both microorganisms and should not be used as an antimicrobial substance on its own, but rather for its other biological and chemical properties.

The experimental mix extract of Arctium lappa root powder and Aloe vera gel is very efficient against Candida albicans, but exhibits a smaller diameter of inhibition for Enterococcus faecalis. Considering these results, further studies are necessary in order to evaluate the antibacterial effect of this product.

Given the fact that endodontic bacteria are organized in biofilms, further investigations must be conducted, to determine the behavior of the experimental product, not only against single microorganisms, but also against complex bacterial communities.

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