



# Physicochemical Evaluation of a Toothpaste Incorporated with Brazilian Red Propolis

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## Authors' contributions

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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## ABSTRACT

The development of new types of toothpastes can be highlighted, which are the most used products for oral hygiene products. In this context, natural products have been incorporated and propolis has stood out because its several biological properties. The present study aimed to evaluate the physicochemical characteristics of the mentioned toothpaste during a period of one year. After this, the physical chemical characterization of the BRP dentifrice was performed and the stability was analyzed on the day of the manipulation, 90, 180 and 365 days after storage. The tests included: macroscopic analysis and determination of pH and mass. Odor and appearance also demonstrated stability, exhibiting a characteristic odor characteristic of propolis and mild mentholate as well as a standard dentifrice aspect. The data showed no statistical significance over time intragroup and intergroup ( $p > 0.05$ ). The pH of the formulation showed slight variations during measurements. The result of the evaluation of the formulation of the dentifrice incorporated with Brazilian red propolis was within the initial expectations for the product, meeting the parameters of the Brazilian regulatory agency and presenting parameters that suggest stability.

*Keywords: Dentifrices; propolis; stability.*

## 1. INTRODUCTION

The use of naturals in dental formulations, for the control of biofilm products, has been growing in recent times. The development of new types of toothpastes can be highlighted, which are the most used products for oral hygiene products [1-3].

Among the various natural products used in dental formulations, propolis stands out. It is a resonant complex collected by *Apis mellifera* bees from different parts of the plant. Propolis has several pharmacological properties, including those of dental interest, such as antimicrobial and anti-inflammatory activity [4,5]. In Brazil, among the 13 types of propolis, stands out Brazilian propolis (BRP). This type can be found in the region of Alagoas and some years ago, received the title of geographical indication by the National Institute of Industrial Property [6].

Propolis is a product that has been standing out for its pharmacological properties, especially antimicrobial and anti-inflammatory, in which several products have been launched on the market to combat various conditions [4,5].

In this context, a fluoride toothpaste associated with BRP extract was developed and patented in Brazil (BR1020170110974) in order to be a low-cost alternative for the control of biofilm-related diseases, with clinical and antimicrobial properties being reported by clinical and laboratory studies [2,7,8].

The present study aimed to evaluate the physicochemical characteristics of the

mentioned toothpaste during a period of one year.

## 2. MATERIALS AND METHODS

### 2.1 Brazilian Red Propolis Extract

The extract of red propolis was collected in the city of Marechal Deodoro (Latitude South 9°44.555', Latitude West 35°52.080' and Altitude of 18.1 m above sea level). It was used 150 grams of the extract of red propolis and dissolved in 1L of alcohol of cereals of greater graduation.

### 2.2 Preparation of BRP Dentifrice and Macroscopic Analysis

The extract of Brazilian red propolis at 1% concentration (antimicrobial concentration previously studied) was incorporated into the fluoridated dentifrice (1500 ppm) in the Pharmacotechnical laboratory of the Pharmacy course of the Federal University of Ceará, Brazil (Fig. 1). After, chemical identification of the constituents and of the dentifrice was performed by High Performance Liquid Chromatography (HPLC), being mainly identified the constituents Quercetin, Vestitol and Neovestitol.

After this the physical chemical characterization of the BRP dentifrice was performed and the stability was analyzed on the day of the manipulation, 90, 180 and 365 days after storage. The tests included: macroscopic analysis and determination of pH and mass.

### 2.3 Evaluation of pH

The pH verification of the samples under study was carried out in a potentiometer, previously

calibrated with buffer solutions pH 7.0, 12.0 and pH 4.0. A calibrated pH meter (Sensoglass – SP 1800) was used through a glass electrode. A sample of 5 mL of dentifrice was dispensed in a glass beaker and suspended in 3 parts of distilled water, obtaining a final volume of 20 mL, after stirring, the reading was performed.

## 2.4 Evaluation of Mass

The analysis of the mass of the samples was performed in a semi-analytical balance (Bel S203H), the analyzes were performed in triplicate.

Analysis of organoleptic characteristics: The organoleptic characteristics were evaluated following ANVISA [9] guidelines in different periods of time, using as a comparison parameter the characteristics obtained immediately after handling each batch of experimental toothpaste.

## 3. RESULTS

The main constituents in the extract were identified, Vestitol, Neovestitol and Quercetin (Fig. 2).

Regarding the organoleptic characteristics, the product remained stable during the evaluated periods (D1, D90, D180 and D365). The color of the dentifrice showed to be slightly gray throughout the period as can be seen in Fig. 1. Odor and appearance also demonstrated stability exhibiting a characteristic odor characteristic of propolis and mild mentholate as well as a standard dentifrice aspect.

The pH of the formulation showed slight variations ( $p > 0.05$ ) during measurements as can be seen in Table 1. From the foregoing it has been found that the pH of the dentifrice is suitable for this type of pharmaceutical form, since an acid pH of the product could generate wear on the matrix and because it is a calcium and fluoride dentifrice, it may favor the demineralization process.

The mass variation of the formulations also exhibited stability as shown in Table 2, without significant changes ( $p > 0.05$ ).

## 4. DISCUSSION

Products that include propolis in their formulation are growing in the market. This is due to the

investment made by industries in biotechnology of products derived from bees [10]. As previously mentioned, this interest is a consequence of the various properties that propolis can have as an alternative or complementary therapy in dentistry [11,12].

Formulations with natural products have been used as an alternative to the adverse effects of synthetic drugs, such as antimicrobial resistance. It is known that many of the active molecules are still unexplored [1,13-15].

In the case of Brazilian red propolis, used in the present study to formulate the toothpaste, it has potent antimicrobial activity even at a concentration of 0.1%, this is due to the high concentration of flavonoids and phenolic compounds [1,13-15].

The present dentifrice demonstrated in vitro properties similar to Parodontax dentifrice [8]. Clinically, it already has some properties identified, regarding the antimicrobial activity of *S. mutans*, *Lactobacillus*, gram-negative bacteria and clinical activity regarding the bacterial plaque index and gingival bleeding index [2,7]. In addition, it has been found to reduce inflammatory cytokines from gingivitis [16,17] and has a good acceptance in orthodontic patients [18]. As for the availability of fluoride in saliva, it showed kinetics similar to a commercial toothpaste [19]. However, given the possibility of one day becoming commercial, studies - related to stability - are essential. In the present study, it was noticed that the pH was being changed making it slightly basic, but this is not a concern. According to Tokura et al. [20] the acidic pH leads to a lower diffusion of fluoride in the dental biofilm in the short term, thus influencing the cariogenic activity. In addition, an acidic pH, negatively, influences erosion control [21]. However, further studies should assess the influence of high pH dentifrices on antibiofilm activity.

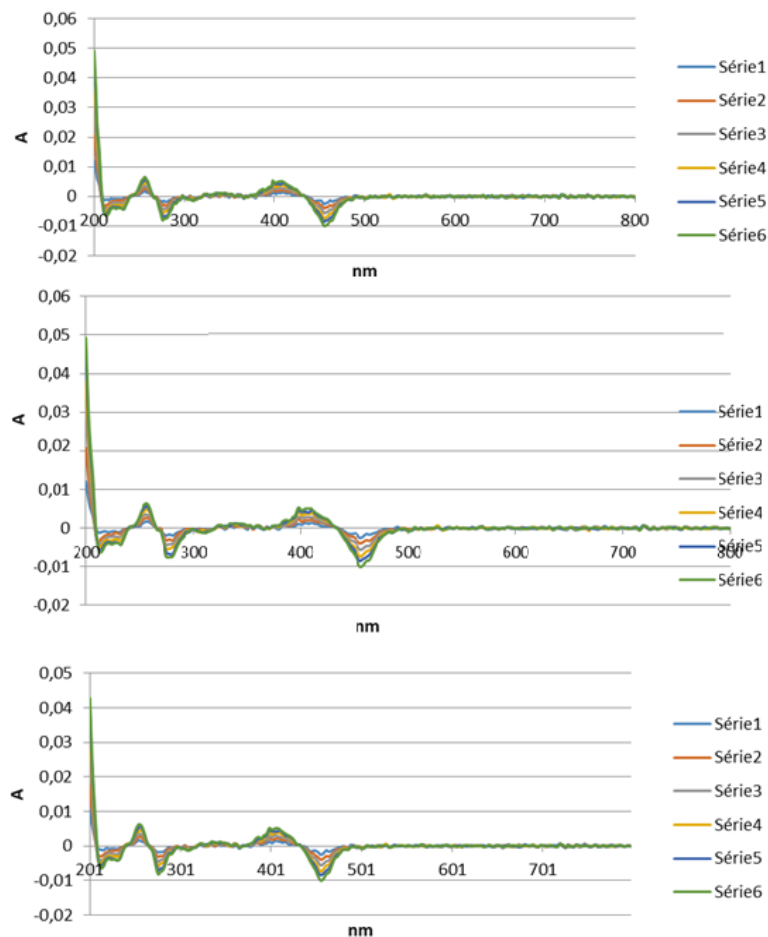
Studies similar to this one are found in the literature. Hilgert et al. [22] evaluated the physicochemical characteristics of whitening dentifrices, considering the mass loss by desiccation, ash content and pH of the dentifrices. The evaluated samples showed different physicochemical characteristics, but the pH in all samples was higher than 7, similar to the present study. The authors report that an alkaline pH tends to cause less change in tooth enamel when compared to dentifrices with an

acidic pH. Santos [23] also carried out a similar study following the ANVISA Cosmetic Products Stability guide [9], where he determined the organoleptic characteristics (color, odor and taste) and physicochemical properties (density, consistency, pH, rheological characteristics and abrasiveness) of a toothpaste. The authors

verified the samples in the periods of 0, 15, 30, 60 and 90 days, and they remained stable. Other studies with natural products also showed stability for up to 90 days, such as the one by Leite [24] in a dentifrice by *Ricinus Communis* and Valones et al., [25] in a *Rosmarinus officinalis* Linn toothpaste.



**Fig. 1. BRP dentifrices after 60 days of production**



**Fig. 2. Chemical characterization by HPLC**

**Table 1. Changes on the pH on the dentifrices**

Formulation	D1	D90	D180	D365	p-value
1	9.53	9.64	10.06	10.31	> 0.05
2	9.52	9.62	10.01	10.52	> 0.05
3	9.63	9.75	10.03	10.45	> 0.05
<b>p-value</b>	> 0.05	> 0.05	> 0.05	> 0.05	

**Table 2. Changes on the mass of the dentifrices**

Formulation	D1	D90	D180	D365	p-value
1	59.71	58.16	55.85	51.12	> 0.05
2	56.13	55.44	54.26	52.17	> 0.05
3	56.77	55.43	54.72	51.33	> 0.05
<b>p-value</b>	> 0.05	> 0.05	> 0.05	> 0.05	

It is known that changes in properties can be a sign of contamination, which reinforces the safety of the product in a period of one year, the maximum time that normally a toothpaste is in an establishment. Studies like these are important, because evaluating the organoleptic characteristics determines the safety and stability of the product, being a control of the chemical and physical quality of the same.

## 5. CONCLUSION

The result of the evaluation of the formulation of the dentifrice incorporated with Brazilian red propolis was within the initial expectations for the product, meeting the parameters of the Brazilian regulatory agency and presenting parameters that suggest stability. However, further studies are still needed in future evaluations, to obtain more detailed information about the product's shelf life.

## CONSENT AND ETHICAL APPROVAL

It is not applicable.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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