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Original Article

In vitro Evaluation of Antimicrobial Activity of Brazilian Red Propolis Containing-dentifrice

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ABSTRACT

Background: This study evaluated the antimicrobial activity of brazilian red propolis (BRP) dentifrice and compare *in vitro*. **Materials and methods:** Strains of *S. mutans* ATCC UA159 were used in the present study. This study compared the antibacterial effects of BRP extract, BRP containing-dentifrice and one antimicrobial commercial dentifrice (Parodontax) against *S. mutans*. For the evaluation of the antimicrobial activity the microdilution method was used in culture broth. The strain was activated by incubation at 37°C overnight in Brain Heart Infusion (BHI) culture medium, in an anaerobic jar. To the wells of the microplates were added 100µL of BHI broth, 20µL of the substances tested (experimental groups), at concentrations ranging from 100 µL/mL to 0.0488 µL/mL, and 80 µL/mL of the standardized microbial suspension. The microplates were incubated for 24 hr in a bacteriological oven at 37°C. Visual inspection of the colour changes and reading in BioTek microplate reader at 570nm was performed to determine the Minimum Bactericidal Concentration (MBC). Mean values and standard deviations were calculated. ANOVA followed by Dunnett's test was performed; *p*-value of less than 0.05 was considered significant. **Results:** BRP extract and BRP containing-dentifrice showed antimicrobial activity against *S. mutans* up to the concentration of 0.3906 µg/mL. The BRP extract and dentifrice showed bactericidal effect at a concentration of 1.5625, but was able to reduce microbial viability at a concentration of up to 0.3906. Parodontax dentifrice showed antimicrobial activity at all dilutions ($p < 0.05$), presenting a bactericidal effect in the same concentration of the extract (1.5625). It was concluded that all the groups observed had antimicrobial activity against *S. mutans*. There was no difference between the propolis extract and the common dentifrice. **Conclusion:** BRP in pure form and processed in a toothpaste shows some antimicrobial activity against *S. mutans* but less than a commercial toothpaste containing herbal extracts and sodium bicarbonate.

Keywords: Propolis, *Streptococcus mutans*, Dentifrice, Biofilm, Dental decay.

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INTRODUCTION

Bioactive molecules of natural products have pharmacological activity and have been widely studied and used by the pharmaceutical industry as an alternative to traditional medicines.[1,2]

The biofilm developed on the dental surface is composed of several species of micro-organisms, initially colonized by gram-positive and aerobic bacteria, but later, there is a sequential colonization of gram-negative and anaerobic micro-organisms. Thus, a constant and effective disorganization of this biofilm is necessary to prevent these oral pathologies.[3,4]

Propolis is a complex mixture formed by a non-toxic resinous material that is collected by *Apis mellifera* bees from different parts of the plant such as shoots, branches, flowers, pollen and tree exudates, associated with the salivary secretions and enzymes of these bees, which are rich in biological properties. This complex mixture is used by bees to provide closure of the beehive and its asepsis through its antimicrobial action. Due to these biological properties, propolis has been used for centuries in alternative medicine, including for oral diseases as a therapeutic alternative.[5,6]

The chemical composition of propolis will depend on the local biodiversity where the hive is located. They are usually composed of 50% resin and vegetable balsam, 30% wax, 10% essential oil and other aromatic compounds, 5% pollen and 5% other substances.[7]

The biological activities of propolis have been widely explored in various fields of medicine as an important resource for the prevention and treatment of oral and systemic diseases.[6-8]

In Brazil there are 13 types of propolis cataloged, where the one found in Alagoas in the Brazilian Northeast, the name brazilian red própolis (BRP) is due to the red coloring coming from the pigments of the plants.[8]

It is known that this type is relatively new and has aroused attention because chemical composition and promising pharmacological properties, especially antimicrobial and anti-inflammatory. In the state of Alagoas, whose botanical origin is *Dalbergia ecastophyllum*. This type is unique to this region, having a high concentration of isoflavonoids in its composition, which made the National Institute of Industrial Property (INPI) grant the title of Geographical Indication to that locality, ensuring the international certificate of only producer of this type of propolis in the world, with assured quality throughout the year.[9-12]

This product has a high medicinal, historical and economic value, and in recent years PVB has had a great commercial expansion, where the interest in the pharmaceutical industry and its production has increased considerably worldwide, especially in Brazil, Japan, China, Russia,

Germany and France. In dentistry researches point promising results in various specialties such as Endodontics, Cariology, Surgery, Preventive Dentistry and Periodontics.[13]

In this context, investments in patents are increasingly widespread in capitalism, because the entire operation of the system is related to innovation and scientific and technological advancement. For this, advance is essential to the existence of intellectual property rights through patent protection.[14] No reports of the use of incorporated dentifrice with BRP were found in the literature, thus the application for a patent of invention was deposited under protocol BR1020170110974. The objective of the present study was to evaluate *in vitro* the antimicrobial activity of a dentifrice of BRP against *Streptococcus mutans (S. mutans)*.

MATERIALS AND METHODS

Bacterial Strains and Culture Medium

Strains of *S. mutans* ATCC UA159 were used in the present study. The strain was activated by incubation at 37°C overnight in Brain Heart Infusion (BHI) culture medium and in anaerobic jar, using the candle method, for a period of 24 hr.

Brazilian Red Propolis Extract and Dentifrice Preparation

The extract of red propolis was collected from the State of Alagoas (Latitude South 9 ° 44.555 ‘, Latitude West 35 ° 52.080’ and altitude of 18.1 m above sea level). It was used 150 g of the extract of red propolis and dissolved in 1L of alcohol of cereals of greater graduation. The extract of BRP at 1% concentration (antimicrobial concentration previously studied) was incorporated into the fluoridated dentifrice (1500ppm). After, chemical identification of the constituents and the dentifrice was performed by High Performance Liquid Chromatography (HPLC).

Microbiological Analysis

An experimental study was carried out in which the potential antimicrobial activity of BRP dentifrice on the standard strain *S. mutans* ATCC UA159 was investigated. All the tests were performed in triplicate and at two different times. The following groups were tested: G1- BRP extract; G2- BRP dentifrice; G3- Parodontax® dentifrice.

For the evaluation of the antimicrobial activity, the micro dilution method was used in culture broth, according to Standard M07-A10 of the Clinical and Laboratory Standard Institute (CLSI, 2015).

After this time, the cultures had their cell density adjusted in sterile 0.85% saline so as to achieve a turbidity equivalent to the 0.5 tube of the Mc Farland scale (approximately 1.5 x 10^8 CFU / mL). The suspension obtained was diluted 100-fold in sterile BHI medium. This suspension was used in the assay (Figure 1).

To the wells of the microplates were added 100µL of BHI broth, 20µL of the tested substances (experimental groups), at concentrations ranging from 100 µL / mL to 0.0488 µL / mL, and 80 µL / mL of the standardized microbial suspension. The microplates were incubated for 24 hr in a bacteriological oven at 37°C. After this period, visual inspection of microbial growth was performed. Due to the intense turbidity of the substances tested, 10 µL of resazurin solution (0.01%) was added to all wells, and the microplates were incubated at 37°C for 2 hr. Visual inspection of the color change and reading in BioTek microplate reader at 570nm was performed to determine the Minimum Bactericidal Concentration (MBC) (Figure 1).

Turbidity control (medium + substance of the experimental groups), growth control (medium + microbial suspension) and control of sterility of the culture medium (medium only) were performed

RESULTS

Figure 1 shows the CBM of the group treated with BRP extract. The extract showed significant antimicrobial activity against *S. mutans* up to the concentration of 0.3906 µg/mL. Serial dilutions were performed in order to show the lowest concentration.

Figure 2 shows the CBM of the group treated with BRP dentifrice. The BRP dentifrice again showed significant antimicrobial activity against *S. mutans* up to the concentration of 0.3906 µg/mL. Serial dilutions were performed in order to show the lowest concentration.

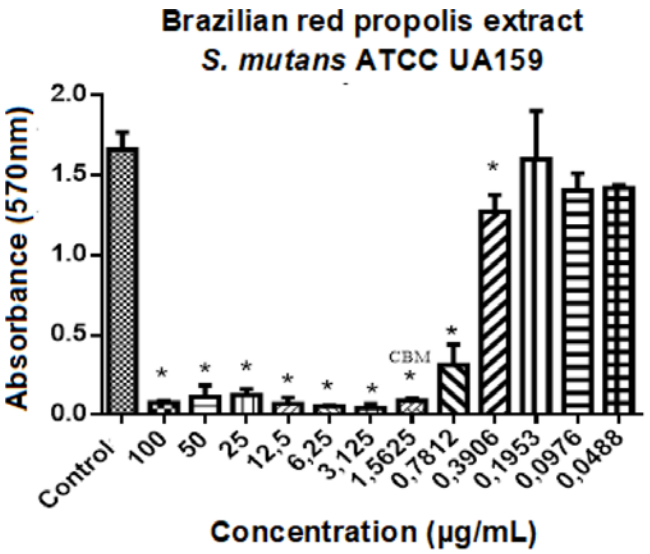

Figure 1: CBM of the Brazilian red propolis extract on S. mutans ATCC UA 159. *P<0.05 compared to the control group (ANOVA and Dunnett tests). All the values are mean ± 0.05 (n =3).

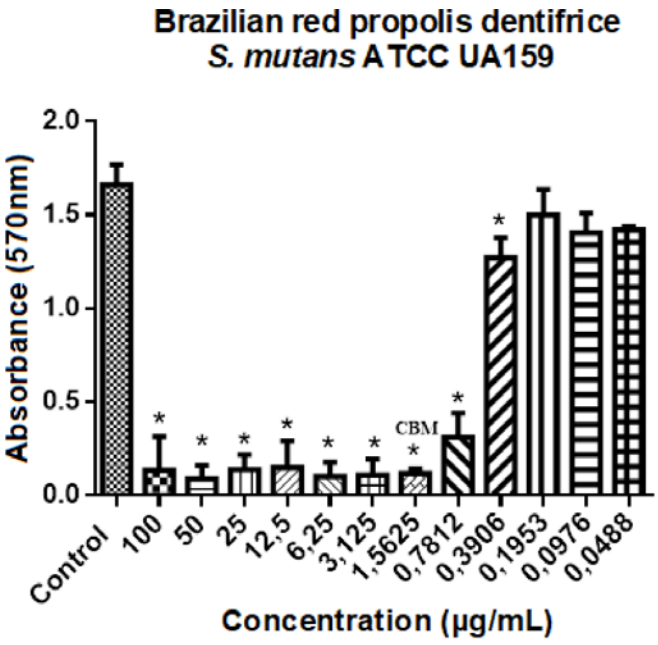

Figure 2: CBM of the Brazilian red propolis dentifrice on S. mutans ATCC UA 159. *P<0.05 compared to the control group (ANOVA and Dunnett tests). All the values are mean ± 0.05 (n =3).

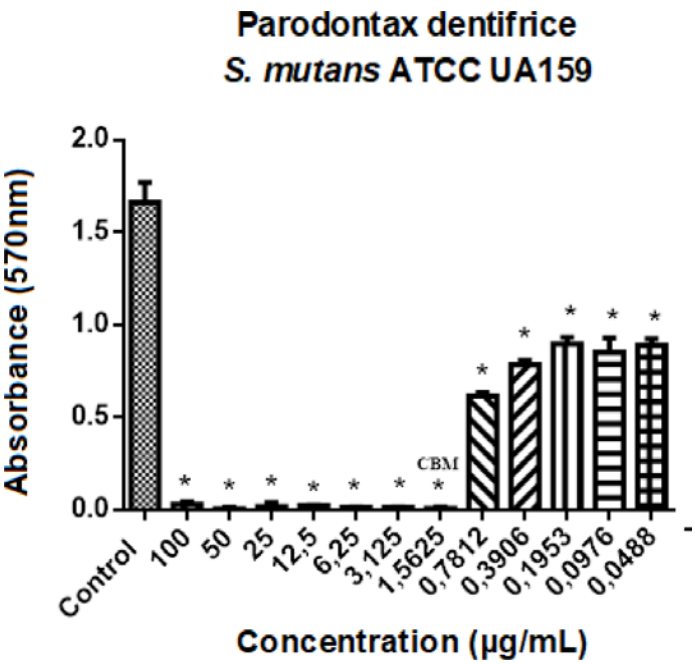

Figure 3: CBM of the Parodontax dentifrice on S. mutans ATCC UA 159. **P*<0.05 compared to the control group (ANOVA and Dunnett tests). All the values are mean ± 0.05 (n =3).

Figure 3 shows the CBM of the group treated with Parodontax dentifrice. Parondontax dentifrice also showed significant antimicrobial activity against *S. mutans* at all concentrations.

DISCUSSION

Resistance to anti-infective chemotherapeutics and the search for substances with biological properties with lower adverse effects has increased interest in natural products. More than one hundred million molecules are cataloged around the world and many are still unexplored.[2,11,15]

The high demand for propolis and the investment in biotechnology of bee products contributed to the launch of several products on the market. Thus, challenges regarding resistance to synthetic antimicrobials increased the search for natural products, as for the derivatives of bees, honey and propolis stand out.[16-18]

Propolis is notable for the great gram-positive and gram-negative antimicrobial spectrum against colonizers of the dental biofilm, such as *S. mutans*, *Lactobacillus*, *P. gingivalis*, *Actinomyces naeslundii*, *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Prevotella intermedia*. This product has potent antimicrobial activity even at the concentration of 0.1%, possessing antimicrobial and anti-inflammatory activity proven *in vitro* and *in vivo*. This is due to high concentration of flavonoids and phenolic compounds.[13-17]

Streptococcus is one of the main micro-organisms that colonize the oral cavity, being pioneering species in the various mouth niches.[19] Cayo *et al*. evaluated the *in vitro* antimicrobial effect of various concentrations of the Peruvian propolis against *S. mutans* (ATCC 25175). At the same 10% concentration of propolis was sufficient for inhibition of halos.[20]

Fernandes Jr *et al*. analyzed the minimum inhibitory concentration of the propolis alcoholic extract found in Botucatu-SP, Mossoró-RN and Urubici-SC on species isolated from human clinical infections (*Staphylococcus aureus*, *Escherichia coli*, Enterococcus sp., *Pseudomonas aeruginosa* and *Candida albicans*). In the analysis, there was a higher sensitivity for gram- positive bacteria when compared to gram-negative bacteria.[21]

Grenho *et al*. believe that the various infections associated with biomaterials require new strategies to overcome this problem. The authors studied the association of nanohydroxyapatite with green propolis (25%) and red (25%) in the prevention of bacterial growth against *S. aureus* as well as cytotoxicity. The results showed that propolis had antimicrobial activity and was not cytotoxic to cell fibroblasts.[22]

Koru *et al*. evaluated the minimum inhibitory concentration (MIC) and MBC of propolis samples (10%) in four different regions of Turkey and Brazil against nine species of anaerobic bacteria using the dilution method of agar. The analysis showed that the different extracts had better activity in gram-positive than gram-negative bacteria and flavonoids were the most found components.[23]

Simões *et al*. evaluated *in vitro* and *ex vivo* the action of different concentrations of propolis extracts and compared with oral antiseptics using the saliva of individuals. The extracts at concentrations of 11%, 20% and 30% of propolis marketed in Bahia were compared to Periogard, Listerine, Malvatricin and Parodontax. In this study, solutions prepared with propolis had the same antimicrobial action independent of concentration and presented the same pharmacological action of commercial antiseptics.[24]

According to Silva *et al*. the lack of standardization of methodologies in products incorporated with propolis limits its use of industry, especially food and pharmaceutical. The authors evaluated *in vitro* several biological activities of extracts of propolis 2%, red, green and brown from different Brazilian regions. The extracts were prominent in the antimicrobial activity mainly against *S. aureus*, *E. coli* and *C. albicans*, especially the red propolis extract, type 13, for antimicrobial, antiparasitic and antioxidant activities.[25]

It was concluded that all the groups observed had antimicrobial activity against *S. mutans*. There was no difference between the propolis extract and the dentifrice incorporated with the extract. However, there was difference in the common dentifrice group.

CONCLUSION

It was concluded that all the groups observed had antimicrobial activity propolis extract, the dentifrice incorporated with the extract and the common dentifrice. The Paradontax dentifrice obtained the best results. against *S. mutans*. There was no difference between the propolis extract, the dentifrice incorporated with the extract and the common dentifrice. The Parodontax dentifrice had MBC equivalent to those of the test substance.

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