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DESENVOLVIMENTO, EFICÁCIA CLÍNICA E MICROBIOLÓGICA DE DENTIFRÍCIO INCORPORADO COM PRÓPOLIS VERMELHA BRASILEIRA

JOÃO HILDO DE CARVALHO FURTADO JÚNIOR

FORTALEZA
2019

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Tese apresentada ao Programa de Pós-graduação em Desenvolvimento e Inovação em Medicamentos da Universidade Federal do Ceará, como parte das exigências para a obtenção do título de Doutor em Desenvolvimento e Inovação Tecnológica em Medicamentos.

Orientadora: Profa Dra Marta Maria de França Fonteles (Universidade Federal do Ceará).

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JOÃO HILDO DE CARVALHO FURTADO JÚNIOR

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Esta Tese foi submetida como parte dos requisitos necessários à obtenção do GRAU DE DOUTOR em Desenvolvimento e Inovação Tecnológica em Medicamentos, outorgado pela Universidade Federal do Ceará, e encontra-se à disposição dos interessados na Biblioteca Setorial da referida Universidade.

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RESUMO

Desenvolvimento, eficácia clínica e microbiológica de dentifrício incorporado com própolis vermelha brasileira. João Hildo de Carvalho Furtado Júnior. Orientadora: Profa. Dra. Marta Maria de França Fonteles. Tese de Doutorado em Desenvolvimento e Inovação Tecnológica em Medicamentos. Universidade Federal do Ceará, 2019.

A própolis é um complexo resinoso com diversas atividades biológicas documentadas na literatura, diversos são os produtos derivados do própolis e a cada dia depósitos de patentes são realizados. A cavidade bucal é bastante complexa, e em indivíduos com fatores retentivos, como pacientes ortodônticos, ocorre um maior acúmulo do biofilme dentário. Objetivou-se realizar um estudo prospectivo de patentes odontológicas sobre própolis. Desenvolver, avaliar in vitro e clínico a eficácia de um dentifríco a base de extrato de própolis vermelha brasileira (PVB). Trata-se de um estudo desenvolvido em três etapas. Na primeira etapa foi realizado um estudo prospectivo de patentes sobre própolis e Odontologia em bancos de propriedade intelectual. Na segunda etapa, verificou-se in vitro a atividade antimicrobiana do extrato e dentrifricio de PVB, e comparou ao dentifrício Parodontax. Cepas de *Streptococcus mutans* (*S.mutans*), foram utilizadas e o método de microdiluição em caldo de cultura foi o escolhido. A terceira etapa, trata-se de um ensaio clínico, onde um total de 92 participantes, livres de cárie, foram randomizados em 2 grupos: Grupo I –Dentífricio fluoretado comum e Grupo II – Dentifricio PVB. Cada grupo utilizou os dentifrícios por um período de 28 dias, três vezes diariamente. Exame gengival foi realizado para avaliação do índice de sangramento marginal (ISM) e saliva foi coletada no *baseline* (T0) e após 28 dias (T28) para contagens de unidades formadoras de colônia (UFC) de bactérias gram-negativas e *S.mutans*. Os dados de ISM e UFC (log10) foram expressos em forma de média e desvio-padrão, submetidos ao teste de normalidade de Kolmogorov-Smirnov e comparados por meio dos testes de Wilcoxon e Mann-Whitney. Foram encontrados 62 produtos de própolis depositados em bancos de propriedade intelectual no mundo. No estudo in vitro o extrato e dentifrício de própolis mostraram eficácia contra *S.mutans* até a concentração de 0,3906 ug/mL e o dentifrício Parodontax mostrou atividade antimicrobiana em todas as diluições. Não houve assim diferença entre o extrato de própolis e o dentifrício incorporado com o extrato. Na fase clínica, os dois grupos tiveram redução do ISM estatisticamente significante, não havendo diferença na análise inter-grupo. Durante o período de tratamento o dentifrício de PVB demonstrou melhor atividade clínica e antimicrobiana. Futuros estudos são necessários para identificar melhores efeitos para estabelecer o uso do dentifricio no controle do biofilme dentário.

Palavras-chave: Própolis. Biofilme. Produtos naturais. Saliva.

ABSTRACT

Development, clinical evaluation and microbiological of brazilian red própolis containing-dentifrice. João Hildo de Carvalho Furtado Júnior. Supervisor: Professor PhD Marta Maria from França Fonteles. Doctoral thesis in Development and Technological Innovation in Medicines. Federal University of Ceara, 2019.

Introduction: Propolis is a resinous complex with several biological activities documented in the literature, several are products derived from propolis and every day patent deposits are made. The buccal cavity is quite complex, and in individuals with retentive factors, such as orthodontic patients, a greater accumulation of the dental biofilm occurs. Objectives: To carry out a prospective study of dental patents on propolis. To develop, evaluate in vitro and clinical the efficacy of a dentifrice based on Brazilian red propolis extract (PVB). Material and Methods: This is a three-stage study. In the first stage a prospective patent study on propolis and dentistry in intellectual property banks was carried out. In the second step, the antimicrobial activity of the extract and dentrifricium of PVB was verified in vitro, and compared to the dentifrice Parodontax. Strains of Streptococcus mutans (S.mutans) were used and the microdilution method in culture broth was chosen. The third stage was a clinical trial, in which a total of 92 caries-free participants were randomized into two groups: Group I - Common fluoridated dentifrice and Group II - Dentifrice PVB. Each group used the toothpaste for a period of 28 days, three times daily. Gingival examination was performed to evaluate the marginal bleeding index (ISM) and saliva was collected at the baseline (T0) and after 28 days (T28) for counts of colony forming units (CFU) of gram-negative bacteria and S.mutans. The ISM and UFC (log10) data were expressed as mean and standard deviation, submitted to the Kolmogorov-Smirnov normality test and compared using the Wilcoxon and Mann-Whitney tests. Results: There were found 62 products of propolis deposited in intellectual property banks in the world. In the in vitro study the propolis extract and dentifrice showed efficacy against S.mutans up to the concentration of 0.3906 µg/mL and the Parodontax dentifrice showed antimicrobial activity at all dilutions. There was thus no difference between the propolis extract and the dentifrice incorporated with the extract. In the clinical phase, the two groups had statistically significant ISM reduction, and there was no difference in the intergroup analysis. During the treatment period the PVB dentifrice showed better clinical and antimicrobial activity. Future studies are needed to identify better effects to establish the use of dentifrice in the control of dental biofilm.

Key-words: Propolis. Biofilm. Natural products. Saliva.

LISTA DE ILUSTRAÇÕES

ILUSTRAÇÕES REFERENTES AO CAPÍTULO 1

ILUSTRAÇÕES REFERENTES AO CAPÍTULO 2

ILUSTRAÇÕES REFERENTES AO CAPÍTULO 3

LISTA DE TABELAS

TABELAS REFERENTES AO CAPÍTULO 1

TABELAS REFERENTES AO CAPÍTULO 2

TABELAS REFERENTES AO CAPÍTULO 3

LISTA DE ABREVIATURAS E SIGLAS

BRP	Brazilian Red Propolis
BHI	Brain Heart Infusion
CFU	Colony Forming Unit
CLSI	Clinical and Laboratory Standard Institute
CONSORT	Consolidated Standards of Reporting Trials
F	Flúor
GBI	Gingival Bleeding Index
HPLC	High Performance Liquid Chromatography
ICDAS	International Caries Detection and Assessment System
INPI	Instituto Nacional de Propriedade Industrial
ISM	Índice de Sangramento Marginal
L	Litro
MBC	Minimum Bactericidal Concentration
mL	mililitro
MSB	*Mitis salivarius bacitracin*
pH	Potencial hidrogeniônico
ppm	partes por milhão
PVB	Própolis Vermelha Brasileira
S. mutans	*Streptococcus mutans*
STROBE	Strengthening the Reporting of Observational Studies in Epidemiology
UFC	Unidades Formadoras de Colônias

SUMÁRIO

1. INTRODUÇÃO

A cavidade bucal agrega diversas comunidades de microorganismos que residem nas diversas superfícies como biofilme. Normalmente essas comunidades de vírus, bactérias, entre outros coexistem em equilíbrio. A saúde bucal está diretamente relacionada com esse equilíbrio, onde uma dieta balanceada e boa higiene oral são os principais fatores para essa manutenção. Quando o ambiente bucal sofre alterações, esse ecossistema é desequilibrado resultando em mudanças entre os microrganismos e o biofilme, aumentando assim o risco de disbioses. (MARSH; ZAURA, 2017)

A grande diversidade e complexidade da microbiota oral em diferentes áreas é estabelecida pelas características ambientais da cavidade, tais como alta umidade, temperatura relativamente constante (34 a 36°C), pH próximo da neutralidade e disponibilidade de nutrientes. Fatores como a idade, hábitos alimentares, hormônios, fluxo salivar, higienização, alcoolismo e outros determinam variabilidade da composição desta microbiota oral. (MOURA *et al*., 2007; LINS *et al*., 2013)

As doenças periodontais reúnem um grupo de doenças caracterizadas, principalmente, por gengivite e periodontite, que resultam da interação entre os biofilmes supra e subgengival e a resposta imunoinflamatória gerada pelo hospedeiro. A gengivite é uma alteração bucal dos tecidos moles frequente em pacientes sob tratamento ortodôntico, sendo uma consequência do acúmulo de placa nos braquetes. Caracteriza-se como sendo uma inflamação da gengiva marginal de caráter reversível. Clinicamente pode-se observar uma gengiva vermelha e edemaciada com sangramento espontâneo ou após sondagem. Além disso o uso de aparelho ortodôntico aumenta a retenção de bactérias cariogênicas causando um desequilíbrio da microbiota oral e desmineralização ao redor dos braquetes. (SAMAH *et al*., 2014)

A cárie e doença periodontal são as principais doenças bucais, onde o biofilme dentário é um dos principais determinantes biológicos comuns para o desenvolvimento de ambas as doenças. Diversos fatores podem modular essas doenças, em especial a qualidade da higiene oral e hábitos alimentares. Além disso, sabe-se que o uso de aparelhos fixos facilitam áreas de estagnação de placa dentária, aumentando a susceptibilidade de desenvolvimento de cárie dentária ao redor dos braquetes e gengivite. (JEPSEN *et al*., 2017; PITTS *et al*., 2017)

O biofilme se desenvolve sobre a superfície dentária e é composto de diferentes espécies de microorganismos, inicialmente formados por bactérias gram-positivas e aeróbias, mas posteriormente, há uma colonização sequencial de microorganismos gram negativos e anaeróbios. O tecido gengival marginal começa a desenvolver uma resposta inflamatória provocada pelas toxinas liberadas dos microorganismos presentes no biofilme. Essa resposta acontece quando são liberadas substâncias no organismo, como a histamina, além de ocorrer produção de substâncias inflamatórias que aumentam a permeabilidade dos vasos sanguíneos. A manutenção prolongada do processo inflamatório nos tecidos, designada inflamação crônica, pode promover sua destruição e perda óssea. Desta forma é necessário que haja uma desorganização constante e eficaz deste biofilme para prevenir essa condição clínica, já que o biofilme é o fator etiológico principal no desenvolvimento da gengivite. (SOUZA *et al*., 2013)

Os braquetes atuam como áreas retentivas de alimentos na superfície dentária e são regiões de alta colonização de determinados microrganismos presentes no biofilme dentário, como os *Streptococcos mutans* favorecendo seu crescimento e desenvolvimento. Além disso, sua presença na boca também dificulta a higiene oral. (SOUZA *et al*., 2013)

A própolis é um complexo resinoso responsável pelo selamento das colméias de abelhas da espécie *Apis mellifera*, sendo proveniente da coleta das mesmas nos vegetais. Atualmente existem catalogados 200 tipos de compostos identificados na própolis, a partir de amostra geográficas diferentes e da diversidade botânica. A caracterização química da própolis padronizou constituintes como: ácido fenólico prenilado, lignanas, terpenos e alcoóis terpênicos, além de derivados p-curmarinicos. (ANAUATE NETTO *et al*., 2013)

Os contituintes químicos do extrato de própolis variam de acordo com a vegetação ao redor da colméia. Normalmente, as própolis são compostas por 50% de resina e bálsamo vegetal, 30% de cera, 10% de óleo essencial e compostos aromáticos, 5% de pólen e 5% de outras substâncias (ARAUJO *et al*., 2011). Diversos são os tipos de própolis e sua classificação e cor variam de acordo com a posição geográfica. No Brasil, existe o registro de 13 tipos diferentes de própolis que variam com as propriedades físico-químicas e localização geográfica. A mais recente, que é a própolis vermelha, foi classificada como tipo 13 devido sua composição química única que é conhecida por seu alto conteúdo de isoflavonóide, em especial o neovestitol e vestitol. (SILVA *et al*., 2013)

A própolis vermelha brasileira (PVB) se destaca devido à quantidade de isoflavonóides, especialmente o vestitol e neovestitol, a biodiversidade brasileira favorece a constituição

química. Diversas são as propriedades farmacológicas documentadas como a antioxidante, antimicrobiana, antifúngica, antiviral, anti-parasitária, anti-inflamatória, entre outras. Pesquisas indicam que em baixas concentrações (0,1-1,0%) a própolis já possui atividade antimicrobiana e anti-inflamatória. (FRANCHIN *et al*., 2016; AZEVEDO *et al*., 2018; PORTO *et al*., 2018)

A PVB é exclusiva da região de Marechal Deodoro- AL, o que fez o Instituto Nacional de Propriedade Industrial (INPI) conceder o título de Indicação Geográfica da região, assegurando a esse estado o certificado internacional de único produtor desse tipo de própolis no mundo, com qualidade independente do tempo e clima. (NOGUEIRA *et al*., 2007; SILVA *et al*., 2008; FREIRES *et al*., 2016; FRANCHIN *et al*., 2016; SALATINO; SALATINO, 2018)

A origem botânica da PVB é a *Dalbergia ecastophyllum* e é encontrada no Nordeste brasileiro (FREIRES *et al*., 2013). A própolis tem sido objeto de intensos estudos farmacológicos e químicos nos últimos 30 anos. Em diferentes lugares do mundo é indicada para melhorar a saúde e prevenir doenças. Atualmente, é disponível em várias formas farmacêuticas como cápsulas, extratos, enxaguatório bucal e na forma de pó. Possui propriedades antibacteriana, anti-inflamatória, antifúngica, antiviral, antioxidante, antitumoral dentre outras. (LUSTOSA *et al*., 2008)

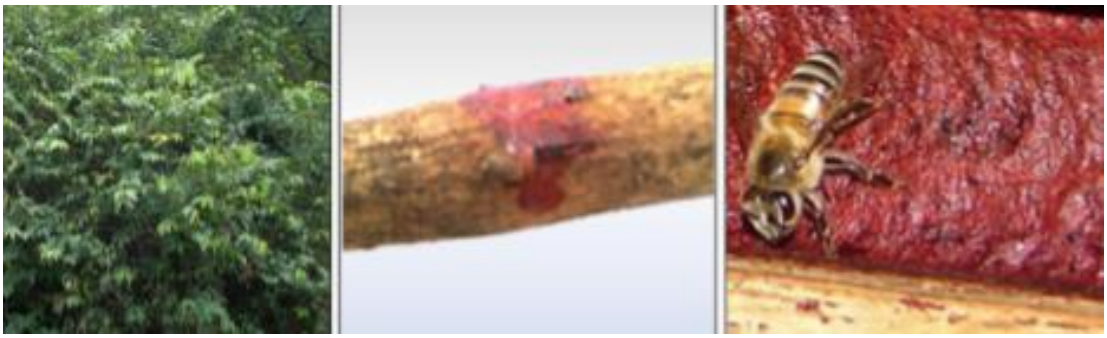

Figura 1: Origem botânica (*Dalbergia ecastophyllum*) e própolis vermelha brasileira.
Fonte: Freires *et al*. (2016).

O Neovestitol e Vestitol, isoflavonóides, são os principais compostos bioativos encontrados no extrato de PVB. Os mesmos além da atividade antimicrobiana possuem a capacidade de modular processos inflamatórios. (BUENO SILVA *et al*., 2013)

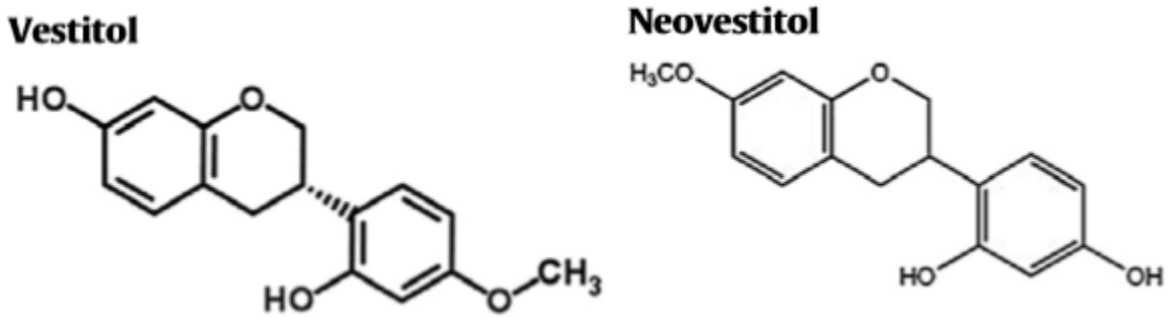

Figura 2: Forma molecular da própolis vermelha brasileira.
Fonte: Freires *et al*. (2016).

Diversos são os estudos que reportam o uso de dentifrícios e enxaguatórios no controle da gengivite, sendo a clorexidina o de maior escolha (CAGETTI *et al*., 2015). A mesma tem um largo espectro antimicrobiano, porém são documentados diversos efeitos colaterais relacionados ao uso prolongado, como alteração do paladar, manchamento dentário, recolonização, irritação da mucosa, entre outros. (LOBO *et al*., 2014; GÓES *et al*., 2016)

A saliva é uma excelente fonte de biomarcadores, sendo útil na identificação e monitoramento de diversas condições orais e sistêmicas. É um material biológico de fácil coleta e não-invasivo. Sendo assim, mudanças na composição desses biomarcadores podem ser utilizadas na decisão diagnóstica e identificação de doenças, particularmente em estudos de eficácia clínica. (MOURA *et al*., 2007; GIANOBILE *et al*., 2009)

No caso da cárie dentária, existem vários indicadores de risco, tanto diretamente relacionados à doença, como a exposição ao açúcar e consequente alteração da microbiota, bem como os fatores moduladores, como aspectos socioeconômicos. A saliva é um material biológico, sendo um excelente biomarcador para doenças sistêmicas e orais, especialmente em relação à cárie dentária, onde aproximadamente 108 microrganismos podem ser contados em 1 mL de saliva. Pesquisas associadas à cárie utilizam contagens de *S. mutans* como biomarcadores de desequilibrio da microbiota. (MARSH et al., 2016; KAUR et al., 2013)

Diversos são os estudos sobre a própolis em diversas áreas, como Medicina, Odontologia e Química. Em Odontologia estudos apontam resultados promissores em Endodontia, Cariologia, Cirurgia, Odontologia Preventiva e Periodontia. (PORTO *et al*., 2018)

Não foram encontrados na literatura relatos da utilização de dentifricio incorporado com própolis vermelha, desse modo foi depositada o pedido de uma patente de invenção sob

protocolo BR1020170110974. Em consequência do exposto, teria uma boa utilização nos pacientes ortodônticos com a finalidade de controle de placa através de uma possível atividade antimicrobiana desse dentifricio.

2. OBJETIVOS

2.1 OBJETIVO GERAL

Desenvolver um dentifrício com extrato de própolis vermelha brasileira. Avaliar in vitro e in vivo a eficácia do mesmo em bactérias do biofilme cariogênico e periodontal.

2.2 OBJETIVOS ESPECÍFICOS

- Realizar um estudo documental sobre patentes de produtos de própolis em bancos de propriedade intelectual no mundo;
- Avaliar a atividade antimicrobiana in vitro da PVB sobre cepas de *S. mutans*;
- Comparar in vitro a atividade antimicrobiana sobre cepas de *S. mutans* do dentifrício de PVB com dentifrício comercial;
- Verificar a estabilidade do dentifrício após incorporação de PVB.
- Avaliar clinicamente a atividade antimicrobiana de *S. mutans* e bactérias gram-negativas na saliva de adolescentes;
- Verificar a redução do índice de sangramento marginal (ISM) após o uso do dentifrício de PVB.

3. CAPÍTULO 1

FOLHA DE ROSTO

Este capítulo é referente ao artigo publicado no periódico **Recents Patents on Biotechnology**.

.

PROPOLIS AND ITS DENTAL APPLICATIONS: A TECHNOLOGICAL PROSPECTION

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PROPOLIS AND ITS DENTAL APPLICATIONS: A TECHNOLOGICAL PROSPECTION

Abstract: *Background:* Propolis is a resinous complex produced by *Apis mellifera* L. bees whose variety of pharmacological properties is resulting from the complexity of its composition. In dentistry, propolis is used on the prevention of oral diseases such as dental caries and gingivitis. Prospective studies in intellectual property banks are important to increase market competitiveness and thus generate new products in the various research areas. In this way, investments in patents play an important role in the technological and economic development of a country. *Objective:* To evaluate patents with dental products containing propolis, on intellectual property banks. *Method:* The research was conducted in 10 banks of intellectual property, including since the first deposits up to 2016. Relevant information that describes the invention in the patent document were collected, processed and described. *Results:* The search performed in 62 patents using propolis in the dental topic. World Intellectual Property Organization (WIPO) has the largest number of filing patents (83.60%) and the National Institute of Industrial Property (INPI) in third place with 4 patents (6.55%). Built-in dental cream with propolis was the most patented product. The first patent date of 1998, followed by an increase in the number of deposits in the last 20 years. Most of the patents are A61K code (51) for medical, dental or hygienic purposes. *Conclusion:* This study has shown that propolis is a promising bioactive component in dental products, especially for use in Cariology and Periodontology. Although there has been significant progress in applications of propolis, the field of dental products is still a growing area and it is important to encourage innovation and development of new products incorporating propolis based on knowledge of its composition and therapeutic properties.

Descriptors: Propolis; Dentistry; Dental Applications; Intellectual Property; Patents.

1. INTRODUCTION

Propolis is a natural complex resinous synthesized by bees of the species *Apis mellifera* L. as material for sealing of hives, derived from products collected from different parts of plants (leaves, flowers, bud, exudates, etc.) according to the plant diversity found around the beehive [1].

This material gets salivary secretions and wax used as protection against animals and microorganisms. The resin is characterized as a complex chemical composition composed of about 300 to 400 different chemical compounds and vary with the location and type of plant where it was collected having as main constituents prenyled phenolic acid, flavonoids, steroids, lignans, terpenes and terpenic alcohols, in addition to p-curmarinics derivatives [2-4]. The variety of pharmacological properties of propolis is a result of the complexity of its dependent composition on the number of phenolic compounds, especially flavonoids, responsible for some activities such as anti-inflammatory, antibacterial, antiviral and antioxidant. The ability of propolis to inhibit the growth of microorganisms is the most popularly known pharmacological activity and scientifically proven [5-9].

The therapeutic properties of the various types of propolis have been explored in different biological areas. In dentistry, propolis is an important source of oral disease preventions as caries and gingivitis. Several studies prove the therapeutic effect of propolis extract on microbial inhibition of cariogenic agents and periodontal diseases; in addition, it is a compound with low toxicity and adequate biocompatibility. Currently, the Brazilian red propolis has a distinct chemical composition and broad therapeutic action [10-12].

The use of natural products for medicinal purposes dates from old age and is essential in the emergence of new products and medicines. In ancient Greece, propolis has been used as a disinfectant and antiseptic for skin and oral infections. In the dental area, investment in natural products research has increased constantly to the discoveries of new bioactive substances and complex with therapeutic activity. The application based on knowledge of the properties of propolis aims to add economic value to raw propolis, development and technological innovation of new drugs [1, 13-17].

In Brazil, the production of propolis presents data around 100 tons per year and its highest percentage for export had high prices in foreign trade as a major source of income, both in raw form as in manufactured goods. The added value is due to the increased interest in Brazilian propolis extract, inserted in the context of international food trade [18]

The interest and investment made by universities and industries in research to innovation and technology grows every day. When these searches generate a product, it can be patented, being deposited in a Bank and so the inventor and its group will have exclusive rights to the product for a certain period, which varies according to the legislation of the country. For something to be patented, you must have the following characteristics: innovation, technology, and marketing [19-21]

Investments in patents are increasingly widespread in capitalism because the entire operation of the system is related to innovation, scientific and technological advancement. For this, advance is essential to the existence of intellectual property rights through patent protection. Thus, the right to intellectual property carries an important role in economic development of a country [20]

The intellectual property institutions and banks are responsible for deposits and patent grants. Each country has the responsibility and its bank legislation varies according to the

nation. Technological prospecting seeks to identify the main changes and emerging technological guidelines, development routes and potential impacts in the future, through predefined methods and approaches for mapping technologies. Prospective studies on intellectual property are important for improving the competitiveness of the market and thus generate new products in the various research areas. Despite the important source of information, these studies are little used and often unknown in academia [14, 22]

To facilitate searches, patents have an international classification system since 1971 that distributes for codes, known as IPC (International Patent Classification), which ranks the inventions according to the branches of industry, or of human activity and distributes the products in technological areas of classes (A – Human Necessities; B –Processing, Transport Operations; C – Chemistry, Metallurgy; D – Textiles, Paper; E – Fixed Constructions; F – Mechanical Engineering, Lighting, Heating, Weapons, Explosion; G – Physics and H – Electricity), where each class has its division into subclasses. This classification aims to establish an effective search tool and easily accessible, broadening the dissemination of the product [23].

The aim of this research was to conduct a study of technological prospecting based on the patents of dental products with propolis in different banks of intellectual property due to the growing interest about the activities of the different types of therapy propolis and intense marketing. This paper summarizes patents concerning conventional and new methods for dental formulations using propolis with therapeutic purpose and its various applications in dentistry.

2. MATERIALS AND METHOD (FOR RESEARCH ARTICLES ONLY)

Elaboration of the prospective study

The survey was conducted through collection, treatment and analysis of extracted information from patent documents selected. The documentary research was conducted from July to September 2016, including since the first patent deposits until the current time. The searches were directed with access to patents deposited on 10 banks of intellectual property used as references in the world:

1. National Institute of Industrial Property (INPI) – Brazil;
2. Canadian Intellectual Property Office – Canada;
3. German Patent and Trademark Office – Germany;
4. Japanese Patent Office – Japan;
5. Office for harmonization in the internal market – Europa;
6. Spanish Patent and Trademark Office – Spain;
7. Swiss Federal Institute of Intellectual Property – Switzerland;
8. Unites States Patent and Trademark Office (USPTO) – United States;
9. World Intellectual Property Organization (WIPO) – Europa;
10. World Trade Organization (WTO) – Europa.

Search strategy

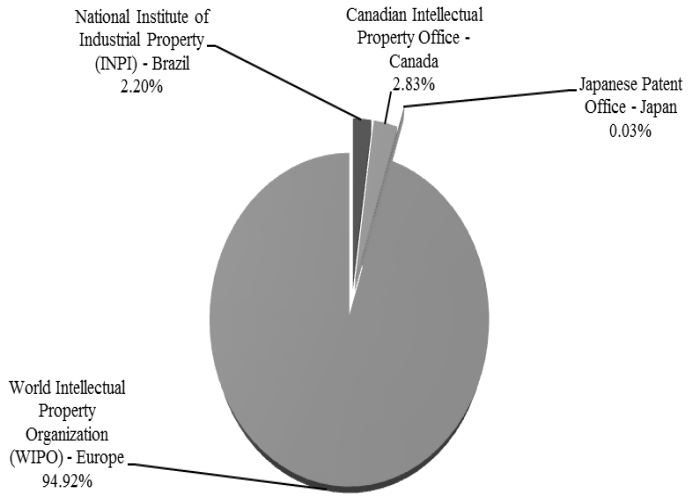
For the preparation of the survey, we conducted a mapping of patent applications related to applications of propolis in dentistry using the keyword ‘propolis’ in the search field with the exploratory reading of titles and summaries, as a criterion for inclusion of the patents found. Relevant information that describes the invention in the patent document was selected and graphics were generated in Microsoft Excel® program for analysis of descriptive statistics. The

data represent the distribution of the number of patents by banks, the types of patented products, the annual evolution of deposits, the main application areas of patent documents and types according to IPC.

3. RESULTS AND DISCUSSIONS (FOR RESEARCH ARTICLES ONLY)

The initial search resulted in 2859 patents found with the term 'propolis', followed by the stage title and summary read to selection patents directed to odontological applications with a total of 62 documents.

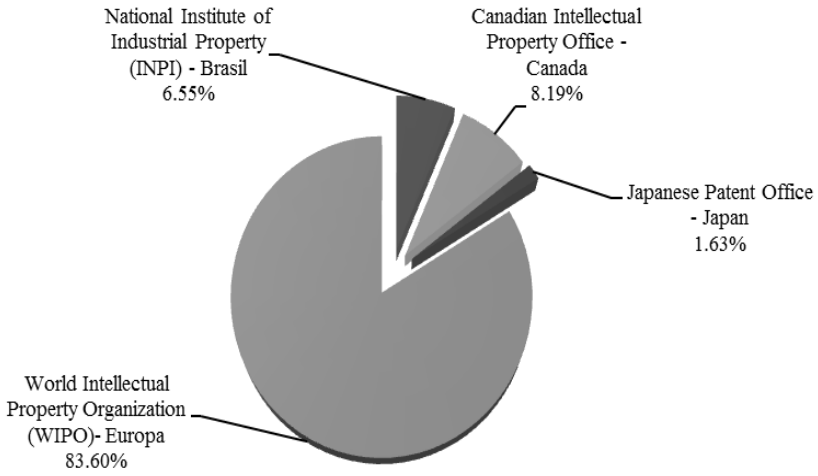
According to graphic 1, the World Intellectual Property Organization (WIPO) was the bank with the largest number of filing patents for products with propolis (94.92%) in various areas of application.



Graphic 1. Distribution of patents found in intellectual property banks with the term '*Propolis*'.

The Patent Office German Patent and Trademark Office (Germany), Harmonization workshop of the internal market (Europe), Spanish Office and patent marks (Spain), Swiss Federal Institute of Intellectual Property (Switzerland), Unites States Patent and Trademark Office (United States) and World Trade Organization (WTO) (Europe) did not provide patents that include the word propolis.

The first patent, in Brazil, with propolis formulations for use in dentistry was deposited in 2001 at the INPI (PI 0105471-6) [24], about antiseptic formulations of propolis toothpaste intended to oral hygiene. Regarding patent deposits in the dental area, Europe stands out with the highest number of patents in the dental area, followed by a much smaller number by Canada. Brazil is in third place with 4 patents deposited for dental use (mouthwashes, PI 0105471-6 [24]; aseptic gel implants (BR1020130184926) [25], dental varnish (BR1020120104415) [26]; intracanal medication, PI (0506243-8) [27], ahead of Asian countries, which are traditionally users of propolis as natural medicinal product and finally Japan with only a patent (Chart 2).



Graphic 2. Distribution of patents deposited with the term '*Propolis*' for dental applications.

In table 1, there are the types of patented products for use in dentistry. It is observed that embedded dental cream with propolis was the product with the largest number of deposits found in greater concentration in the patent database World Intellectual Property Organization (WIPO).

Table 1: Description of intellectual property banks and its product type with Propolis for patented dental applications.

Intellectual Property Bank	Patented Product Type	Quantity of Patents
National Institute of Industrial Property (INPI)	Antiseptic formulation	1
	Dental Varnish	1
	Gel	1
	Processes/Methods	1
Canadian Intellectual Property Office - Canada	Desensitizing	1
	Pasta for prophylaxis	2
	Rinse	1
	Non-specified	1
Japanese Patent Office - Japan	Cake	1
World Intellectual Property Organization (WIPO)- Europa	Toothpaste	25
	Candy	2
	To rinse	1
	Dental adhesive	1
	Non-specified	17
	Solution for Alveoli	1
	Product for dental bleaching	2
	Oil for lesions	1
	Local anesthetic	1
	Rinse	1

.

Graphic 3 shows the annual evolution of patent deposits of products with propolis for dental purposes in intellectual property banks, with the first deposit made in 1998 in WIPO

(patent number 02102970) [28], about a dentifrice formulation incorporated with alcoholic tincture of propolis for treatment of deep caries deposited by the Medical University of the State of Ryazan in Russia, identified by the classification code A61k 6/02 related to the use of preparations for artificial teeth, for filling or for covering of teeth. There is an increase in the number of deposits, with a highlight from 2012 to 2014, with a total of 18 patents, especially in 2013, with 7 deposits.

Graphic 3. Quantity of patents deposited and registered per year with the term '*Propolis*' for dental applications in the intellectual
property banks accessed.

In table 2, there is the classification of patented products in banks of intellectual property over the years (1998-2015). The most prominent classification code in patent documents selected was the class A61K (Preparations for medical, dental or hygienic purposes), followed by class A61Q (cosmetic products or formulations for personal hygiene) indicating that the most patent documents selected is related to Section A (Human Needs) of IPC (International Patent Classification). These results were expected, since the application of propolis is related to its pharmacological and therapeutic properties, due to its chemical composition.

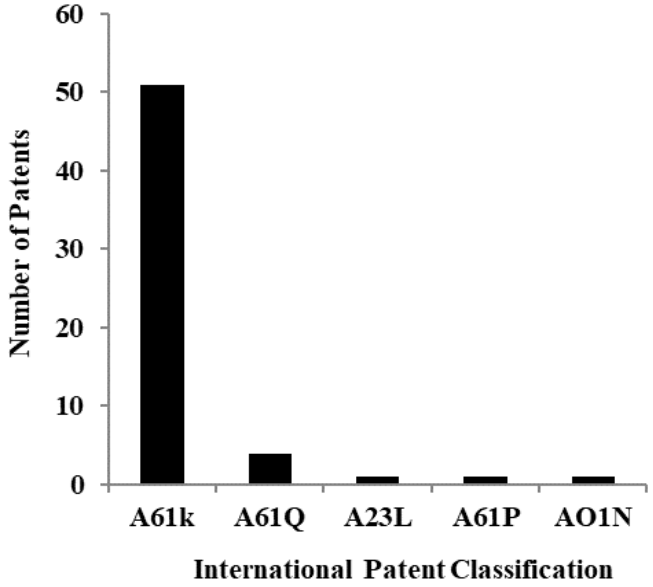
Table 2. Quantity of Propolis patents in dental products by an international ranking code of patents.

Intellectual Property Bank	Year of deposit	Classification	Quantity
National Institute of Industrial Property (INPI)- Brazil	2013	A61K	1
	2009	A61K	1
	2008	A61k	2
Canadian Intellectual Property Office - Canada	2009	A61K	2
	2008	A61K	1
	2006	A61K	1
	2001	A61K	1
Japanese Patent Office - Japan	2004	A61K	1

World Intellectual Property Organization (WIPO) - Europe	2015	A61K	3
	2015	A61Q	1
	2015	A23L	1
	2015	AO1N	1
	2014	A61K	3
	2014	A61Q	1
	2014	A23L	1
	2013	A61K	7
	2012	A61K	7
	2011	A61K	2
	2010	A61K	3
	2009	A61K	1
	2008	A61K	2
	2006	A61K	3
	2006	A61Q	2
	2005	A61K	1
	2004	A61K	4
	2003	A61K	1
	2001	A61K	1
	2000	A61K	1
	2000	A61P	1
	1999	A61K	2
	1998	A61K	3

A61K: Prescriptions for medical, dental or hygienic purposes; A61P: Specific therapeutic activity of chemical compounds or medicinal presets; A61Q: Cosmetic products or formulations for personal hygiene; A23L: Preservation of foods or foods in general.

Graphic 4 indicates that patent deposits using propolis in dental products are more focused on Human Need section (A), and the classification presented the largest number of patents A61K (51), a category which includes patents for preparations for medical, dental or hygienic purposes.



Graphic 4. Distribution of patents deposited with the term '*Propolis*' for dental applications by International Patent Classification Codes (IPC).

Legend: A61K: Prescriptions for medical, dental or hygienic purposes; A61Q: Cosmetic products or formulations for personal hygiene; A23L: Preservation of foods or foods in general; A61P: Specific therapeutic activity of chemical compounds or medicinal presets; A01N: Preservation of organisms of humans or animals or plants or their parts.

The patents were analyzed to find a complete print and logic of trends in research related to applications of propolis in dental products in the area. The items checked in each of the records of filing patents allowed patenting trends to generate that applied to A61k class with the dental cream formulation incorporated with propolis most patented. It is noteworthy that is to be expected that in the last 18 months, the number of patents submitted found in the search is less than reality since many deposits are still in the period of confidentiality.

According to the results, the number of formulations containing propolis and deposited patents has been increasing over the years due to current technological level investments in odontological area. Except for Brazil, in other countries and continents assessed, propolis collection by bees is basically the exudate of the poplar buttom (genus *Populus*), resulting in a greater uniformity in its chemical constitution and therefore a more limited therapeutic activity. On the other hand, propolis from the Americas is obtained through various plants collected, which extends its therapeutic potential. This fact can justify the little interest in patents with propolis in banks, as the Japanese Patent Office and the Canadian Intellectual Property Office [29].

When patent deposits in the dental area is analyzed, Brazil is in third place with 4. However, a dearth of patents with the opotherapic product (therapeutic preparations obtained from glands, tissues and animal secretions) in dentistry, where most of the patents of propolis for dental use is deposited in the World Intellectual Property Organization (WIPO) [30].

Thus, there is a situation in which Brazil, developing country, features a large amount of research and technological innovation with propolis-based products. However, there are few patents deposited. Several authors emphasize that the lack of interest of some researchers to patent their inventions gives priority to publish articles, by the ignorance in the procedures for conducting the deposit of patents and the lack of public guidelines in universities [18, 29,30].

Most patented products incorporated propolis. They are for purposes in Cariology or Periodontics. This can be explained by the fact of propolis stand out as antimicrobial activity and they are bacteria-related diseases. Particularly, the antibiotic effect of propolis can be explained by two different mechanisms: the first is regulated by flavonone pinocembrin by the flavanol galagina and phenylethyl ester of caffeic acid with action in inhibiting the bacterial RNA polymerase and the second with the flavonoids (caffeic acid, benzoic acid, cinnamic acid) causing damage to walls or membranes of microorganisms. In dentistry, studies demonstrate the activity of propolis against various cariogenic bacteria and periodontal [2,11].

In the search conducted, patent deposits summed up to five classification types. A61K code is related to products with medical, dental and hygienic purposes; A61Q is related to cosmetics and personal hygiene items; foodstuffs A23L; A01N preservation of bodies of humans or animals or plants or parts thereof biocides and A61P therapeutic activity of chemical compounds or medicinal. These results were expected since the main use of propolis is related to pharmacological and therapeutic properties, due to its complex chemical composition [22].

The discussion in relation to intellectual property is small in Brazil but it has been gaining notoriety and treated in different institutions directing its importance and influence on the country's economy, since Brazil is currently the third largest producer of propolis in the world and presents great potential for technological investment [29]. It is known that law N 9,279, of 14th

May 1996, which deals with the patent legislation was a reference on the theme in Brazil. Another reference was the law N 10,973, from 2nd December 2004, which deals with measures to promote innovation and scientific and technological research, authorizing that the union, the states and municipalities and funding agencies encourage the interaction between companies for research-oriented entities and technology [14, 22, 31, 32]. With the creation of the innovation law in 2004, the research institutes were required to create the core of technological innovation, a strategic policy to encourage the deposit of patents and the mediators with companies [33,34].

Technological prospection studies are considered a relatively recent activity and are currently being used to assist decision-making within a context of changes, especially with regard to the globalization of the economy and the acceleration of advances technology [22].

4. CONCLUSION

Patent analysis about propolis in dental application products on intellectual property banks has shown the growing importance and this natural product emerging from this material in the area, concluding that the majority is related to the area of microbiology for use in Cariology and Periodontology, as well as the great interest of the countries of Europe Canada as major depositors in the area followed by Brazil in 3rd position on patenting.

Europe represented by the World Intellectual Property Organization (WIPO) has the largest number of patent applications than other banks. It can be noticed that in Brazil the interest in relation to the protection of intellectual property in opotherapic products is still small, despite propolis being a natural product of important commercial production that is gaining notoriety in research and in developing medicines.

There is an important presence of universities and research institutes in the world patenting propolis, indicating an opportunity for University-enterprise cooperation in research and development.

5. CURRENT & FUTURE DEVELOPMENTS

At present, the possibility of using propolis in formulations with therapeutic purpose is one of the areas of concern and research that has been excelling in the field of dentistry. In order to treat patients combined with the increase or the appearance of oral diseases stimulates the search for natural products with diverse biological properties, as well as by propolis in its complex chemical composition.

Future patents will likely deal with technological innovations and should add a global demand with the biological properties of propolis with costs that bring all these pharmaceutical technologies to global reach. The guides for these applications vary according to each country, especially for its production and marketing of propolis. For example, Japan has higher added value for import because of the interest in brazilian propolis.

Although there has been significant progress in applications of propolis as evidenced by the number of patent applications and patents issued in the last 20 years, the field of dental products is still a growing area and it is important to innovative encourage of new products with the incorporation of propolis and its different types.

CONFLICT OF INTEREST

The authors state that the content of this article has no conflict of interest.

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REFERENCES

1- Sforcin JM. Biological properties and therapeutic applications of propolis. Phytotherapy Research 2016; 30: 894–905.
2- Anauate Netto C, Marcucci MC, Paulino N, Anido-Anido A, Amore R, Mendonça S, et al. Effects of Typified propolis on mutans streptococci and lactobacilli: a randomized clinical trial. Brazilian Dental Science 2013; 16(2): 31-6.
3- Dias L, Pereira AP, Estevinho LM. Comparative study of diffent portuguese samples of própolis: pollinic, sensorial, physicochemical, microbiological characterization and antibacterial activity. Food and Chemical toxicology 2012; 50(12): 4246-53.
4- Silva TMS, Santos FP, Rodrigues AE, Silva EMS, Silva GS, Novais JS, Santos FAR, Camara CA. Phenolic compounds, melissopalynological, physicochemical analysis and antioxidant activity of jandaira (Melipona subnitida) honey. Journal of Food Composition and analysis 2013; 29(1): 10-8.
5- Chirumbolo S. Anti- inflammatory property of propolis. Journal of Clinical of Biochemistry Nutrition 2015; 56(2): 163–4.
6- Dota KFD, Consolaro MEL, Svidzinski TIE, Bruschi ML. Antifungal activity of Brazilian propolis microparticles against yeasts isolated from vulvovaginal candidiasis. Evidence-Based Complementary Alternative Medicine 2011; 201953.
7- Yildirim A, Duran GG, Duran N, Jenedi K, Bolgul BS, Miraloglu M, Muz M. Antiviral Activity of Hatay Propolis Against Replication of Herpes Simplex Virus Type 1 and Type 2. Medical Science Monitor 2016; 9(22): 422-30.
8- Frozza CO, Garcia CS, Gambato G, de Souza MD, Salvador M, Moura S, Padilha FF, Seixas FK, Collares T, Borsuk S, Dellagostin OA, Henriques JAP, Roesch-Ely M. Chemical characterization: antioxidant and cytotoxic activities of Brazilian red propolis. Food and Chemical Toxicololy 2013; 52: 137–42.
9- Aguiar SC, Zeoula LM, Franco SL, Peres LP, Arcuri PB, Forano E. Antimicrobial activity of Brazilian propolis extracts against rumen bacteria *in vitro*. World Journal of Microbiology and Biotechnology 2013; 29(10): 1951-59.
10-Paula AMB, Gomes RT, Santiago WK, Dias RS, Cortes ME, Santos VR. “Susceptibility of oral pathogenic bacteria and fungi to brazilian green propolis extract.” Pharmacologyonline 2006; 3(1): 467–73.
11-Liberio SA, Pereira ALA, Araujo MJAM, Dutra RP, Nascimento FRF, Monteiro Neto V, *et al*. The potential use of propolis as a cariostatic agent and its actions on mutans group streptococci,” Journal of Ethnopharmacology 2009; 125(1): 1–9.
12-Freires IA, Alencar SM, Rosalen PL. A Pharmacological Perspective on the Use of Brazilian Red Propolis and Its Isolated Compounds Against Human Diseases. European Journal of Medicinal Chemistry 2016; 110(14): 267-79.
13-Zabaiou N, Fouachea A, Troussona A, Barona S, Zellaguid A, Lahouelc M, Lobaccaroa JMA. Biological properties of propolis extracts: Something new from an ancient product. Chemistry and physics of lipids 2017; 207: 214-22.
14-Borba AM, Macedo M, Walter LRF. Alternative dentistry with medicinal plants in Chapada dos Guimarães – Mato Grosso – Brazil. South Brazilian Dentistry Journal 2008; 5(1): 43-50.

15-Lobo PLD, Fonteles CSR, Marques LARV, Fechine FV, Fonseca SGC, Carvalho CBM, Moraes MEA.The efficacy of three formulations of Lippia sidoides Cham. Essential oil in the reduction of salivary Streptococcus mutans in children with caries: A randomized, double-blind, controlled study. Phytomedicine 2014; 21(9): 1043-7.

16-Araujo Filho WR, Romeiro HLM, Cabreira MS. The use of phytotherapics in endodontics. Revista Brasileira de Odontologia Militar 2013; 29(1): 36-43.

17-Lobo PLD, Fonteles CSR, Carvalho CBM, Nascimento DF, Fonseca SGC, Fechine FV, *et al*. Dose-response evaluation of a novel essential oil against Mutans streptococci in vivo. Phytomedicine 2011; 18(4): 551-6.

18-Machado BAS, Cruz LS, Nunes SB, Guez MAU, Padilha FF. Prospective study of propolis and technologies correlated under the focus on patent documents deposited in Brazil. Innovation, Technology and Managemen Journal 2012; 2(3): 221-35.

19-Ferreira AA, Guimarães ER, Contador JC. Patent as a competitive tool and as a source of technological information. Management & Production 2009; 16(2): 209-21.

20-Lima JAA. Digressions on Intellectual Property as agent of development, Innovation and Strategy. Forum HSM of Strategy 2006; 10(93): 45-55.

21-Ministry of Development, Industry and Foreign Trade. National Institute of Industrial Property. Manual for the patent filer. Patent Directory, Brasília- DF. 2015.

22-Mueller SPM, Perucchi V. Universities and the production of patents: topics of interest to the student of technological information. Perspectives in Information Science 2014; 19(2): 15-36.

23-Machado BAS, Silva RPD, Barreto GDA, Costa SS, Silva DFD, Brandão HN, Rocha JLC, Dellagostin OA, Henriques JAP, Umsza-Guez MA, Padilha FF. Chemical Composition and Biological Activity of Extracts Obtained by Supercritical Extraction and Ethanolic Extraction of Brown, Green and Red Propolis Derived from Different Geographic Regions in Brazil. Plos One 2016; 11(1): e0145954.

24-Ribeiro MCM. Anhanguera Educacional ltda. (br/sp) / Foundation for Research Support of the State of São Paulo - FAPESP (BR / SP). Propolis formulations for dental use. PI0105471-6, 2001.

25-Vieira JS, Huttner EA. Propolis gel as a filler, antimicrobial and deodorant for use inside dental implants. BR1020130184926, 2013.

26-Ezequiel Dias Foundation, Research Support Foundation of the State of Minas Gerais - FAPEMIG, Scoaris DO, Bastos EMAF, Ligório Filho S. Pharmaceutical formulation of green propolis for human use in dentistry. BR1020120104415, 2012.

27-Franco SL, Hidalgo MM, Bersani CA, Victoriano FR, State University of Maringá. Process of obtaining propolis extract for dental use and formulations of propolis for dental use. PI0506243-8, 2005.

28-Kurjakina NV. Method for treating deep dental caries. WIPO 02102970, 1998.

29-Lustosa ABG, Nunes LCC, Randau KP, Rolim Neto PJ. Propolis: updates on chemistry and pharmacology. Revista Brasileira de Farmacognosia 2008; 18(3): 447-54.

30-Pereira AS, Silva FRM, Aquino Neto FR. Propolis: 100 years of research and its future prospects. New Chemistry 2002; 25(2): 321-6.

31-Haase H, Araújo EC, Dias J. Patent innovations: requirements for new university functions. Brazilian Journal of Innovation 2005; 4(2): 12-22.

32-Dagnino RD, Silva RB. The patents of public universities. Economy & Technology Joural 2009; 18(5): 169-72.

33-Assunção, E. The Brazilian patent system and universities in the 1990s. Rio de Janeiro: INPI. 2000.

34-Castro BS, Souza GC. The role of the Nuclei of Technological Innovation (nits) in Brazilian universities. Liinc in Journal 2012; 8(1): 125-40.

4. CAPÍTULO 2

FOLHA DE ROSTO

Este capítulo é referente ao artigo submetido ao periódico **BMC Complementary and Alternative Medicine**.

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IN VITRO EVALUATION OF ANTIMICROBIAL ACTIVITY OF BRAZILIAN RED PROPOLIS CONTAINING-DENTIFRICE

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IN VITRO EVALUATION OF ANTIMICROBIAL ACTIVITY OF BRAZILIAN RED PROPOLIS CONTAINING-DENTIFRICE

Abstract

Background: The aim of this study was to evaluate the stability of brazilian red propolis (BRP) dentifrice and compare the antibacterial effects of BRP extract, BRP containing-dentifrice and one antimicrobial commercial dentifrice (Parodontax) against *S. mutans* in vitro. Methods: Strains of *S. mutans* ATCC UA159 were used in the present study. 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 (106 CFU/mL). The microplates were incubated for 24 hours 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. Parodontax dentifrice showed antimicrobial activity at all dilutions ($p < 0,05$) 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. The Parodontax dentifrice obtained the best results. Conclusions: 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, Microbiology, Toothpaste.

BACKGROUND

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, in the constant search for safe products with pharmacological activity [George and Kasliwal et al., 2017; Valadas et al., 2018].

The oral cavity is quite complex, containing several communities of microorganisms that reside on different surfaces. Usually these communities of virus, bacteria, among others coexist in equilibrium. Oral health is related to this balance, but when the oral environment undergoes changes, this ecosystem is unbalanced resulting in dysbioses [Marsh and Zaura, 2017].

The biofilm developed on the dental surface is composed of several species of microorganisms, initially colonized by gram-positive and aerobic bacteria, but later, there is a sequential colonization of gram-negative and anaerobic microorganisms. Thus, a constant and effective disorganization of this biofilm is necessary to prevent these oral pathologies [Souza et al., 2013].

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 [Lustosa et al., 2008; Salatino and Salatino, 2018].

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 [De Araujo et al., 2011].

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 [Nogueira et al., 2007; Salatino and Salatino, 2018].

Propolis has been the subject of intense pharmacological and chemical studies in the last 30 years. In several parts of the world it is indicated to improve health and prevent diseases. Studies demonstrate the therapeutic effect of propolis extract on microbial inhibition of cariogenic microorganisms, besides presenting as a clinical option of low toxicity [Silva et al.,

2013]. Currently, there are several pharmaceutical forms which this product is delivered, such as capsules, extracts, gels, dentifrices, sprays, mouthwash and in the form of powder. They have antibacterial, anti-inflammatory, antifungal, antiviral, antioxidant and antitumoral properties among others [Lustosa et al., 2008; Salatino and Salatino, 2018].

In Brazil there are 13 types of propolis cataloged, where the one found in Alagoas in the Brazilian Northeast, the name brazilian red propolis (BRP) is due to the red coloring coming from the pigments of the plants [Nogueira et al., 2007].

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. It is found in the Brazilian northeast, a region rich in biodiversity, mainly on the coast of 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 th e 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 [Silva et al., 2008; Silva et al., 2013; Freires et al., 2016; Franchin et al., 2016; Salatino and Salatino, 2018].

This product has a high medicinal, historical and economic value, and in recent years BRP has had a great commercial expansion, where the interest in the pharmaceutical industry and its commercial 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 [Porto et al., 2018].

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 (Furtado Jr et al., 2018). Dentifrices with bioactive molecules have been studied in in vitro research as antimicrobial agents. 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 the stability and antimicrobial activity of the BRP dentifrice against *Streptococcus mutans* (*S.mutans*).

METHODS

Brazilian red propolis extract and dentifrice preparation

The extract of red propolis was collected from the city of Marechal Deodoro/AL (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 96º greater graduation. The extract of Brazilian red propolis at 1% concentration (antimicrobial concentration previously studied) was incorporated into the fluoridated dentifrice (1500ppm) with $CaCO_3$ in the Pharmacotechnical laboratory of the Pharmacy course of the Federal University of Ceara, Brazil. After, chemical identification of the constituents and the dentifrice was performed by High Performance Liquid Chromatography (HPLC), being mainly identified the constituents Quercetin, Vestitol and Neovestitol.

Preparation of BRP dentifrice

For the preparation of BRP dentifrices, commercial fluoride dentifrices were used as pharmaceutical bases. These were incorporated with BRP extract resulting in a pharmaceutical product with a final concentration of 1% of the extract.

After this the physical chemical characterization of the BRP dentifrice was performed and the stability was analyzed on the day of the manipulation, 30 and 60 days after storage. The tests included: macroscopic analysis and determination of pH and mass.

Macroscopic Analysis

The formulations were evaluated for their organoleptic characteristics with regard to color, odor and appearance. The pH verification of the samples under study was carried out in potentiometer previously calibrated with buffer solutions pH 7.0 and pH 4.0. The formulations were diluted in distilled water (10%, m/m). The analysis of the mass of the samples was performed in a semi-analytical balance, the analyzes were performed in triplicate.

Figure 1: BRP dentifrices after 60 days of production.

Bacterial strains and culture medium

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

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.

Microbiological tests were carried out at the Laboratory of Research in Applied Microbiology (LabMicro) at the Federal University of Ceara. All the tests were performed in triplicate and at two different times. The following groups were tested: G1- Brazilian red propolis extract; G2- Brazilian red propolis 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 108 CFU/mL). The suspension obtained was diluted 100-fold in sterile BHI medium, resulting in a culture with approximately 106 CFU / mL. This suspension was used in the assay.

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 (106 CFU / mL). The microplates were incubated for 24 hours 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 hours. 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

Regarding the organoleptic characteristics, the product remained stable during the evaluated periods (D1, D30 and D60). The color of the dentifrice showed to be slightly gray throughout the period as can be seen in figure 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 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.

Table 1: Changes on the pH on the dentifrices.

Formulation	D1	D30	D60
1	9.65	9.74	10.12
2	9.64	9.73	10.11
3	9.67	9.78	10.15

The mass variation of the formulations also exhibited stability as shown in Table 2.

Table 2: Changes on the mass of the dentifrices.

Formulation	D1	D30	D60
1	59.74	58.82	56.41
2	57.45	56.91	54.55
3	56.85	55.78	54.42

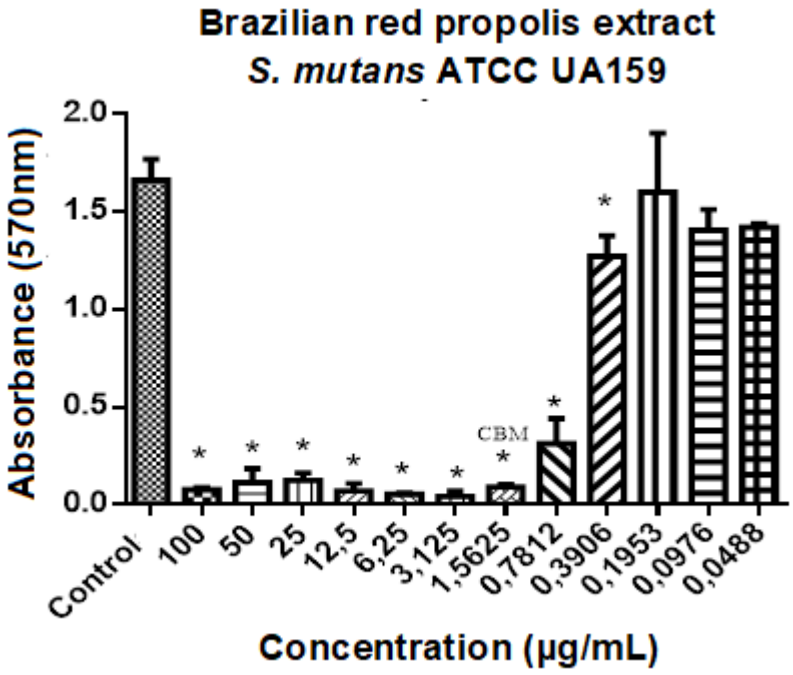

Figure 2: CBM of the Brazilian red propolis extract on *S. mutans* ATCC UA159.

Figure 2 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.

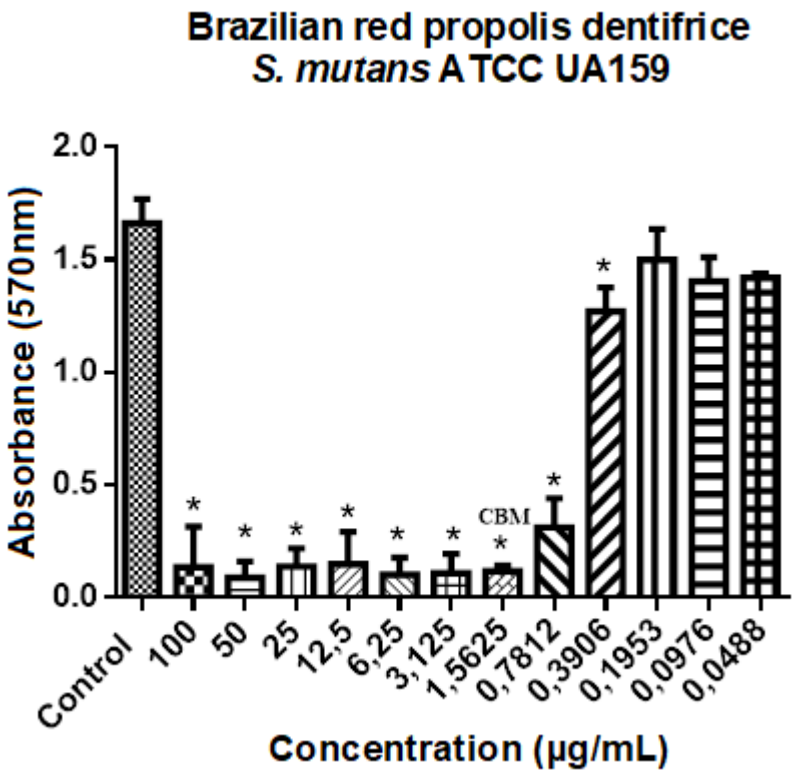

Figure 3: CBM of the Brazilian red propolis dentifrice on *S. mutans* ATCC UA159.

Figure 3 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.

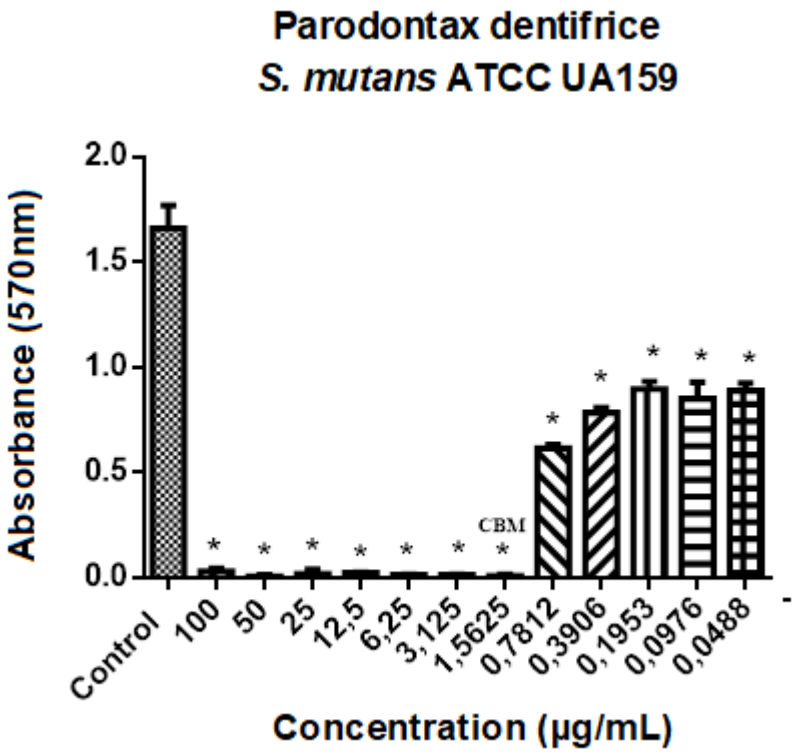

Figure 4: CBM of the Parodontax dentifrice on *S. mutans* ATCC UA159.

Figure 4 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 synthetic antimicrobials 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 [Lobo et al., 2014; Chinsembu 2016; Freires and Rosalen, 2016; Valadas et al., 2018].

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 [Chinsembu, 2016; Nascimento et al., 2017].

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. (Freires et al., 2016, Chinsembu 2016). 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 [Freires et al., 2016; Porto et al., 2018].

Streptococcus is one of the main microorganisms that colonize the oral cavity, being pioneering species in the various mouth niches [Manji et al., 2018]. Fernandes Jr et al. (2016) 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.

Grenho et al. (2000) 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.

Koru et al. (2007) evaluated the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (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 with MIC ranging from 4 to 512µ / mL and MBC 8 to 512µ / mL.

Simões et al. (2008) 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.

According to Silva et al. (2017) 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, with MIC between 31.5 to > 1000µ/mL.

CONCLUSIONS

It was concluded that the BRP dentifrice showed stability during the 60 days observed. 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. The Parodontax dentifrice obtained the best results.

REFERENCES

George RM, Kasliwal AV. Effectiveness of Propolis, Probiotics And Chlorhexidine on Streptococcus Mutans And Candida Albicans: An In-Vitro Study. IOSR Journal of Dental and Medical Sciences (IOSR-JDMS). 2017; 16(3):15–8.

Valadas LAR, Gurgel MF, Mororó JM, Fonseca SGC, Fonteles CSR, de Carvalho CBM et al. Dose-response evaluation of a copaiba-containing varnish against streptococcus mutans in vivo. Saudi Pharmaceutical Journal. 2018.

Marsh PD, Zaura, E. Dental biofilm: ecological interactions in health and disease. Journal of Clinical Periodontology. 2017; 44: S12-S22.

De Souza TMPA, Freires IA, Lima DMB, Jovito VC, Almeida LFD, Castro RC. Aderência in vitro de *Streptococcus mutans* à Superfície de Braquetes Ortodônticos Metálicos e de Policarbonato. Revista Brasileira de Ciências da Saúde. 2013;17(1):91-6.

Lustosa SR, Galindo AB, Nunes LCC, Randau KP, Rolim PJN. Revista Brasileira de Farmacognosia. 2008; 18(3):447-54.

Salatino A. Brazilian Red Propolis: Legitimate Name of the Plant Resin Source. Med Crave. 2018;5:435–41.

De Araújo YLFM, De Mendonça LS, Orellano SC, De Araújo ED. Comparação entre duas técnicas utilizadas no teste de sensibilidade antibacteriana do extrato hidroalcoólico de própolis vermelha. Scientia Plena. 2011;7:1-4.

Nogueira MA, Diaz MG, Tagami PM, Lorcheide J. Atividade microbiana de óleos essenciais e extratos de própolis sobre bactérias cariogênicas. Revista de Ciências Farmacêuticas Básica e Aplicada.2009; 28:93-7.

Bueno-Silva B, Alencar SM, Koo H, Ikegaki M, Silva GV, Napimoga MH. Anti-inflammatory and antimicrobial evaluation of neovestitol and vestitol isolated from Brazilian red propolis. Journal of Agricultural and Food Chemistry. 2013; 61(19):4546-50.

Silva BB, Rosalen PL, Cury JA, Ikegaki M, Sousa VC, Esteves A, Alencar MS. Chemical composition and botanical origin of red propolis, a new type of Brazilian propolis. Evidence-based complementary and alternative medicine. 2008;5(3):313-6.

Freires IA, De Alencar SM, Rosalen PL. A pharmacological perspective on the use of Brazilian Red Propolis and its isolated compounds against human diseases. European Journal of Medicinal Chemistry. 2016;110:267–79.

Franchin M, Colón DF, Da Cunha MG, Castanheira FVS, Saraiva ALL, Bueno-Silva B, Alencar SM, Cunha TM. Neovestitol, an isoflavonoid isolated from Brazilian red propolis, reduces acute and chronic inflammation: involvement of nitric oxide and IL-6. Scientific Reports. 2016; 6:36401.

Porto ICCM, De Almeida DCC, Costa GVCO, Donato TSS, Nunes LM, Do Nascimento TG et al. Mechanical and aesthetics compatibility of Brazilian red propolis micellar nanocomposite as a cavity cleaning agent. Celerino de Moraes Porto et al. BMC Complementary and Alternative Medicine. 2018;18(219):1 -17.

Furtado Jr JH, Valadas LAR, Mendonça KS, de Oliveira Filho RD, Gadelha LMU, Fiallos NM. A Technological Prospection. Recent Patents on Biotechnology. 2018;12(4):288-96.

Lobo PLD, Fonteles CSR, Marques LARV, Fechine FV, Fonseca SGC, Carvalho CBM et al. The efficacy of three formulations of *Lippia sidoides Cham*. essential oil in the reduction of salivary Streptococcus mutans in children with caries: A randomized, double-blind, controlled study. Phytomedicine. 2014;21(9):1043-7.

Chinsembu KC. Plants and other natural products used in the management of oral infections and improvement of oral health. Acta Tropica. 2016;154:6-18.

Freires IA, Rosalen PL. How Natural Product Research has Contributed to Oral Care Product Development? A Critical View. Pharm Res. 2016;33(6):1311-7.

Do Nascimento TG, Da Silva PF, Azevedo LF, Da Rocha LG, Porto ICCM, Lima e Moura TFA et al. Polymeric Nanoparticles of Brazilian Red Propolis Extract: Preparation, Characterization, Antioxidant and Leishmanicidal Activity. Nanoscale Research Letters. 2016; 11(1):301.

Manji F, Dahlen G, Fejerskov O. Caries and Periodontitis: Contesting the Conventional Wisdom on Their Aetiology. Caries Research. 2018; 52(6):548–64.

Cayo C, Quijandría L, Ramos J. Evaluación in vitro del efecto antibacteriano del propóleo sobre cultivos de Streptococcus mutans (ATCC 25175). Ciencia y Desarrollo. 2016;19(2):19-24.

Fernandes Jr A, Lopes MMR, Colombari V, Monteiro ACM, Vieira EP. Atividade antimicrobiana de própolis de Apis mellifera obtidas em três regiões do Brasil. Ciência Rural. 2006;36(1):294-7.

Grenho L, Barros J, Ferreira C, Santos VR, Monteiro FJ, Ferraz MP et al. In vitro antimicrobial activity and biocompatibility of propolis containing nanohydroxyapatite. Biomedical Materials. 2015;10(2):025004.

Koru O, Toksoy F, Acikel CH, Tunca YM, Baysallar M, Guclu AU et al. In vitro antimicrobial activity of propolis samples from different geographical origins against certain oral pathogens. Anaerobe. 2007;13(3):140-5.

Simões CC, Araújo DB, Araújo RPC. Estudo in vitro e ex vivo da ação de diferentes concentrações de extratos de própolis frente aos microrganismos presentes na saliva de humanos. 2008;18(1):84-9.

Silva RPD, Machado BAS, Barreto GA, Costa SS, Andrade LN, Amaral RG et al. Antioxidant, antimicrobial, antiparasitic, and cytotoxic properties of various Brazilian propolis extracts. Plos One. 2017; 12(3):e0172585.

5. CAPÍTULO 3

FOLHA DE ROSTO

Este capítulo é referente ao artigo submetido ao periódico Journal of Clinical Periodontology.

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CLINICAL AND MICROBIOLOGICAL EVALUATION OF BRAZILIAN RED PROPOLIS CONTAINING-DENTIFRICE IN ORTHODONTIC PATIENTS

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CLINICAL AND MICROBIOLOGICAL EVALUATION OF BRAZILIAN RED PROPOLIS CONTAINING-DENTIFRICE IN ORTHODONTIC PATIENTS

Clinical Relevance

Scientific rartionale for study: Caries and gingivitis are diseases modulated by biofilm and a preventive strategy would be the use of formulations with bioactive molecules, such as propolis. Principal findings: This study evaluated clinically and microbiologically a dentifrice incorporated with brazilian red propolis. Patients under orthodontic treatment and with gingivitis used the same for 4 weeks. The product proved to be effective for the control of gingivitis and decrease of oral bacteria, and may in the future be an alternative in the prevention of caries and gingivitis. Practical implications: The use of dentifrice with propolis may have implications for the prevention and biofilm control.

ABSTRACT

Aim: To evaluate the efficacy of a dentifrice of brazilian red iropolis (BRP) in adolescents under orthodontic treatment. Materials and Methods: This is a randomized clinical trial. A total of 92 participants, free of caries, were randomized into 2 groups, the first received fluoride dentifrice and the second fluoride dentifrice incorporated with BRP. The gingival bleeding index (GBI) was recorded and saliva was collected in the baseline (D0) and after 28 days (D28) for microbiological analysis. Data from MBI and Colony Forming Units (CFU) (log_{10}) were expressed as mean and standard deviation. Results: The two groups reduced GBI significantly, with no difference in intergroup analysis. In the intra-group analysis, it was observed that G2 ($p < 0.001$) had a significant reduction for gram-negative bacteria and in the inter-group analysis there was significance ($p < 0.001$) when compared to G1. For *S.mutans* bacteria it was observed that only G2 had a statistically significant reduction ($p < 0.001$), in the intergroup analysis there was significance ($p = 0.006$) of the G2 group when compared to G1. Conclusions: BRP dentifrice demonstrated better clinical and microbiological activity. Future studies are needed to identify better effects to establish the use of dentifrice in biofilm control.

Descritores: Biofilm; Propolis; Saliva.

INTRODUCTION

The oral cavity aggregates diverse communities of microorganisms that reside on the various surfaces as a biofilm. Usually these communities of viruss, bacteria, among others coexist in balance. Oral health is directly related to this balance, where a balanced diet and good oral hygiene are the main factors for this maintenance. However, when the oral environment undergoes changes, this ecosystem is unbalanced resulting in changes between the microorganisms and the biofilm, thus increasing the risk of dysbiosis (Marsh & Zaura, 2017).

Gingivitis is considered an inflammatory disease of microbiological origin. Dental caries is defined as dysbiosis caused by frequent exposure of sugars, resulting in demineralization of dental tissues. It is a consensus that the dental biofilm is the major biological determinant for the development of both diseases (Figuero et al. 2007, Sanz et al. 2017).

The use of brackets due to orthodontic treatment is one of the factors that favor the retention of the dental biofilm, due to the difficulty of hygiene and plaque accumulation, as a

consequence changes in pH and development of caries lesions and gingivitis are frequent (Longoni et al. 2017). The use of orthodontic appliances also increases the retention of cariogenic bacteria, favoring their growth and development, thus causing an imbalance of the oral microbiota and demineralization around the brackets (Samah et al. 2014). It is known that Streptococcus mutans (*S.mutans*) are one of the main organisms involved in the cariogenic biofilm, due to its acidogenic and acidic properties have an important role of virulence in caries pathogenesis (Klein et al. 2009, Souza et al. 2013).

The biofilm develops on the dental surface and is composed of different species of microorganisms, initially formed by gram-positive and aerobic bacteria, but later, there is a sequential colonization of gram-negative and anaerobic microorganisms. The marginal gingival tissue begins to develop an inflammatory response caused by the toxins released from the microorganisms present in the biofilm. This response occurs when substances are released into the body, such as histamine, in addition to producing inflammatory substances that increase the permeability of blood vessels. Prolonged maintenance of the inflammatory process in the tissues, named chronic inflammation, can promote bone destruction and bone loss. Thus, it is necessary that there is a constant and effective disorganization of this biofilm to prevent these pathological conditions (Souza et al. 2013).

One strategy to aid in the mechanical removal of plaque in these patients is through the addition of an antimicrobial agent with antiplate action in dental formulations, especially dentifrices. Ideally, these agents have the antimicrobial activity to assist in plaque control and inflammation of the gum, and anti-caries action to prevent or reduce frequent demineralization around the brackets. In addition, it is appropriate for the product to have a good taste and not cause side effects (Herrera et al. 2017).

One of the complementary methods used as an indicator to verify changes in the microbiota is through saliva (Castro et al. 2016). Saliva is a biological material that is easy to collect and non-invasive, which can identify different genera and species as it comes into contact with all surfaces of the oral cavity, such as teeth, gums, tongue. Through salivary samples, changes in the oral microbiota can be monitored. Because of this, saliva can identify more microorganisms than the dental biofilm (Gomar-Vercher et al. 2014, Eriksson et al. 2017).

Propolis is a complex, non-toxic resinous mixture, collected from plant exudates by *Apis mellifera* bees, where the biological properties are related to geographical locations and botanical origin (Salatino & Salatino, 2018).

The pharmacological benefits 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. There are 13 types of propolis classified according to their physicochemical properties and geographic origin, where Brazilian red propolis (BRP) stands out. BRP is the 13th type, where the red coloration is due to the pigments of the plants (Nogueira et al. 2007, Salatino & Salatino, 2018).

This type is relatively new and has drawn attention to its promising chemical composition and pharmacological properties, especially antimicrobial and anti-inflammatory properties. It is found in the Brazilian northeast, a region rich in biodiversity, mainly on the coast of the state of Alagoas, city of Marechal Deodoro, whose botanical origin is *Dalbergia ecastophyllum*. This type is unique to this region, possessing isoflavonoids in its composition, which made the National Institute of Industrial Property (INPI) grant the title of Geographical Indication of the region, assuring to that city the international certificate of only producer of this type of propolis in the world, with quality independent of time and climate (Silva et al. 2008, Freires et al. 2016, Franchin et al. 2016, Salatino & Salatino, 2018).

This product has a high medicinal, historical and economic value, and in recent years BRP has had an interest in the pharmaceutical industry and its commercial production considerably increased in several countries, such as Brazil, Japan, China, Russia, Germany and France. Therefore, both nationally and internationally the product is expanding. Several studies on propolis in several areas, such as Medicine, Dentistry and Chemistry. In Dentistry, studies point to promising results in Endodontics, Cariology, Surgery, Preventive Dentistry and Periodontics (Porto et al. 2018).

Both diseases, caries and gingivitis can progress slowly throughout life and can be controlled through non-invasive interventions (Manji et al. 2018). Dentifrices with bioactive molecules have been studied in in vitro research as antimicrobial agents. 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. As a consequence of the above, it would have a good use in orthodontic patients for the purpose of plaque control through a possible antimicrobial activity of this dentifrice. Therefore, the objective of this research was to evaluate clinically and microbiologically the efficacy of a dentifrice incorporated with BRP extract in adolescents with signs of gingivitis.

MATERIALS AND METHODS

Type and location of the study

It is a longitudinal, parallel, randomized, double-blind, controlled clinical trial and adhered to the CONSORT checklist. In order to improve the study methodology, the rules of the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) were followed. The clinical phase occurred in the city of Aracati- CE, a city where only 0.8% of the population has public fluoridated water coverage (Soares et al. 2014).

Ethical aspects, Population and Sampling

This study was approved by the Ethics Committee of the Federal University of Ceara (approval number 1.552.749), according to resolution 466/12 of research with human beings and with the Declaration of Helsinki under ethical principles for medical research involving human beings .

The sample was designed to demonstrate the statistical superiority of the dentifrice containing red propolis extract in relation to the common dentifrice in the treatment of gingivitis, considering a power of 90% ($\beta = 0,10$) and a significance level of 5% ($\alpha = 0.05$), based on the gingival bleeding index (GBI) measured at the end of the treatment, defined as the primary outcome. For this, it was established that the minimum difference between the effects of the two treatments to be detected would correspond to 0.12 points in the GBI, considering a standard deviation of this variable estimated at 0.16 points. This difference represents a reduction of around 32% in the GBI, according to previous studies. In addition, it was defined that the allocation rate would be 1, that is, the groups would have equal sizes. Thus, using the expression proper to studies of statistical superiority and considering that the primary outcome is a quantitative variable, the sample size needed to satisfy the above requirements was calculated in 38 subjects in each group. To this value, 20% were added to cover possible follow-up losses, so that the final sample size was estimated in 46 patients in each group.

An active search was conducted in public elementary and middle schools for the selection of participants. After signing the informed consent of those responsible and the consent of the

participants, 92 adolescents 12 to 16 years of age, of both genders, free of caries (ICDAS II = 0), users of fixed orthodontic appliance and with visible plaque index were selected.

Adolescents with a history of allergies such as asthma, urticaria, rhinitis, sinusitis or intra oral soft tissue injury were excluded from the study. None of the participants underwent antibiotic treatment up to 3 months prior to the initiation of the study nor during the course of this clinical trial.

BRP extract and dentrifice preparation

The extract of BRP was collected from the city of Marechal Deodoro (South Latitude 9°44.555', West Latitude 35°52.080' and altitude of 18.1 m above sea level), a region with a geographical indication granted by the National Institute of Industrial Property, in the state of Alagoas, Brazil. It was used 150 grams of the extract of red propolis and dissolved in 1 L of cereal alcohol of 96º greater graduation. The extract of BRP 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 Ceara, Brazil. After chemical identification of the constituents by High Performance Liquid Chromatography (HPLC), being mainly identified the constituents Quercetin, Vestitol and Neovestitol, the dentifrices were formulated with the same taste, color and odor.

Application of treatment

The participants were randomly distributed into two groups: Group test (G1) - Fluoride commercial dentifrice and Group control (G2) – Fluoride dentifrice with BRP. In the G1 there were 46 participants, as well as in the control group. Saliva collection was performed for microbiological analysis and the Gingival Bleeding Index (GBI) using the WHO periodontal probe and buccal mirror before starting treatment (D0) and on the last day (D28).

The dentifrices were stored in equal tubes to keep confidential the type of treatment applied to both the researchers involved in the clinical trial and the participants, thus guaranteeing the double-blind study. All participants received toothbrush of the same brand, with a straight handle, small head and soft bristles, and the toothpaste of the treatment. Standardized oral hygiene instruction was conducted through a single instructor for all participants. To reinforce the instructions, all participants received the recommendations to be followed in writing.

Saliva collection and microbiological analysis

Initially, each patient chewed one piece of a 3 × 3 cm plastic film (Parafilm®) for 60 s to stimulate the production of saliva and release the bacteria from the dental biofilm. Saliva was collected using a plastic device and stored in sterile microcentrifuge tubes (Eppendorf®), which were stored in a polystyrene box containing ice and analyzed in the microbiology laboratory within 2 hours of collection.

The saliva of each patient was collected in two moments (baseline and 28 days after starting treatment). Participants were instructed not to eat or drink at least 2 hours before collection of saliva, so that the circadian circus influence was minimized, samples were collected under the same conditions, operated between 9:00 and 11:00 a.m.

A volume of 0.1 mL of each sample was transferred to a sterile hemolysis tube containing 0.9 mL of saline. This procedure was repeated twice, establishing dilutions of 1:10 and 1: 100. A volume corresponding to 10µL of each dilution was seeded in Agar mitis bacitracin (MSB) and MacConkey Agar in triplicate for evidence of *S. mutans* and gram-negative bacteria.

The plates were incubated at 37 ° C for 48 hours in microaerophilic jars and placed in an oven. After this period, colonies with gram - morphological characteristics were counted. Bacteria were expressed as CFU/mL of saliva.

Clinical analysis

The patients were submitted to the gingival bleeding index (GBI) test on all teeth by a single examiner. The mesial, buccal, distal and lingual surfaces were evaluated. Through a WHO probe, the presence of gingival bleeding was evaluated before treatment started (D0) and 4 weeks later (D28).

Statistical Analysis

Clinical data were expressed as absolute and percentage frequency and compared using the chi-square test. The GBI and CFU (log_{10}) data were expressed as mean and standard deviation, submitted to the Kolmogorov-Smirnov normality test and compared using the Wilcoxon and Mann-Whitney tests (SPSS, 20.0; $p < 0.05$).

RESULTS

Table 1 shows the analysis of gram-negative and *S. mutans* CFU in the groups treated with fluoridated dentifrice and BRP at different dilutions. For gram-negative bacteria it was observed in the intra-group analysis of the first dilution (1:10) that G1 had a statistically significant increase for gram-negative bacteria ($p = 0.001$) and G2 showed reduction ($p < 0.001$). The inter-group analysis showed significance ($p < 0.001$) in the G2 group when compared to G1. For *S.mutans* CFU it was observed that in the intra-group analysis of the first dilution there was a statistically significant reduction for *S.mutans* in G2 ($p < 0.001$), in the inter-group analysis there was significance ($p = 0.001$) of the G2 group when compared to G1.

In the second dilution (1:100) it was found that for gram-negative bacteria in the intra-group analysis, G1 had a statistically significant increase ($p = 0.005$) and G2 showed reduction ($p < 0.001$). The inter-group analysis showed significance ($p < 0.001$) of the G2 group when compared to G1. For *S.mutans* bacteria it was observed that in the intra-group analysis that only G2 had a statistically significant reduction ($p = 0.002$), in the intergroup analysis there was significance ($p = 0.019$) of the group of G2 when compared to G1 (table 1).

Table 1: Data corresponding to intra and inter groups analysis of colony forming units (CFU) of Gram-negative bacteria and *S.mutans* and mean of groups at different dilutions and different times.

	Toothpaste	D0	D28	*p*-Value[a]	Δ	*p*-Value[b]
Dilution 1:10 Gram -	**Commercial**	0,95±0,63	1,42±0,79	***0,001***	0,47±0,78	***<0,001***

	Propolis	1,17±0,62	0,56±0,52	***<0,001***	-0,60±0,54	
S. mutans	**Commercial**	1,06±0,62	1,06±0,83	0,869	0,01±0,73	***0,001***
	Própolis	0,99±0,62	0,46±0,46	***<0,001***	-0,54±0,58	
Dilution 1:100						
Gram -	**Commercial**	0,97±0,68	1,44±0,83	***0,005***	0,46±0,88	***<0,001***
	Propolis	1,22±0,55	0,60±0,50	***<0,001***	-0,61±0,55	
S. mutans	**Commercial**	0,95±0,53	1,05±0,82	0,421	-0,02±0,23	***0,019***
	Propolis	0,87±0,58	0,58±0,26	***0,002***	0,12±0,33	

[a]Wilxocon test; [b]Mann-Whitney test (mean ± DP). Measured as CFU (log_{10}).

Table 2 shows the gingival bleeding index (GBI) and the mean of the analysis of counts of Gram-negative colony forming units and *S.mutans* in the groups treated with fluoridated dentifrice and BRP at different times. The two groups had statistically significant GBI reduction, and there was no significant difference in the intergroup analysis. For gram-negative bacteria it was observed that in the intra-group analysis, G1 had a statistically significant increase (p = 0.003) and G2 reduction (p <0.001). In the inter-group analysis we can see a significantly statistical difference (p <0.001) of the G2 group when compared to G1. For *S.mutans* bacteria it was observed that in the intragroup analysis that only G2 had a statistically significant reduction for *S.mutans* (p <0.001), in the intergroup analysis there was significance (p = 0.006) of the G2 group when compared to G1.

Table 2: Comparisons of GBI in the two groups treated with common toothpaste and BRP, measured on 0 (D0) and 28 (D28):

	Group	**D0**	**D28**	**p-Valor[a]**	**Δ**	**p-Valor[b]**
GBI	**Commercial toothpaste**	38,35±19,39	27,24±14,40	***0,001***	-11,11±19,88	0,135
	Propolis	37,94±18,68	20,59±16,45	***<0,001***	-17,35±12,77	
Gram -	**Commercial Toothpaste**	3,05±0,66	3,50±0,77	***0,003***	+0,45±0,84	***<0,001***
	Propolis	3,27±0,54	2,66±0,49	***<0,001***	-0,61±0,54	
S. mutans	**Commercial Toothpaste**	3,03±0,51	3,09±0,81	0,612	-0,06±0,69	***0,006***
	Propolis	2,95±0,57	2,62±0,28	***<0,001***	-0,33±0,50	

Compararion between different groups at different times. Wilxocon Test[a], Mann-Whitney Test[b] Data are expressed in CFU (log_{10}) as mean ± SD.

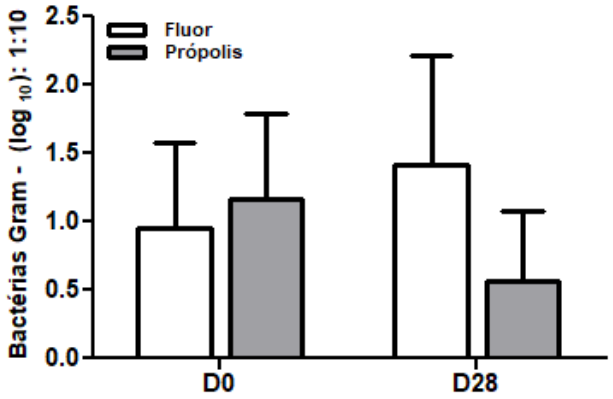


Figure 1: Salivary reduction of colony forming units (CFU) log_{10} of gram-negative bacteria in the different groups and times in the analysis of the first dilution (1:10).

Figure 1 shows the log reduction (CFU/mL) of colony forming units of gram-negative bacteria in the different groups and times of the first dilution (1:10).

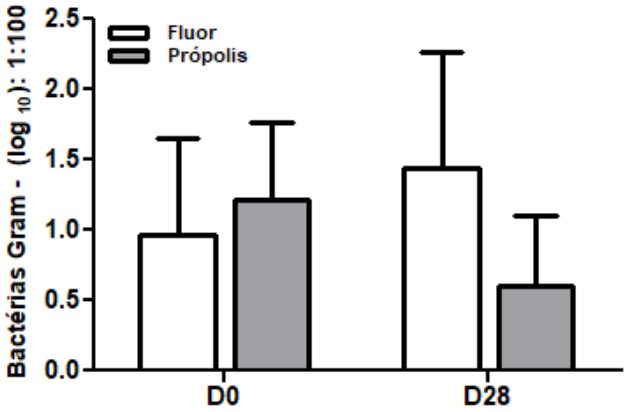


Figure 2: Salivary reduction of colony forming units (CFU) log_{10} of gram-negative bacteria in the different groups and times in the analysis of the second dilution (1:100).

Figure 2 shows the log reduction (CFU/mL) of colony forming units of gram-negative bacteria in the different groups and times of the second dilution (1: 100).

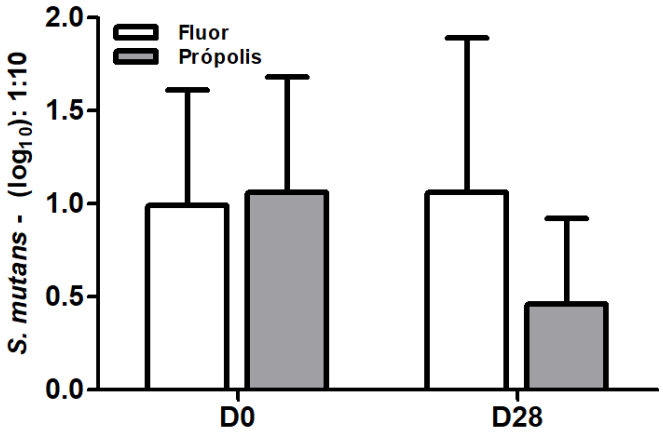


Figure 3: Salivary reduction of colony forming units (CFU) log_{10} of S.mutans bacteria in the different groups and times in the analysis of the first dilution (1:10).

Figure 3 shows the log reduction (CFU / mL) of the colony forming units of *S.mutans* bacteria in the different groups and times of the first dilution (1:10).

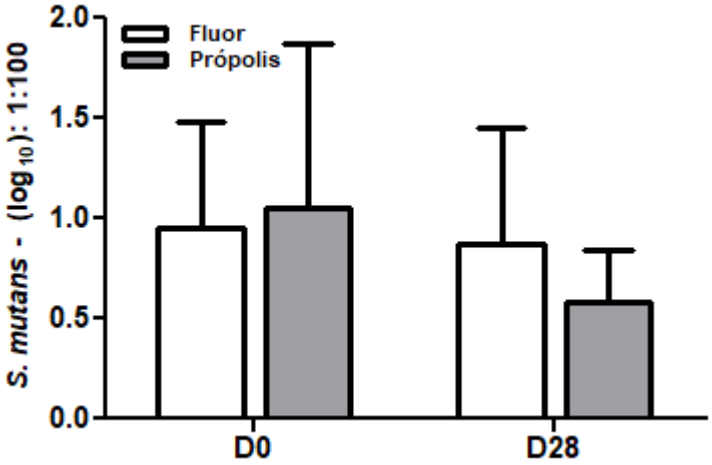


Figure 4: Salivary reduction of colony forming units (CFU) log_{10} of *S.mutans* bacteria in the different groups and times in the analysis of the first dilution (1: 100).

Figure 4 shows the log reduction (UFC/mL) of the colony forming units of S.mutans bacteria in the different groups and times of the second dilution (1: 100).

DISCUSSION

Resistance to synthetic antimicrobials and the search for substances with pharmacological properties with lower adverse effects have been caused the increased interest in natural products. More than one hundred million molecules are cataloged around the world and many are still unexplored. The high demand for propolis and the modernization of analytical devices contributed to the launch of several products in the market (Lobo et al. 2014, Chinsembu 2016, Freires & Rosalen 2016, Nascimento et al. 2017).

Propolis is distinguished by the broad gram-positive and gram-negative antimicrobial spectrum against colonizers of the oral biofilm, such as *S.mutans*, *Lactobacillus*, *P. gingivalis*,

Actinomyces naeslundii, *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Prevotella intermedia* (Freires et al. 2016, Chinsembu 2016).

BRP has potent antimicrobial activity even at the concentration of 0.1%, with antimicrobial and anti-inflammatory activity proven in vitro and in vivo. This is due to the high concentration of flavonoids and phenolic compounds (Freires et al. 2016, Porto et al. 2018). In this study in the 1% concentration the group treated with propolis presented better clinical and microbiological results when compared to fluoride dentifrice.

Caries and periodontal disease are the main oral diseases, where the dental biofilm is one of the main biological determinants common for the development of both diseases. Several factors can modulate these diseases, especially the quality of oral hygiene and eating habits. In addition, it is known that the use of fixed appliances facilitates areas of dental plaque stagnation, increasing the susceptibility of demineralization around brackets and gingivitis (Jepsen et al. 2017, Pitts et al. 2017).

Cagetti et al. (2015) report that gingivitis is the most prevalent type of periodontal disease in adolescents and that it increases with frequency and severity at puberty. In a randomized double-blind clinical trial, the authors compared the effects of a fluoride dentifrice (control group) with a dentifrice containing fluoride, triclosan, cetylpyridinium chloride and essential oils (test group) for plaque control and gingival inflammation after four weeks of use. The dentifrice test showed better anti-plaque results.

Every day new strategies for improving the toothpaste and control of the microbiota, for example Adams et al. (2017) conducted a clinical trial where they evaluated a dentifrice with enzymes and proteins for the control of supra gengival plaque for four weeks. The dentifrice test was compared to a fluoridated dentifrice control and the plaque was collected for analysis of the microbiome, where it was observed better results in the dentifrice test.

Streptococcus is one of the microorganisms most found in the oral cavity, being the pioneer species after the dental eruption. Species such as *Veillonella*, *Haemophilus*, *Neisseria* spp., *Prevotella* and *Fusobacterium* are normally found in dental plaque, tongue and saliva, when this balance is broken oral diseases can be developed. The results showed that the BRP dentifrice presented a statistically significant reduction at the end of the treatment (D28) for *S.mutans* ($p < 0.001$) and gram-negative bacteria ($p < 0.001$). The group treated with fluoridated dentifrice showed increase of bacteria in the salivary findings without S.mutans ($p = 0.612$) and statistically significant for gram-negative bacteria ($p = 0.003$).

Escribano et al. (2016) performed a systematic review on the efficacy of various chemical agents in plaque control. In the case of dentifrices, triclosan and chlorhexidine presented greater effects, for mouthwashes, essential oils and chlorhexidine are the most outstanding in the market.

Gingivitis and periodontitis may be previns controlling the supragingival biofilm. However, in situations such as the use of orthodontic appliances may require the use of a product that improves the mechanical removal of the biofilm. At the beginning of the study, all participants had gingivitis and gingival inflammation. At the end of the clinical trial, both groups had a reduction in the gingival bleeding index (MBI), fluoride dentifrice ($p = 0.01$) and BRP dentifrice ($p < 0.001$), and there was no statistical difference in the intergroup analysis ($p = 0.135$).

Figuero et al. (2017) performed a systematic review on the effect of mechanical and chemical plaque control in the fight against oral diseases such as caries and gingivitis. The authors reported that the addition of fluoride is significant for caries, while antimicrobials are significant for gingivitis. In addition motivational programs and supervised brushing show significant effect on plaque reduction, this explains the reduction of GBI in both groups of the present study because all participants had educational brushing activities prior to the start of the

clinical trial. The authors add that the simultaneous use of chemical agents for mechanical plaque control in the simultaneous management of gingivitis and caries is still limited in evidence and the indication of a particular product should be based on individual risk and need.

Herrera et al. (2017) evaluated the clinical and microbiological effects of a dentifrice of cetylpyridinium chloride (CPC) and mouthwash in patients under orthodontic treatment. During three months the patients were evaluated monthly for plaque index and gingival examination. No significant changes were identified in the microbiological analysis. The authors argue that mechanical control is essential in the prevention of caries and periodontal disease, reinforcing once again the findings of the present study.

Dental biofilm removal, oral hygiene practices and professional intervention are essential to eliminate dental biofilm, and retentive factors are the critical elements in the treatment of gingivitis (Montero et al. 2017).

Goes et al. (2016) evaluated in a randomized clinical trial the clinical efficacy of a mouthwash containing 1% extract of Matricaria chamomilla L. (MTC) in dental plaque reduction and gingivitis in patients under orthodontic treatment. Participants were allocated to three groups, the first using 15 mL placebo, the second chlorhexidine 0.12% (CHX), and the third 1% TCM, immediately after brushing for two weeks. Patients using chlorhexidine and the test rinse have reduced plaque and inflammation.

It is known that CHX is effective in the reduction of gingival inflammation, however its efficacy is reduced in the long term and several adverse effects are associated with continuous use, such as altered taste, tooth staining, calculus formation, mucosal irritation, others. Due to these factors, the prolonged use of this drug is not indicated (Goes et al., 2016).

The mechanical removal of the plaque through brushing contributes to the maintenance of gingival health, but there is great evidence that in situations of greater risk, the use of antimicrobial substances besides brushing contributes to plaque control and prevents gingivitis (Cagetti et al., 2015).

The etiologies of dental caries and periodontal diseases are independent. However, some factors are common to both diseases, such as the presence of biofilm (Sanz et al., 2017). The present dentifrice reduced bacteria related to both diseases, besides being fluoridated (1500 ppm F), being able to be a strategy for the prevention and control of both diseases.

Although there is no consensus in the literature, data suggest that in patients with retention, such as in orthodontic appliances, the association of fluoridated dentifrice with antimicrobial agents may be more effective than fluoride dentifrice use alone (Cury, 2002). In the inter-group comparison of the present study, the propolis dentifrice had superior results when compared to the fluoride dentifrice in relation to the reduction of *S.mutans* ($p = 0.006$) and gram-negative bacteria ($p < 0.001$).

During the treatment period BRP dentifrice demonstrated better antimicrobial activity against S. mutans, gram negative bacteria and reduction of the marginal bleeding index. Future studies are needed to identify better effects to establish the use of dentifrice in the control of dental biofilm.

REFERENCES

Marsh P. D., & Zaura E. (2017). Dental biofilm: Ecological Interactions in Health and Disease. J Clin Periodontol, 44, 12–22. doi: 10.1111/jcpe.12679.

Figuero, E., Nóbrega, D.F., García-Gargallo, M., Tenuta, L. M., Herrera, D., Carvalho, J. C. (2017). Mechanical and chemical plaque control in the simultaneous management of gingivitis

and caries: a systematic review. J Clin Periodontol, 44 (Suppl. 18): 116–134. doi: 10.1111/jcpe.12674.

Sanz, M., Beighton, D., Curtis, M. A., Cury, J., Dige, I., Dommisch, H., Ellwood, R., Giacaman, R., Herrera, D., Herzberg, M. C., Kononen, E., Marsh, P. D., Meyle, J., Mira, A., Molina, A., Mombelli, A., Quirynen, M., Reynolds, E., Shapira, L., Zaura, E. (2017). Role of microbial biofilms in the maintenance of oral health and in the development of dental caries and periodontal diseases. Consensus report of group 1 of the Joint EFP/ORCA workshop on the boundaries between caries and periodontal disease. J Clin Periodontol, 44, 5–11. doi: 10.1111/jcpe.12682.

Longoni, N. J., Lopes, B. M. V., Freires, I. A., Dutra, K. L., Franco, A., Paranhos, L. R. (2017). Self – ligating versus conventional metallic brackets on Streptococcus mutans retention: A systematic review. European journal of dentistry, 11, 537 – 547. doi: 10.4103/ejd.ejd_132_17.

Klein, I. M., Duarte, S., Xião, J., Mitra, S., Foster, T. H., Koo, H. (2009). Structural and Molecular Basis of the Role of Starch and Sucrose in Streptococcus mutans Biofilm Development. Applied and Environmental Microbiology, 75, 837–841. doi: 10.1128/AEM.01299-08.

De Souza, T.M.P.A., Freires, I.A., Lima, D.M.B., Jovito, V.C., Almeida, L.F.D., Castro, R.C. (2013). Aderência in vitro de *Streptococcus mutans* à Superfície de Braquetes Ortodônticos Metálicos e de Policarbonato. Revista Brasileira de Ciências da Saúde, 17, 91 – 96. doi: 10.4034/RBCS.2013.17.01.12.

Herrera D., Escudero, N., Pérez, L., Otheo, M., Cañete-Sánchez, E., Pérez, T., Alonso, B., Serrano, J., Palma, J. C., Sanz, M., Martín, C. (2017). Clinical and microbiological effects of the use of a cetylpyridinium chloride dentifrice and mouth rinse in orthodontic patients: a 3-month randomized clinical trial. European Journal of Orthodontics, 2017, 1–10. doi:10.1093/ejo/cjx096.

Castro, R. J., Herrera, R., Giacaman, R. A. (2016). Salivary protein characteristics from saliva of carious lesion-free and high caries adults. Acta Odontológica Latinoamericana, 29, 178–185. doi: S1852-48342016000200011.

Gomar – Vercher, S., Cabrera-Rubio, R., Mira, A., Montiel-Company, J. M., Almerich-Silla, J. M. (2014). Relationship of children's salivary microbiota with their caries status: a pyrosequencing study. Clinical Oral Investigations, 18. doi 10.1007/s00784-014-1200-y.

Eriksson, L., Holgerson, P.L., Johansson, I. (2017) Saliva and tooth biofilm bacterial microbiota in adolescents in a low caries community. Scientific reports, 7, 5861. doi: 10.1038/s41598-017-06221-z.

Salatino, A., (2018). Brazilian Red Propolis: Legitimate Name of the Plant Resin Source. Med Crave: Step into the world of research, 5, 435-441. doi: 10.15406/mojfpt.2018.06.00139.

Nogueira, M. A., Diaz., M. G., Tagami, P. M., Lorscheide, J., (2007). Atividade microbiana de óleos essenciais e extratos de própolis sobre bactérias cariogênicas. Revista de Ciências Farmacêuticas Básica e Aplicada, 28, 93 – 97. DOI: lil – 485206.

Silva, B. B., Rosalen, P. L., Cury, J.A., Ikegaki, M., Sousa, V. C., Esteves, A., Alencar, M. S. (2008). Chemical Composition and Botanical Origin of Red Propolis, a New Type of Brazilian Propolis. ECAM, 5, 313–316. doi:10.1093/ecam/nem059.

Freires, I. A., De Alencar S.M., Rosalen P.L. (2016). A pharmacological perspective on the use of Brazilian Red Propolis and its isolated compounds against human diseases. European Journal of Medicinal Chemistry, 110, 267 – 279. doi: 10.1016/j.ejmech.2016.01.033.

Franchin, M., Colón, D. F., Da Cunha, M. G., Castanheira, F. V. S., Saraiva, A. L. L., Bueno-Silva, B., Alencar, S. M., Cunha, T. M., (2016). Neovestitol, an isoflavonoid isolated from Brazilian red propolis, reduces acute and chronic inflammation: involvement of nitric oxide and IL-6. Scientific Reports, 6, 36401. doi: 10.1038/srep36401.

Porto, I. C. C. M., De Almeida, D. C. C., Costa, G. V. C. O., Donato, T. S. S., Nunes, L. M., Do Nascimento, T. G., Oliveira, J. M. S., Da Silva, C. B., Dos Santos, N. B., Leite, M. L. A. S., Basílio-Júnior, I. D., Dornelas, C. B., Escobro, P. B., Fonseca, E. J. S., Kamiya, R. U. (2018). Mechanical and aesthetics compatibility of Brazilian red propolis micellar nanocomposite as a cavity cleaning agent. BMC Complementary and Alternative Medicine, 18, 219. doi: 10.1186/s12906-018-2281-y.

Soares, C.C.S., Simões, C.A.D., Freire, M.C.M., Narvai, P.C., Frazão, P., Pereira, P.Z., Souza, T.A.C. Projeto Vigifluor – Manual de Credenciamento do Agente do Programa Vigifluor; 2014.

Manji, F., Dahlen, G., Fejerskov, O. (2018). Caries and Periodontitis: Contesting the Conventional Wisdom on Their Aetiology. Caries Research, 52. 548–564. doi: 10.1159/000488948.

Lobo, P. L. D., Fonteles, C. S. R., Marques, L. A. R. V., Jamacaru, F. V. F., Fonseca, S. G. D. C., De Carvalho, C. B. M., De Moraes, M. E. A. (2014). The efficacy of three formulations of *Lippia sidoides* Cham. essential oil in the reduction of salivary Streptococcus mutans in children with caries: A randomized, double-blind, controlled study. Phytomedicine, 21, 1043–1047. doi: 10.1016/j.phymed.2014.04.021.

Chinsembu, K. C., (2016). Plants and other natural products used in the management of oral infections and improvement of oral health. Acta Tropica, 154, 6–18. doi: 10.1016/j.actatropica.2015.10.019.

Freires, I. A., Rosalen P. L., (2016). How Natural Product Research has Contributed to Oral Care Product Development? A Critical View. Pharmaceutical Research 33,:1311-1317. doi: 10.1007/s11095-016-1905-5.

Do Nascimento, T. G., Da Silva, P. F., Azevedo, L. F., Da Rocha, L. G., Porto I. C. C. M., Lima e Moura, T. F. A., Basílio-Júnior, I. D., Grillo, L. A. M., Dornelas, C. B., Fonseca, E. J. S., Oliveira, E. J., Zhang, A. T., Watson, D. G. (2016). Polymeric Nanoparticles of Brazilian Red Propolis Extract: Preparation, Characterization, Antioxidant and Leishmanicidal Activity. Nanoscale Research Letters, 11, 301. doi 10.1186/s11671-016-1517-3.

Jepsen, S., Blanco J., Buchalla, W., Carvalho, J.C., Dietrich, T., Dorfer, C., Eaton, K.A., Figuero, E., Frencken, J.E., Graziani, F., Higham, S.M., Kocher, T., Maltz, M., Ortiz-Vigon, A., Schmoeckel, J., Sculean, A., Tenuta, L.M.A., Van Der Veen, M.H., Machiulskiene V. (2017). Prevention and control of dental caries and periodontal diseases at individual and population level: consensus report of group 3 of joint EFP/ORCA workshop on the boundaries between caries and periodontal diseases. J Clin Periodontol, 44, 85–93. doi: 10.1111/jcpe.12687.

Pitts, N. B., Zero, D. T., Marsh, P. D., Ekstrand, K., Weintraub, J. A., Ramos-Gomez, F., Tagami, J., Twetman, S., Tsakos, G., Ismail, A., (2017). Dental caries. Nature Reviewa Disease Primers, 3, 17030. doi: 10.1038/nrdp.2017.

Cagetti, M. G., Strohmenger, L., Basile, V., Abati, S., Mastroberardino, S., Campus, G., (2015). Effect of a toothpaste containing triclosan, cetylpyridinium chloride, and essential oils on gingival status in schoolchildren: A randomized clinical pilot study. Quintessence International, 46, 5, 437 – 445. doi: 10.3290/j.qi.a33530.

Adams S. E., Arnold, D., Murphy, B., Carroll, P., Green, A. K., Smith, A. M., Marsh, P. D., Chen, T., Mariott, R. E., Brading, M. G., (2017). A Randomised clinical study to determine the effect of a toothpaste containing enzymes and proteins on plaque oral microbiome ecology. Scientific Reports, 7, 43344. DOI: 10.1038/srep 43344.

Escribano, M., Figuero, E., Martín, C., Tobías, A., Serrano, J., Roldán, S., Herrera, D., (2016). Efficacy of adjunctive anti-plaque chemical agents: a systematic review and network meta-analyses of the Turesky modification of the Quigley and Hein plaque index. J Clin Periodonto, 43, 1059-1073. doi: 10.1111/jcpe.12616.

Figuero, E., Nobrega, D. F., García – Gargallo, M., Tenuta, L. M. A., Herrera, D., Carvalho, J. C., (2017). Mechanical and chemical plaque control in the simultaneous management of gingivitis and caries: a systematic review. J Clin Periodontol, 44, 116–134. doi: 10.1111/jcpe.12674.

Herrera, D., Escudero, N., Pérez, L., Otheo, M., Cañete-Sánchez, E., Pérez, T., Martín, C. (2017). Clinical and microbiological effects of the use of a cetylpyridinium chloride dentifrice and mouth rinse in orthodontic patients: a 3-month randomized clinical trial. European Journal of Orthodontics, 1–10 doi:10.1093/ejo/cjx096.

Montero, E., Iniesta, M., Rodrigo, M., Marin, M., Figuero, E., Herrera, D., Sanz, M. (2017). Clinical and Microbiologial Effects of the Adjunctive use of Probiotics in the Treatment of Gingivitis: A Randomized Controlled Clinical Trial. J Clin Periodontol, 44, 708-716. doi: 10.1111/jcpe.12752.

Goes, P., Dutra C.S., Lisboa M. R., Gondim D.V., Leitão R., Brito G.A., Rego R.O., (2016). Clinical efficacy of a 1% Matricaria chamomile L. mouthwash and 0.12% chlorhexidine for gingivitis control in patients undergoing orthodontic treatment with fixed appliances. Journal of Oral Science, 58, 4, 569-574. doi: 10.2334/josnusd.16-0280.

Cagetti, M. G., Strohmenger, L., Basile, V., Abati, S., Mastroberardino, S., Campus, G., (2015). Effect of a toothpaste containing triclosan, cetylpyridinium chloride, and essential oils on gingival status in schoolchildren: A randomized clinical pilot study. Quintessence International, 46, 5, 437 – 445. doi: 10.3290/j.qi.a33530.

Cury, J. A. (2002). Dentifrícios: Como escolher e como indicar. In: APCD. (Org.). Odontologia. São Paulo: Artes Médicas - Divisão Odontológica, 4, 281-295.

6. DISCUSSÃO

O desenvolvimento de materiais odontológicos com produtos naturais ainda é bastante limitado, praticamente se resumindo às formulações tópicas e sem muitos ensaios clínicos publicados nas últimas décadas. No entanto, vários estudos documentaram a atividade farmacológica de produtos naturais contra o biofilme dentário, especialmente o cariogênico. (FREIRES; ROSALEN, 2016)

A resistência a antimicrobianos sintéticos e a busca por substâncias com propriedade farmacológica com menores efeitos adversos fez com que aumentasse o interesse por produtos naturais. Mais de cem milhões de moléculas estão catalogadas em todo o mundo e muitas ainda estão inexploradas. A alta demanda pela própolis e modernização de dispositivos analíticos contribuiu para o lançamento de diversos produtos no mercado. Atualmente a maioria dos ensaios clínicos randomizados em Odontologia estão voltados para o desempenho de materiais restauradores e com muitos vieses na amostra. Na atual fase da Odontologia minimamente invasiva estudos com materiais e alternativas preventivas são importantes, por isso destaca-se a necessidade constante de desenvolvimento de novos materiais preventivos. No desenvolvimento de produtos é importante todo um estudo sobre os produtos viáveis de utilização e estudos in vitro que validem previamente o uso clínico, assim como na presente pesquisa, que se iniciou com um estudo documental de patentes previamente a escolha do tipo de produto a ser desenvolvido e estudos laboratoriais necessários. (LOBO *et al*., 2014; CHINSEMBU, 2016; FREIRES; ROSALEN, 2016; LEVEY et al., 2017; NASCIMENTO *et al*., 2017)

A alta demanda pela própolis e o investimento na biotecnologia de produtos derivados de abelhas contribuiu para o lançamento de diversos produtos no mercado. Sendo assim, desafios quanto à resistência aos antimicrobianos sintéticos aumentaram as buscas por produtos naturais, quanto aos derivados das abelhas se destacam o mel e própolis, onde o desenvolvimento e patenteamento de produtos é uma realidade cada vez maior. A prospecção de patentes é algo importante e indica como o mercado se movimenta. (CHINSEMBU, 2016; NASCIMENTO *et al*., 2017; FURTADO JÚNIOR et al., 2018)

A própolis se destaca pelo amplo espectro antimicrobiano gram – positivo e gram – negativo contra colonizadores do biofilme dentário, como *S.mutans*, *Lactobacillus, P. gingivalis, Actinomyces naeslundii, Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Prevotella intermedia*. (FREIRES *et al*., 2016, CHINSEMBU, 2016).

Existem várias razões para o desenvolvimento de produtos com propriedades antimicrobianas, uma vez que cresce a cada dia a resistência microbianas, toxicidade e altos custos de diversos materiais. O principal agente antimicrobiano na Odontologia é a clorexidina, que quando utilizada por um período prolongado causa coloração do dente, alteração do paladar e perde sua capacidade farmacológica, ocorrendo a recolonização de bactérias como *S. mutans*. (VALE *et al*., 2014)

A própolis vermelha brasileira apresenta potente atividade antimicrobiana mesmo na concentração de 0,1% isso é devido a alta concentração de flavonoides e compostos fenólicos (FREIRES *et al*., 2016, PORTO *et al*., 2018). Nesse estudo na concentração de 1% o grupo tratado com própolis apresentou melhores resultados clínicos e microbiológicos quando comparado ao dentifrício fluoretado.

Cagetti *et al*. (2015) relatam que a gengivite é o tipo de doença periodontal mais prevalente em adolescentes e que a mesma aumenta com frequência ou severidade na puberdade. Em um ensaio clínico duplo-cego randomizado, os autores compararam os efeitos de um dentifrício fluoretado (grupo controle) com um dentifrício contendo flúor, triclosan, cloreto de cetilpiridínio e óleos essenciais (grupo teste) para o controle da placa dentária e inflamação gengival após quatro semanas de uso. O dentifrício teste demonstrou melhores resultados anti-placa.

A cada dia novas estratégias para melhoria dos dentifrícios e controle da microbiota, por exemplo Adams *et al*. (2017) realizaram um ensaio clínico onde avaliaram um dentifrício com enzimas e proteínas para o controle de placa supra-gengival durante quatro semanas. O dentifricio teste foi comparado a um dentifrício fluoretado controle e a placa foi coletada para análise do microbioma, onde observou-se melhores resultados no dentifrício teste.

Os *Streptococcus* são um dos microorganismos mais encontrados na cavidade oral, sendo as espécies pioneiras após a erupção dentária. Espécies como Veillonella, Haemophilus, Neisseria spp., Prevotella e Fusobacterium são normalmente encontradas na placa dentária, língua e saliva, quando esse equilíbrio é quebrado doenças bucais podem ser desenvolvidas. (MANJI *et al*. 2018) O dentifrício de própolis vermelha brasileira apresentou uma redução estatisticamente significante no final do tratamento (D28) para *S.mutans* ($p< 0,001$) e bactérias gram-negativas ($p< 0,001$), quando comparado ao D0. O grupo tratado com dentífricio fluoretado mostrou aumento de bactérias nos achados salivares sem singnificância para *S.mutans* ($p=0,612$) e estatisticamente significante para bactérias gram-negativas ($p=0,003$).

A gengivite e periodontite podem ser prevenidas controlando o biofilme supragengival. Entretanto, em situações como o uso de aparelhos ortodônticos podem exigir o uso de um produto que melhore a remoção mecânica do biofilme. No início desse estudo, todos os participantes apresentavam gengivite e inflamação gengival, ao final do ensaio clínico ambos os grupos tiveram redução no índice de sangramento marginal (ISM), dentifricio comercial fluoretado (p= 0,01) e Dentifricio de Própolis vermelha brasileira (p<0,001), não havendo diferença estatística na análise inter-grupo (p=0,135).

Figuero et al. (2017) realizaram uma revisão sistemática sobre o efeito do controle de placa mecânica e químico no combate à doenças bucais como cárie e gengivite. Os autores relataram que a adição de flúor é significativa para cárie, enquanto antimicrobianos são significativos para a gengivite. Além disso programas motivacionais e escovação supervisionada mostram efeito significativo na redução da placa, isso explica a redução do ISM em ambos os grupos do presente estudo, pois todos os participantes tiveram atividades educacionais de escovação previamente ao início do ensaio clínico. Os autores acrescentam que o uso simultâneo de agentes químicos para controle de placa mecânica no manejo simultâneo de gengivite e cárie ainda é limitado em evidências e a indicação de determinado produto deve ser baseada no risco e necessidade individual.

Goes et al. (2016) avaliaram através de um ensaio clínico randomizado a eficácia clínica de um enxaguatório bucal contendo extrato de 1% de *Matricaria chamomilla* L. (MTC) na redução da placa dentária e gengivite em pacientes sob tratamento ortodôntico. Os participantes foram alocados em três grupos, o primeiro utilizou 15 mL de placebo, o segundo clorexidina 0,12% (CHX) e o terceiro MTC a 1%, imediatamente após a escovação durante duas semanas. Os pacientes que utilizaram clorexidina e o enxaguatório teste tiverem redução de placa dentária e inflamação gengival.

Sabe-se que a CHX é eficaz na redução da inflamação gengival, entretanto sua eficácia reduz a longo prazo e diversos efeitos adversos estão associados ao uso contínuo, como a alteração no paladar, manchamento dos dentes, formação de cálculo, irritação da mucosa, entre outros. Devido à esses fatores não se indica o uso prolongado da mesma. (GOES *et al*., 2016)

A remoção do biofilme dentário, as práticas de higiene bucal e a intervenção profissional são essenciais para eliminar o biofilme dentário, além disso os fatores retentivos são os elementos críticos no tratamento da gengivite (MONTERO *et al*., 2017).A remoção mecânica da

placa através da escovação contribui para a manutenção da saúde gengival, entretanto existem grandes evidências que em situações de maior risco, o uso de substâncias antimicrobianas além da escovação contribuem no controle da placa e previnem a gengivite. (CAGETTI *et al*., 2015)

As etiologias da cárie dentária e das doenças periodontais são independentes. Entretanto alguns fatores são comuns às duas doenças, como a presença do biofilme (SANZ *et al*., 2017). O presente dentifrício reduziu bactérias relacionadas às duas doenças, além de ser fluoretado (1500 ppm F), podendo ser uma estratégia para a prevenção e controle de ambas as doenças.

Apesar de não haver consenso na literatura, dados sugerem que em pacientes com retenção, como em aparelhos ortodônticos, a associação do dentifrício fluoretado com substâncias antimicrobianas pode ser mais eficaz que apenas o uso do dentifrício fluoretado (CURY, 2002). Na comparação inter-grupo do presente estudo o dentifrício de própolis teve achados superiores quando comparado ao dentifrício fluoretado em relação a redução de *S.mutans* ($p = 0,006$) e bactérias gram-negativas ($p< 0,001$).

Sabe-se que em estudos in vivo, as condições da cavidade bucal e saliva, podem influenciar na atividade de certo produto. Na fase clínica percebeu-se que o PVB mesmo não estando nas condições ideais de um estudo in vitro, teve excelentes resultados.

Durante o período de tratamento o dentifrício de PVB 1% demonstrou melhor atividade antimicrobiana contra *S.mutans*, bactérias gram-negativas e na redução do índice de sangramento marginal. Apesar da cárie dentária e gengivite terem suas prevalências diminuídas no mundo, ainda há grandes desigualdades em países subdesenvolvidos, sendo ainda um desafio para os programas de saúde pública de países em desenvolvimento pelo alto consumo de carboidratos, baixa exposição ao flúor, e dificuldade no acesso a serviços de saúde bucal. Sendo assim, programas preventivos devem ser constantemente estimulados, especialmente na saúde pública, para a diminuição dessas condições bucais.

7. CONSIDERAÇÕES FINAIS

A prospecção de produtos derivados de abelhas é uma realidade que aumenta a cada dia. Na análise dos depósitos de patentes foi crescente o número de produtos derivados da própolis que a indústria lança diariamente no mercado. Pode-se perceber que no Brasil o interesse em relação à proteção da propriedade intelectual em produtos opoterápicos ainda é pequeno, apesar de a própolis ser um produto natural de importante produção comercial que vem ganhando notoriedade em pesquisas e no desenvolvimento de medicamentos.

No estudo in vitro realizado observou-se não haver diferença da atividade antimicrobiana entre o extrato e o dentifrício de PVB. No estudo in vivo, o dentrifricio de própolis vermelha brasileira mostrou melhores resultados clínicos e microbiológicos quando comparado ao dentifrício controle. Futuros estudos longitudinais, multicêntricos, são necessários para estabelecer o uso do dentifrício como alternativa no controle do biofilme.

REFERÊNCIAS BIBLIOGRÁFICAS

ADAMS, S.E. et al. A Randomised clinical study to determine the effect of a toothpaste containing enzymes and proteins on plaque oral microbiome ecology. **Scientific Reports**, v. 7, p.43344, 2017.

DE AGUIAR, S.C. et al. Antimicrobial activity of Brazilian propolis extracts against rumen bacteria in vitro. **World Journal of Microbiology and Biotechnology**, v. 29, n. 10, p. 1951-1959, 2013.

ANAUATE NETTO, C. *et al*. Effects of typified propolis on mutans streptococci and lactobacilli: a randomized clinical trial. **Brazilian Dental Science**, v.16, n.2, p.31–36, 2013.

ARAUJO FILHO, W.R.; ROMEIRO, H.L.M.; CABREIRA, M.S. The use of phytotherapics in endodontics. **Revista Brasileira de Odontologia Militar, v.29, n.1, p.36-43,** 2013.

ASSUNÇÃO, E. **O sistema de patentes e as universidades brasileiras nos anos 90**. Rio de Janeiro: INPI, 2000.

AZEVEDO, L.F. *et al*. Polymeric nanoparticle systems loaded with red propolis extract: a comparative study of the encapsulating systems, PCL-Pluronic versus Eudragit® E100-Pluronic. **Journal of Apicultural Research**, v. 57, n. 2, p. 255-270, 2018.

BORBA, A. M.; MACEDO, M.; WALTER, L. R. F. Alternative dentistry with medicinal plants in Chapada dos Guimarães-Mato Grosso, Brazil. **South Braz Dent J**, v. 5, n. 1, p. 43-9, 2008.

BUENO-SILVA, B. *et al*. Anti-inflammatory and antimicrobial evaluation of neovestitol and vestitol isolated from Brazilian red propolis. **Journal of Agricultural and Food Chemistry,** v. 61, n.19, p. 4546-4550, 2013.

CAGETTI, M. G. *et al*. Effect of a toothpaste containing triclosan, cetylpyridinium chloride, and essential oils on gingival status in schoolchildren: A randomized clinical pilot study. **Quintessence International**, v. 46, n. 5, p. 437-445, 2015.

CASTRO, B.S.; SOUZA, G.C.O papel dos Núcleos de Inovação Tecnológica (NITs) nas universidades brasileiras. **Liinc em Revista**, v.8, n.1, p. 125-140, mar. 2012.

CASTRO, R. J.; HERRERA R.; GIACAMAN, R. A. Salivary protein characteristics from saliva of carious lesion-free and high caries adults. **Acta Odontológica Latinoamericana**, v.29, n.2, p.178-185, 2016.

CAYO, C.; QUIJANDRÍA, L.; RAMOS, J. Evaluación in vitro del efecto antibacteriano del propóleo sobre cultivos de Streptococcus mutans. **Ciencia y Desarrollo**, v. 19, n. 2, p. 19-24, 2016.

CHINSEMBU, K. C. Plants and other natural products used in the management of oral infections and improvement of oral health. **Acta Tropica**, v. 154, p. 6-18, 2016.

CHIRUMBOLO, S. Anti-inflammatory property of propolis. **Journal of clinical biochemistry and nutrition**, v. 56, n. 2, p. 163-163, 2015.

CURY, J.A. **Dentifrícios: Como escolher e como indicar**. In: APCD. (Org.). Odontologia. São Paulo: Artes Médicas - Divisão Odontológica, 4 ed., p. 281-295.

DAGNINO, R.D.; SIVA, R.B. As patentes das universidades públicas. **Economia & Tecnologia**, v.18, n.5, p. 169-172, jul. 2009.

DIAS, L.G.; PEREIRA, A.P.; ESTEVINHO, L.M. Comparative study of different Portuguese samples of propolis: pollinic, sensorial, physicochemical, microbiological characterization and antibacterial activity. **Food and Chemical Toxicology**, v. 50, n. 12, p. 4246-4253, 2012.

DOTA, K.F.D. *et al*. Antifungal activity of Brazilian propolis microparticles against yeasts isolated from vulvovaginal candidiasis. **Evidence-Based Complementary and Alternative Medicine**, v. 2011, 2011.

ERIKSSON, L.; HOLGERSON, P. L.; JOHANSSON, I. Saliva and tooth biofilm bacterial microbiota in adolescents in a low caries community. **Scientific reports**, v. 7, n. 1, p. 5861, 2017.

ESCRIBANO, M. *et al*. Efficacy of adjunctive anti-plaque chemical agents: a systematic review and network meta-analyses of the Turesky modification of the Quigley and Hein plaque index. **J. Clin. Periodonto.**, v. 43, n. 12, p. 1059-1073, 2016.

FERNANDES JÚNIOR, A. *et al*. Atividade antimicrobiana de própolis de Apis mellifera obtidas em três regiões do Brasil. **Ciência Rural**, Santa Maria, v. 36, n. 1, p. 294-297, 2006.

FERREIRA, A. A.; GUIMARÃES, E. R.; CONTADOR, J. C. Patent as a competitive tool and as a source of technological information. **Gestão & Produção**, v. 16, n. 2, p. 209-221, 2009.

FIGUERO, E. *et al*. Mechanical and chemical plaque control in the simultaneous management of gingivitis and caries: a systematic review. **J. Clin. Periodonto.**, v. 44, p. S116-S134, 2017.

FRANCHIN, M. *et al*. Neovestitol, an isoflavonoid isolated from Brazilian red propolis, reduces acute and chronic inflammation: involvement of nitric oxide and IL-6. **Scientific Reports**, v. 6, p. 36401, 2016.

FREIRES, I. A.; ALENCAR, S. M.; ROSALEN, P. L. A pharmacological perspective on the use of Brazilian Red Propolis and its isolated compounds against human diseases. **European Journal of Medicinal Chemistry**, v. 110, p. 267-279, 2016.

FREIRES, I. A.; ROSALEN, P. L. How natural product research has contributed to oral cara product developmente? A critical view. **Pharm Res.**, v. 33, n. 6, p. 1311-1317, 2016.

FROZZA, C.O.S. *et al*. Chemical characterization, antioxidant and cytotoxic activities of Brazilian red propolis. **Food and chemical toxicology**, v. 52, p. 137-142, 2013.

FURTADO JÚNIOR, J. H. *et al*. Propolis and its Dental Applications: A Technological Prospection. **Recent Patents on Biotechnology**, v. 12, n. 4, p. 288-296, 2018.

GEORGE, R. M.; KASLIWAL, A. V. Effectiveness of propolis, probiotics and chorhexidine on Streptococcus mutans and Candida albicans: an in vitro study. **Journal of Dental and Medical Sciences**, v. 16, n. 3, p.15-18, 2017.

GIANNOBILE, W.V. *et al*. Saliva as a diagnostic tool for periodontal disease: current state and future directions. **Periodontology**, v. 50, n. 1, p. 52-64, 2000.

GOES, P. *et al*. Clinical efficacy of a 1% Matricaria chamomile L. mouthwash and 0.12% chlorhexidine for gingivitis control in patients undergoing orthodontic treatment with fixed appliances. **Journal of Oral Science**, v. 58, n. 4, p. 569-574, 2016.

GOMAR-VERCHER, S. *et al*. Relationship of children's salivary microbiota with their caries status: a pyrosequencing study. **Clinical Oral Investigations**, v. 18, n. 9, p. 2087-2094, 2014.

GRENHO, L. *et al*. In vitro antimicrobial activity and biocompatibility of propolis containing nanohydroxyapatite. **Biomedical Materials**, v. 10, n. 2, p. 025004, 2015.

HAASE, H.; ARAÚJO, E.C.; DIAS, J. Patent innovations: requirements for new university functions. **Brazilian Journal of Innovation**, v.4, n.2, p.12-22, 2005.

HERRERA, D. *et al*. Clinical and microbiological effects of the use of a cetylpyridinium chloride dentifrice and mouth rinse in orthodontic patients: a 3-month randomized clinical trial. **European Journal of Orthodontics**, v. 20, n. 5, p. 465-474, 2017.

JEPSEN, S. *et al*. Prevention and control of dental caries and periodontal diseases at individual and population level: consensus report of group 3 of joint EFP/ORCA workshop on the boundaries between caries and periodontal diseases. **J. Clin. Periodonto.**, v. 44, p. S85-S93, 2017.

KAUR, R. *et al*. Salivary levels of Bifidobacteria in caries-free and caries-active children. **Int J Paediatr Dent.**, v. 23, n. 1, p. 32-38, 2013.

KLEIN, I. M. *et al*. Structural and molecular basis of the role of starch and sucrose in Streptococcus mutan biofilm development. **Applied and Environmental Microbiology**, v. 75, n. 3, p. 837-841, 2009.

KORU, O. *et al*. In vitro antimicrobial activity of propolis samples from different geographical origins against certain oral pathogens. **Anaerobe**, v. 13, n. 3-4, p. 140-145, 2017.

LEVEY, C.; INNES, N.; SCHWENDICKE, F.; LAMONT, T.; GOSTEMYER, G. Outcomes in randomised controlled trials in prevention and management of carious lesions: a systematic review. **Trials,** v.18, n.1, p.515.

LIBÉRIO, S.A. *et al*. The potential use of propolis as a cariostatic agent and its actions on mutans group streptococci. **Journal of ethnopharmacology**, v. 125, n. 1, p. 1-9, 2009.

LINS, R. *et al*. Avaliação clínica de bochechos com extratos de Aroeira (Schinus terebinthifolius) e Camomila (Matricaria recutita L.) sobre a placa bacteriana e a gengivite. **Rev. Bras. Pl. Med.**, v.15, n.1, p.112-120, 2013.

LIMA, J.A.A.. Digressions on Intellectual Property as agent of development, Innovation and Strategy. **Forum HSM of Strategy**, v.10, n.93, p.45-55, 2006.

LOBO, P.L.D. *et al*. Dose–response evaluation of a novel essential oil against Mutans streptococci in vivo. **Phytomedicine**, v. 18, n. 7, p. 551-556, 2011.

LOBO, P. L. D. *et al*. The efficacy of three formulations of Lippia sidoides Cham. essential oil in the reduction of salivary Streptococcus mutans in children with caries: A randomized, double-blind, controlled study. **Phytomedicine**, v. 21, n. 9, p. 1043-1047, 2014.

LONGONI, N.J. *et al*. Self-ligating versus conventional metallic brackets on Streptococcus mutans retention: A systematic review. **European Journal of Dentistry**, v. 11, n. 4, p. 537, 2017.

LUSTOSA, S.R. *et al*. Própolis: atualizações sobre a química e a farmacologia. **Revista Brasileira de Farmacognosia**, v. 18, n. 3, p. 447, 2008.

MACHADO, B.A.S. *et al*. Chemical composition and biological activity of extracts obtained by supercritical extraction and ethanolic extraction of brown, green and red propolis derived from different geographic regions in Brazil. **PLoS One**, v. 11, n. 1, p. e0145954, 2016.

MACHADO, B.A.S. *et al*. Prospective study of propolis and technologies correlated under the focus on patent documents deposited in Brazil. **Innovation, Technology and Managemen Journal,** v.2, n.3, p.221-235, 2012.

MANJI, F.; DAHLEN, G.; FEJERSKOV, O. Caries and Periodontitis: Contesting the conventional wisdom on their aetiology. **Caries Research**, v. 52, n. 6, p. 548-564, 2018.

MARSH, P.; BEIGHTON, D.; DEVINE, D.A. Influence of saliva on the oral microbiota. **Periodontology 2000**, v. 70, n. 1, p. 80-92, 2016.

MARSH, P. D.; ZAURA, E. Dental biofilm: ecological interactions in health and disease. **Journal of Clinical Periodontology**, v. 44, p. S12-S22, 2017.

MONTERO, E. *et al*. Clinical and microbiological effects of the adjunctive use of Probiotics in the treatment of Gingivitis: A randomized controlled clinical trial. **J. Clin. Periodonto.**, v. 44, n. 7, p. 708-716, 2017.

MOURA, S.A.B *et al*. Valor Diagnóstico da Saliva em Doenças Orais e Sistêmicas: Uma Revisão de Literatura. **Pesq Bras Odontoped Clin Integr**, v. 7, n. 2, p. 187-194, 2007.

MUELLER, S.P.M.; PERUCCHI, V. Universities and the production of patents: topics of interest to the student of technological information. **Perspectives in Information Science**, v.19, n.2, p.15-36, 2014.

NASCIMENTO, T.G. *et al*. Polymeric Nanoparticles of Brazilian Red Propolis Extract: Preparation, Characterization, Antioxidant and Leishmanicidal Activity. **Nanoscale Research Letters**, v. 11, n. 1, p. 301, 2016.

NOGUEIRA, M. A. *et al*. Atividade microbiana de óleos essenciais e extratos de própolis sobre bactérias cariogênicas. **Revista de Ciências Farmacêuticas Básica e Aplicada**, v. 28, n. 1, p. 93-97, 2009.

PAULA, A.M.B. *et al*. Susceptibility of oral pathogenic bacteria and fungi to brazilian green propolis extract. **Pharmacologyonline**, v. 3, p. 467-473, 2006.

PEREIRA, A.S.; SEIXAS, F.R.M.S.; AQUINO NETO, F.R. de. Propolis: 100 years of research and future perspectives. **Química Nova**, v. 25, n. 2, p. 321-326, 2002.

PITTS, N. B. *et al*. Dental caries. **Nature reviews Disease primers**, v. 3, p. 17030, 2017.

PORTO, I. C. C. M. *et al*. Mechanical and aesthetics compatibility of Brazilian red propolis micellar nanocomposite as a cavity cleaning agent. **BMC Complementary and Alternative Medicine**, v. 18, n. 1, p. 219, 2018.

SALATINO, A; SALATINO, M.L.F. Brazilian red propolis: Legitimate name of the plant resin source. **Med Crave., v.**6, n. 1, p. 21–22.

SAMAH, A.; NORA, A.; NASIR, A.; ALI, A.; MOATAZBELLAH, A.; NASSER, A.; *et al*. The effect of orthodontic therapy on periodontal health: a review of the literature. **International Journal of Dentistry**, 595048, 2014.

SANZ, M. *et al*. Role of microbial biofilms in the maintenance of oral health and in the development of dental caries and periodontal diseases. Consensus report of group 1 of the Joint EFP/ORCA workshop on the boundaries between caries and periodontal disease. **J. Clin. Periodonto.**, v. 44, p. S5-S11, 2017.

SFORCIN, J.M. Biological properties and therapeutic applications of propolis. Phytotherapy research, v. 30, n. 6, p. 894-905, 2016.

SILVA, B. *et al*. Chemical composition and botanical origin of red propolis, a new type of Brazilian propolis. **Evidence-based Complementary and Alternative Medicine**, v. 5, n. 3, p. 313-316, 2008.

SILVA, R. P. D. *et al*. Antioxidant, antimicrobial, antiparasitic, and cytotoxic properties of various Brazilian propolis extracts. **Plos One**, v. 12, n. 3, p. e0172585, 2017.

SILVA, T.M.S. *et al*. Phenolic compounds, melissopalynological, physicochemical analysis and antioxidant activity of jandaíra (Melipona subnitida) honey. **Journal of Food Composition and analysis**, v. 29, n. 1, p. 10-18, 2013.

SIMÕES, C. C. *et al*. Estudo in vitro e ex vivo da ação de diferentes concentrações de extratos de própolis frente aos microrganismos presentes na saliva de humanos. **Revista Brasileira de Farmacognosia,** v. 18, n. 1, p. 84-89, 2008.

SOARES, C.C.S. *et al*. **Projeto Vigifluor – Manual de Credenciamento do Agente do Programa Vigifluor**; 2014.

SOUZA, T. M. P. A. *et al*. Aderência in vitro de Streptococcus mutans à superfície de braquetes ortodônticos metálicos e de policarbonato. **Revista Brasileira de Ciências da Saúde**, v. 17, n. 1, p. 91-96, 2013.

VALADAS, L. A. R. *et al*. Dose-response evaluation of a copaiba-containing varnish against streptococcus mutans in vivo. **Saudi Pharmaceutical Journal**, 2018.

VALE, G.C. *et al*. Recolonization of Mutans Streptococci after application of chlorhexidine gel. **Brazilian Dental Journal**, v. 25, n. 6, p. 485-488, 2014.

YILDIRIM, A. et al. Antiviral activity of hatay propolis against replication of herpes simplex virus type 1 and type 2. **Medical science monitor: international medical journal of experimental and clinical research**, v. 22, p. 422, 2016.

ZABAIOU, N. *et al*. Biological properties of propolis extracts: Something new from an ancient product. **Chemistry and physics of lipids**, v. 207, p. 214-222, 2017.

APÊNDICE I

FICHA DE ANAMNESE
DADOS PESSOAIS E EXAME DENTÁRIO

NOME:_____
IDADE_____ DATA DE NASCIMENTO_____

NOME DO PAI_____
NOME DA MÃE_____
RESPONSÁVEL LEGAL _____
ENDEREÇO_____
TELEFONE PARA CONTATO_____

ESTADO DE SAÚDE GERAL

FAVOR LER E RESPONDER COM ATENÇÃO.

1) O seu filho ou filha se encontra sob tratamento médico? SIM NÃO

Especifique. Caso a sua resposta tenha sido SIM._____

2) O seu filho ou filha tem alguma doença crônica? SIM NÃO

Especifique. Caso a sua resposta tenha sido SIM. _____

3) O seu filho ou filha está tomando algum medicamento (remédio)? SIM NÃO

Especifique. Caso a sua resposta tenha sido SIM. _____

4) O seu filho ou filha tem algum tipo de doença alérgica? SIM NÃO

Especifique. Caso a sua resposta tenha sido SIM. _____

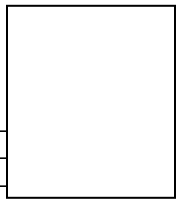
5) O seu filho ou filha já apresentou alergia a algum tipo de medicamento? SIM NÃO
Identifique o(s) medicamento(s). Caso sua resposta tenha sido SIM. _____

5) O seu filho ou filha já esteve hospitalizado (a)? SIM NÃO

Especifique o motivo. Caso a sua resposta tenha sido SIM. _____

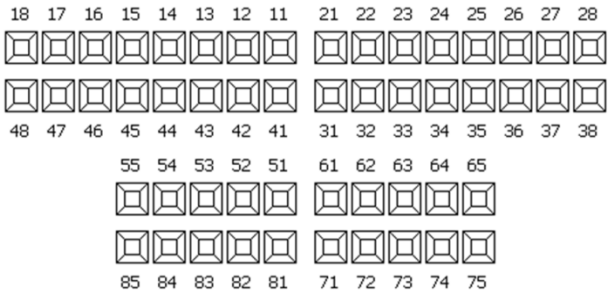
Afirmo que as informações acima são verdadeiras.
Data_____
Assinatura _____
RG: _____

Testemunha1: _____
Testemunha2:_____
Pesquisador:_____



EXAME DENTÁRIO

NOME _____

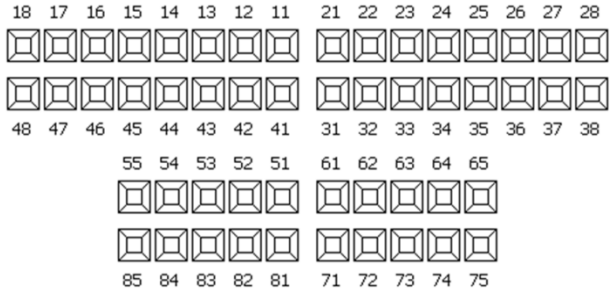


VISITA 01

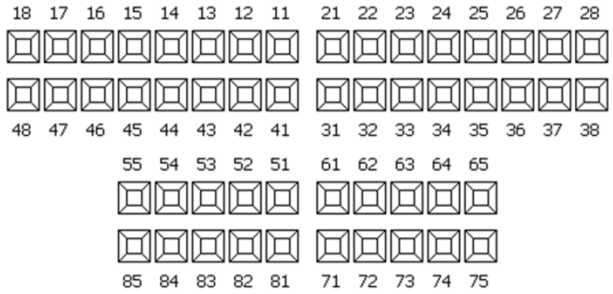
ÍNFLAMAÇÃO GENGIVAL

Critérios e códigos adotados para o IG

Critérios	Códigos
Ausência de inflamação gengival	0
Inflamação leve = pequena modificação de cor e textura	1
Inflamação moderada = gengiva moderadamente avermelhada, vítrea, edemaciada e hipertrófica, com sangramento sob estímulo	2
Inflamação severa = gengiva nitidamente avermelhada, hipertrófica, com tendência ao sangramento espontâneo e tendência à ulceração	3



VISITA 02



ANEXO I

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

Você está sendo convidado pela cirurgiã-dentista Lídia Audrey Rocha Valadas Marques, como participante da pesquisa intitulada "DESENVOLVIMENTO DE DENTIFRÍCIO A BASE DE PRÓPOLIS VERMELHA E AVALIAÇÃO SOBRE A REDUÇÃO DE BACTÉRIAS ORAIS E GENGIVITE EM ADOLESCENTES SOBRE TRATAMENTO ORTODÔNTICO" Você não deve participar contra a sua vontade. Leia atentamente as informações abaixo e faça qualquer pergunta que desejar, para que todos os procedimentos desta pesquisa sejam esclarecidos.

Seu filho ou filha está sendo convidado a participar de um projeto de pesquisa. Sua participação é importante, porém, ele(a) não deve participar contra vontade própria ou contra a sua vontade. Leia com atenção as informações abaixo, sentindo-se livre para fazer qualquer pergunta que desejar, para que não haja dúvida alguma sobre os procedimentos a serem realizados.

a) O objetivo da pesquisa é avaliar a ação de um novo dentifricio (pasta de dente), para auxiliar no tratamento da gengivite

b) Durante o estudo você deverá fornecer informação sobre o estado geral de saúde do seu filho ou filha, bem como possíveis reações alérgicas que ele(a) já possa ter tido

c) A participação neste estudo consistirá de:

- Exame dentário e gengival de seu filho ou filha,
- Utilização de uma pasta de dente feita de um produto natural (Própolis), por 3 vezes ao dia durante 28 dias.
- Coleta de saliva de seu filho ou filha por 2 vezes, uma a cada consulta. Para que seja feita a coleta seu filho ou filha terá que mastigar um pequeno pedaço de "chiclete" por um minuto, e a mesma se encontrará ligada a um pedaço de fio dental para evitar que o seu filho ou filha venha a engolir material. Depois de decorrido o tempo o mesmo será retirado da boca e colocado em um pequeno frasco.

d) A coleta de saliva e o exame bucal NÃO causarão DOR ao seu filho ou filha.

f) Seu filho ou filha **NÃO RECEBERÁ INJEÇÃO** de anestésico local.

g) Após a aplicação do tratamento a saliva recolhida (conforme descrito acima) e analisada para descobrir se o remédio usado nos dentes de seu filho ou filha foi capaz de diminuir a número de bactérias da boca que causam cárie, podendo trazer como beneficio um tratamento para cárie no futuro.

i) Você tem a liberdade de desistir ou interromper a participação do seu filho ou filha neste estudo no momento que desejar, sem necessidade de qualquer explicação.

k) Os resultados obtidos durante este estudo serão mantidos em sigilo. A Faculdade de Odontologia não o identificará por ocasião da exposição e/ou publicação dos mesmos (os dados serão publicados somente em revista científica e/ou congressos científicos não identificando o nome de seu filho ou filha).

l) O surgimento de resfriados ou viroses no dia da pesquisa, com conseqüente uso de medicações por período de tempo limitado, exclui seu filho ou filha do estudo.

m) A participação nessa pesquisa é voluntária e o participante não irá receber nenhum pagamento por isso.

Destacar, ainda no convite, que a qualquer momento o participante poderá recusar a continuar participando da pesquisa e que também poderá retirar o seu consentimento, sem que isso lhe traga qualquer prejuízo. Garantir que as informações conseguidas através da sua participação não permitirão a identificação da sua pessoa, exceto aos

responsáveis pela pesquisa, e que a divulgação das mencionadas informações só será feita entre os profissionais estudiosos do assunto.

Endereço d(os, as) responsável(is) pela pesquisa:

Caso venham a surgir dúvidas ou perguntas, sinta-se livre para contactar a Cirurgiã-dentista. Lidia Valadas (responsável pelo projeto) no telefone (85) 99845353, ou no endereço Capitão Francisco Pedro, 1210 – Rodolfo Teófilo – *CEP* 60430-370, Fortaleza-Ce.

Nome:Lídia Audrey Rocha Valadas Marques
Instituição: Universidade Federal do Ceará
Endereço:Rua Capitão Francisco Pedro, 1210- Rodolfo Teófilo- CEP 60430-370, Fortaleza-Ce
Telefones para contato: 85997361292

ATENÇÃO: Se você tiver alguma consideração ou dúvida, sobre a suaparticipação na pesquisa, entre em contato com o Comitê de Ética em Pesquisa da UFC/PROPESQ – Rua Coronel Nunes de Melo, 1000 - Rodolfo Teófilo, fone: 3366-8344. (Horário: 08:00-12:00 horas de segunda a sexta-feira).
O CEP/UFC/PROPESQ é a instância da Universidade Federal do Ceará responsável pela avaliação e acompanhamento dos aspectos éticos de todas as pesquisas envolvendo seres humanos.

O abaixo assinado _____,___anos, RG:_____, declara que é de livre e espontânea vontade que está como participante de uma pesquisa. Eu declaro que li cuidadosamente este Termo de Consentimento Livre e Esclarecido e que, após sua leitura, tive a oportunidade de fazer perguntas sobre o seu conteúdo, como também sobre a pesquisa, e recebi explicações que responderam por completo minhas dúvidas. E declaro, ainda, estar recebendo uma via assinada deste termo.

Fortaleza, ____/____/___

Nome do participante da pesquisa	Data	Assinatura
Nome do pesquisador	Data	Assinatura
Nome da testemunha (se o voluntário não souber ler)	Data	Assinatura
Nome do profissional que aplicou o TCLE	Data	Assinatura

ANEXO II

TERMO DE ASSENTIMENTO

Você está sendo convidado(a) como participante da pesquisa: **DESENVOLVIMENTO DE DENTIFRÍCIO A BASE DE PRÓPOLIS VERMELHA E AVALIAÇÃO SOBRE A REDUÇÃO DE BACTÉRIAS ORAIS E GENGIVITE EM ADOLESCENTES SOBRE TRATAMENTO ORTODÔNTICO.**

Nesse estudo pretendemos avaliar a diminuição do sangramento da sua gengiva quando você escova os dentes. O motivo que nos leva a estudar esse assunto é a grande presença de gengiva inflamada em pessoas que estão usando aparelho nos dentes e assim busca-se algum produto que diminua isso.

O estudo será composto pelos seguintes procedimentos:

- Exame dos seus dentes e da gengiva
- Utilização de uma pasta de dente feita de um produto natural (Própolis), por 3 vezes ao dia durante 28 dias.
- Coleta de saliva na consulta.

Para participar deste estudo, o responsável por você deverá autorizar e assinar um termo de consentimento. Você não terá nenhum custo, nem receberá qualquer vantagem financeira. Você será esclarecido(a) em qualquer dúvida que tiver e estará livre para participar ou não. O responsável por você poderá também a qualquer momento decidir se você continua ou não na pesquisa. A sua participação é de livre espontânea vontade, e se não quiser participar não vai ser penalizado por isso. Você não terá seu nome divulgado de maneira alguma. Este estudo apresenta risco mínimo isto é, o mesmo risco existente em atividades rotineiras como conversar, tomar banho, ler etc.

No final da pesquisa se você quiser, os resultados sobre o uso da pasta de dente estarão à sua disposição quando finalizada. Seu nome ou o material que indique sua participação não será liberado sem a permissão do responsável por você. Os dados e resultados da pesquisa ficarão arquivados com o pesquisador responsável por um período de 5 anos e, após esse tempo, serão destruídos. Este termo de consentimento encontra-se impresso em duas folhas, sendo que uma será arquivada pelo pesquisador responsável, e a outra será fornecida a você.

Eu, _____, portador(a) do documento de Identidade _____ (se já tiver documento), fui informado(a) dos objetivos do presente estudo de maneira clara e detalhada e esclareci minhas dúvidas. Sei que a qualquer momento poderei solicitar novas informações, e o meu responsável poderá modificar a decisão de participar, se assim o desejar. Tendo o consentimento do meu responsável já assinado, declaro que concordo em participar desse estudo. Recebi uma via deste Termo de Assentimento e me foi dada a oportunidade de ler e esclarecer as minhas dúvidas.

Fortaleza, ____ de _____ de 20____.

_____
Assinatura do(a) menor

_____
Assinatura do(a) pesquisador(a)

Endereço d(os, as) responsável (is) pela pesquisa:

Nome:Lídia Audrey Rocha Valadas Marques
Instituição: Universidade Federal do Ceará
Endereço:Rua Capitão Francisco Pedro, 1210- Rodolfo Teófilo- CEP 60430-370, Fortaleza-Ce
Telefones para contato: 85997361292

ATENÇÃO: Se você tiver alguma consideração ou dúvida, sobre a sua participação na pesquisa, entre em contato com o Comitê de Ética em Pesquisa da UFC/PROPESQ – Rua Coronel Nunes de Melo, 1000 - Rodolfo Teófilo, fone: 3366-8344. (Horário: 08:00-12:00 horas de segunda a sexta-feira).
O CEP/UFC/PROPESQ é a instância da Universidade Federal do Ceará responsável pela avaliação e acompanhamento dos aspectos éticos de todas as pesquisas envolvendo seres humanos.

ANEXO III- PARECER DO COMITÊ DE ÉTICA

UNIVERSIDADE FEDERAL DO CEARÁ/ PROPESQ

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: DESENVOLVIMENTO DE DENTIFRÍCIO A BASE DE PRÓPOLIS VERMELHA E AVALIAÇÃO SOBRE A REDUÇÃO DE BACTÉRIAS ORAIS E GENGIVITE EM ADOLESCENTES SOBRE TRATAMENTO ORTODÔNTICO

Pesquisador: Lídia Audrey Rocha Valadas Marques

Área Temática:

Versão: 1

CAAE: 55395616.6.0000.5054

Instituição Proponente: Departamento de Clínica Odontológica

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 1.520.356

Apresentação do Projeto:

Projeto de pesquisa da mestranda Lídia Audrey Rocha Valadas Marques pautado no efeito antimicrobiano da própolis vermelha. É objetivo desenvolver um dentifrício a base de própolis vermelha e analisar sua eficácia na redução de bactérias cariogênicas e periodontais e do Índice de sangramento marginal em pacientes adolescentes em tratamento ortodôntico. Um total de 100 participantes serão randomizados em dois grupos: Grupo I - Dentifrício manipulado com própolis vermelha associado à escovação e Grupo II Dentifrício

fluoretado comum associado à escovação. Serão realizados exame clínico intra-oral para avaliação e determinação do Índice de Placa (IP) e do Índice Gengival (IG), orientação da escovação e coleta de saliva para análise microbiológica. Todos os resultados serão analisados pelo software GraphPad Prism® versão 5.00 para Windows® (GraphPad Software, San Diego, California, USA, 2007), sendo considerado um índice de significância de 5%.

Objetivo da Pesquisa:

Avaliar clinicamente e microbiologicamente a eficácia de um dentifríco manipulado com extrato de Própolis vermelha em adolescentes, que apresentem gengivite e comparar a um dentifrício fluoretado.

Endereço: Rua Cel. Nunes de Melo, 1000
Bairro: Rodolfo Teófilo **CEP:** 60.430-275
UF: CE **Município:** FORTALEZA
Telefone: (85)3366-8344 **E-mail:** comepe@ufc.br

UNIVERSIDADE FEDERAL DO CEARÁ/ PROPESQ

Continuação do Parecer: 1.520.356

Avaliação dos Riscos e Benefícios:

A pesquisa é de baixo risco e o benefício esperado é a utilização de um dentifrício eficaz que tenha ação antimicrobiana e anti-inflamatória sobre os tecidos gengivais em uma população que apresenta dificuldade de higiene oral em virtude da utilização dos aparatos ortodônticos.

Comentários e Considerações sobre a Pesquisa:

Trata-se de um estudo longitudinal, em paralelo, randomizado, duplo-cego, controlado. Há necessidade de melhor delineamento da metodologia a ser adotada na análise microbiológica.

Considerações sobre os Termos de apresentação obrigatória:

A pesquisadora apresentou a este comitê: projeto, folha de rosto, declaração de concordância, autorização somente da Clínica de Periodontia assinada pela chefia do Departamento de Clínica Odontológica, orçamento, cronograma, currículo, carta de encaminhamento, TCLE, Termo de Assentimento necessitando de adequações.

Recomendações:

Conclusões ou Pendências e Lista de Inadequações:

Não foi apresentado autorização do Laboratório de Farmacotécnica do curso de Farmácia da Universidade Federal do Ceará, onde serão realizados os testes e manipulação do dentifrício proposto.

O Termo de Assentimento não está com linguagem adequada, por vezes, de difícil entendimento, além disso, aborda questões de ressarcimento e indenizações, no entanto não explicita em que situações, quem fará o ressarcimento nem como será. (Conforme resolução 466/12 Item IV.3 alíneas g/h).

Apresentar versão e data da última modificação do Termo de Assentimento.

Considerações Finais a critério do CEP:

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_671999.pdf	21/04/2016 08:51:47		Aceito
Outros	Cartadeapreciacao.pdf	21/04/2016 08:51:23	Lídia Audrey Rocha Valadas Marques	Aceito
Declaração de Pesquisadores	Declaracaodeconcordancia.pdf	21/04/2016 08:50:26	Lídia Audrey Rocha Valadas Marques	Aceito
Orçamento	Orcamento.pdf	21/04/2016	Lídia Audrey Rocha	Aceito

Endereço: Rua Cel. Nunes de Melo, 1000
Bairro: Rodolfo Teófilo **CEP:** 60.430-275
UF: CE **Município:** FORTALEZA
Telefone: (85)3366-8344 **E-mail:** comepe@ufc.br

UNIVERSIDADE FEDERAL DO CEARÁ/ PROPESQ

Continuação do Parecer: 1.520.356

Orçamento	Orcamento.pdf	08:49:10	Valadas Marques	Aceito
Declaração de Instituição e Infraestrutura	Autorizacaodoslocais.pdf	21/04/2016 08:48:11	Lídia Audrey Rocha Valadas Marques	Aceito
Cronograma	Cronograma.pdf	21/04/2016 08:47:35	Lídia Audrey Rocha Valadas Marques	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE.docx	21/04/2016 08:46:14	Lídia Audrey Rocha Valadas Marques	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TALE.docx	21/04/2016 08:45:57	Lídia Audrey Rocha Valadas Marques	Aceito
Projeto Detalhado / Brochura Investigador	Projetopropolis.docx	01/04/2016 19:12:43	Lídia Audrey Rocha Valadas Marques	Aceito
Folha de Rosto	Folha.pdf	01/04/2016 19:10:26	Lídia Audrey Rocha Valadas Marques	Aceito

Situação do Parecer:
Pendente

Necessita Apreciação da CONEP:
Não

FORTALEZA, 28 de Abril de 2016

Assinado por:
FERNANDO ANTONIO FROTA BEZERRA
(Coordenador)

Endereço: Rua Cel. Nunes de Melo, 1000
Bairro: Rodolfo Teófilo **CEP:** 60.430-275
UF: CE **Município:** FORTALEZA
Telefone: (85)3366-8344 **E-mail:** comepe@ufc.br

ANEXO IV- COMPROVANTE DE DEPÓSITO DE PATENTE

República Federativa do Brasil
Ministério da Indústria, Comércio Exterior e Serviços
Instituto Nacional da Propriedade Industrial

(21) BR 102017011097-4 A2

(22) Data do Depósito: 25/05/2017

(43) Data da Publicação Nacional: 18/12/2018

(54) Título: DESENVOLVIMENTO DE DENTIFRÍCIOS INCORPORADOS COM PRÓPOLIS VERMELHA BRASILEIRA ASSOCIADOS A ARGININA,FLÚOR E HIDROXIAPATITA PARA CONTROLE DE MICROORGANISMOS ORAIS

(51) Int. Cl.: A61K 35/644; A61K 31/198; A61P 31/04; A61P 31/02; A61P 1/02.

(71) Depositante(es): UNIVERSIDADE FEDERAL DO CEARÁ.

(72) Inventor(es): JOÃO HILDO DE CARVALHO FURTADO JUNIOR; LIDIA AUDREY ROCHA VALADAS MARQUES; PATRICIA LEAL DANTAS LOBO; SAID GONÇALVES DA CRUZ FONSECA; EDILSON MARTINS RODRIGUES NETO; MARTA MARIA DE FRANÇA FONTELES; ANA CRISTINA DE MELLO FIALLOS; FRANCINEUDO OLIVEIRA CHAGAS.

(57) Resumo: DESENVOLVIMENTO DE DENTIFRÍCIOS INCORPORADOS COM PRÓPOLIS VERMELHA BRASILEIRA ASSOCIADOS A ARGININA, FLÚOR E HIDROXIAPATITA PARA CONTROLE DE MICROORGANISMOS ORAIS. Dentifrícios têm como principal função a prevenção da cárie dentária e gengivite. Entre as diversas espécies, atualmente a Própolis Vermelha Brasileira tem se destacado mundialmente por possuir composição química distinta e ter ampla ação terapêutica em diversas doenças. A própolis vermelha brasileira tem sido testada quanto à sua atividade antimicrobiana sobre as bactérias orais, com bons resultados in vitro e in vivo o que concede credibilidade ao uso clínico do medicamento. Não foram encontradas patentes de dentifrícios com própolis vermelha brasileira, nem associadas com substâncias remineralizadoras como o flúor e a hidroxiapatita.

ANEXO V- COMPROVANTE DE SUBMISSÃO DO ARTIGO DO CAPÍTULO 1

Gmail — João Hildo Carvalho Furtado <jhildocarvalho@gmail.com>

Confirmation of your submission to BMC Complementary and Alternative Medicine - BCAM-D-19-00285

BMC Complementary & Alternative Medicine - Editorial Office <em@editorialmanager.com> — 25 de fevereiro de 2019 19:14
Responder a: BMC Complementary & Alternative Medicine - Editorial Office <jhoanna.hernandez@springernature.com>
Para: Joao Hildo de Carvalho Furtado Junior <jhildocarvalho@gmail.com>

BCAM-D-19-00285
IN VITRO EVALUATION OF ANTIMICROBIAL ACTIVITY OF BRAZILIAN RED PROPOLIS CONTAINING-DENTIFRICE
Joao Hildo de Carvalho Furtado Junior
BMC Complementary and Alternative Medicine

Dear Prof Furtado Junior,

Thank you for submitting your manuscript 'IN VITRO EVALUATION OF ANTIMICROBIAL ACTIVITY OF BRAZILIAN RED PROPOLIS CONTAINING-DENTIFRICE' to BMC Complementary and Alternative Medicine.

The submission id is: BCAM-D-19-00285

Please refer to this number in any future correspondence.

During the review process, you can keep track of the status of your manuscript by accessing the following website:

https://www.editorialmanager.com/bcam/

If you have forgotten your password, please use the 'Send Login Details' link on the login page at https://www.editorialmanager.com/bcam/. For security reasons, your password will be reset.

Best wishes,

Editorial Office
BMC Complementary and Alternative Medicine
https://bmccomplementalternmed.biomedcentral.com/

ANEXO VI- COMPROVANTE DE SUBMISSÃO DO ARTIGO DO CAPÍTULO 2

Gmail — João Hildo Carvalho Furtado <jhildocarvalho@gmail.com>

Manuscript ID CPE-02-19-8181 - Journal of Clinical Periodontology

Jannani Perumal <onbehalfof@manuscriptcentral.com> 25 de fevereiro de 2019 18:44
Responder a: CPEedoffice@wiley.com
Para: jhildocarvalho@gmail.com

25-Feb-2019

Dear Prof. João Furtado Jr,

Your manuscript entitled "CLINICAL AND MICROBIOLOGICAL EVALUATION OF BRAZILIAN RED PROPOLIS CONTAINING-DENTIFRICE IN ORTHODONTIC PATIENTS" has been successfully submitted online. Within the next few days your manuscript will be checked for its compliance with the journal's requirements. If any required part is found missing, you will be notified by email that your manuscript has been unsubmitted and returned to your Author Centre. The email will include detailed explanation of the changes to be made before your manuscript can be resubmitted for review in Journal of Clinical Periodontology.

Your manuscript ID is CPE-02-19-8181.

Please mention the above manuscript ID in all future correspondence or when contacting the editorial office for questions. If there are any changes in your street address or e-mail address, please log in to ScholarOne Manuscripts (formerly known as Manuscript Central) at https://mc.manuscriptcentral.com/jcpe and edit your user information as appropriate.

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Thank you for submitting your manuscript to the Journal of Clinical Periodontology.

Yours sincerely,
Lauren Dawson
Journal of Clinical Periodontology Editorial Office

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ANEXO VII- COMPROVANTE DE PUBLICAÇÃO DO ARTIGO DO CAPÍTULO 3

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Recent Patents on Biotechnology, **2018,** *12,* **000-000** **1**

RESEARCH ARTICLE

Propolis and its Dental Applications: A Technological Prospection

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Abstract: ***Background:*** Propolis is a resinous complex produced by *Apis mellifera* L. bees whose variety of pharmacological properties results from the complexity of its composition. In dentistry, propolis is used on the prevention of oral diseases such as dental caries and gingivitis. Prospective studies in intellectual property banks are important to increase market competitiveness and thus generate new products in the various research areas. In this way, investments in patents play an important role in the technological and economic development of a country.

Objective: To evaluate patents with dental products containing propolis, on intellectual property banks.

Method: The research was conducted in 10 banks of intellectual property, including since the first deposits up to 2016. Relevant information that describes the invention in the patent document were collected, processed and described.

Results: The search performed in 62 patents using propolis in the dental topic. World Intellectual Property Organization (WIPO) has the largest number of filing patents (83.60%) and the National Institute of Industrial Property (INPI) in third place with 4 patents (6.55%). Built-in dental cream with propolis was the most patented product. The first patent date of 1998, followed by an increase in the number of deposits in the last 20 years. Most of the patents are A61K code (51) for medical, dental or hygienic purposes.

Conclusion: This study has shown that propolis is a promising bioactive component in dental products, especially for use in Cariology and Periodontology. Although there has been a significant progress in applications of propolis, the field of dental products is still a growing area and it is important to encourage innovation and development of new products incorporating propolis based on knowledge of its composition and therapeutic properties.

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1. INTRODUCTION

Propolis is a natural complex resinous synthesized by bees of the species *Apis mellifera* L. as material for sealing of hives, derived from products collected from different parts of plants (leaves, flowers, bud, exudates, *etc*.) according to the plant diversity found around the beehive [1].

This material gets salivary secretions and wax used as protection against animals and microorganisms. The resin is characterized as a complex chemical composition composed of about 300 to 400 different chemical compounds and vary with the location and type of plant where it was collected having as main constituents prenyled phenolic acid, flavonoids, steroids, lignans, terpenes and terpenic alcohols, in addition to p-curmarinics derivatives [2-4]. The variety of pharmacological properties of propolis is a result of the complexity of its dependent composition on the number of phenolic compounds, especially flavonoids, re-

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