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Original Article

In vitro antimicrobial evaluation of toothpastes with natural compounds

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ABSTRACT

Objectives: This *in vitro* study evaluated the antimicrobial effects of commercial toothpastes containing natural compounds. **Materials and Methods:** The study groups were divided based on the natural compound present in the toothpaste composition: Sorbitol (I), tocopherol (II), mint (III), cinnamon/mint (IV), propolis/melaleuca (V), mint/açai (VI), mint/guarana (VII), propolis (VIII), negative control (IX), and the positive control (X). The antimicrobial properties of the toothpastes were tested using the disk diffusion method against oral pathogens: *Streptococcus mutans*, *Pseudomonas aeruginosa,* and *Enterococcus faecalis*. The resulting inhibition halos were measured in millimeters. **Results:** The data indicated that the bacteria responded differently to the toothpastes ($P < 0.0001$). The diameters of the inhibition halos against *S. mutans* were in decreasing order of efficacy: Propolis/melaleuca > mint/guarana > mint/açai > sorbitol > tocopherol > cinnamon/mint > propolis > mint ($P < 0.001$ vs. negative control). *E. faecalis* showed variable responses to the dentifrices in the following order of decreasing efficacy: Mint/guarana > propolis > sorbitol > mint/açai > tocopherol > cinnamon/mint > mint = propolis/melaleuca = negative control. The product with the highest antimicrobial activity was mint/guarana, which was significantly different than propolis/melaleuca, mint, cinnamon/mint, and tocopherol and negative control ($P < 0.001$). The statistical analysis indicated that propolis, sorbitol, and mint/açai did not show any differences compared to mint/guarana ($P > 0.05$) and positive control ($P > 0.05$). *P. aeruginosa* was resistant to all dental gels tested including positive control. **Conclusion:** The toothpastes with natural compounds have therapeutic potential and need more detailed searches for the correct clinic therapeutic application. The results from this study revealed differences in the antimicrobial activities of commercial toothpastes with natural compounds.

Key words: Antimicrobial activity, dental gels, dentifrices, natural products, toothpastes

INTRODUCTION

The tendency among world consumers, to seek natural products for a healthier lifestyle has increased the use these compounds in food, cosmetic, and pharmaceutical products.[1] The marketing of these products contributes to an increasing in consumption, and natural dental products are also targets of such marketing with a wide variety of products available in all world. The consumer is often induced to buy these products without being aware of their efficacy[2] with the presence of natural compounds by itself does not guarantee their antibacterial activity in the formulation, for example.

This is particularly important characteristic for toothpastes, which are expected to help in the control of the biofilm, including cariogenic and opportunistic bacteria, such as *Streptococcus mutans*, *Enterococcus faecalis*, and *Pseudomonas aeruginosa*.[3]

Toothpastes have a wide range of pharmaceutical compositions and consistencies, including gel form

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that often incorporates natural compounds. Among the most common natural compounds in toothpastes is mint, which is used mainly for flavor,[4] species of the genus. Mentha are also used for different medicinal purposes as an antiseptic, anti-inflammatory, and antimicrobial agent.[1,2]

The species cinnamon (*Cinnamomum cassia*) has an important antimicrobial activity due to the presence of cinnamaldehyde,[3] while the sugar alcohol - sorbitol is found naturally in different plants; it has a bacteriostatic property,[5] but is considering just a softener and texturing agent in many natural herbal dentifrices.[4]

The use of the açai species (*Euterpe oleracea* and *E. precatoria* Mart.) is very promising.[6,7] They are considered important nutraceuticals agents, and show significant antioxidant properties due to their rich content of flavonoids[7] and anthocyanins,[8] which are known for their anticarcinogenic, anti-inflammatory, and antimicrobial properties.[9,10]

The specie *Paullinia cupana* (guarana) can be used as an antibacterial and antioxidant agent,[11] while the *Melaleuca alternifolia* (tea tree) have excellent medicinal properties, showing anticarcinogenic, anti-inflammatory, antifungal, antiviral, and antibacterial activities.[12]

Due to the presence of flavonoids and phenolic acids, the propolis species have anti-inflammatory, cytotoxic, antiatherogenic, antioxidant, and antimicrobial activities too.[13]

Tocopherols are Vitamin E precursors, belong to the terpenoid group, and they are vitamins, antioxidants, modulators of immune function, and regulators of cell differentiation and proliferation.[14]

Due to the wide variety of plants with activities relevant to dentistry and their presence in dental products, this study aimed to evaluate the *in vitro* antimicrobial activities of toothpastes containing natural compounds.

MATERIALS AND METHODS

Selection and classification of toothpastes for analysis

A total of 11 toothpastes with natural compounds were acquired in drugstores and supermarkets in Ponta Grossa, Paraná, Brazil. Three dentifrices were excluded since they have ingredients with well-known antimicrobial activities that they were not the natural compounds. The remaining eight products composed the sample of this study. The study groups were divided based on the natural compound present in the toothpaste composition: Sorbitol (I), tocopherol (II), mint (III), cinnamon/mint (IV), propolis/melaleuca (V), mint/açai (VI), mint/guarana (VII), and propolis (VIII). The group IX was a toothpaste specially formulated without antimicrobial agents with 1% hydroxyethylcellulose (negative control),[15] and the group X was a dental gel with triclosan and formaldehyde in your pharmaceutical formulation (positive control) [Table 1 and Figure 1].

Microorganisms

The bacterial strains *S. mutans* (ATCC 25175), *P. aeruginosa* (ATCC 27853), and *E. faecalis* (ATCC 29212) were used for the antimicrobial analysis. The mediums were blood agar (*S. mutans*) and Mueller Hinton agar (*P. aeruginosa* and *E. faecalis*) described previably.[16,17]

Microbiological technical for tests

The disk diffusion method was used for the experiment. The samples of toothpaste and saline solution (1:3) were prepared. Inert paper discs of uniform size were embedded in this solution and places in the plates after inoculation. All plates were done in triplicate, and they were incubated at 35–37°C for 16–18 h. At the end of the incubation period, the inhibition halos were measured in millimeters (mm).[15,18]

Statistical analysis

The results were organized and analyzed statistically with the program SPSS® 11.0, SPSS Inc., Chicago, IL, USA. The multiple comparison tests between treatments versus control groups were evaluated by one-way analysis of variance (ANOVA), and the posttests Bonferroni and Tukey with a significance level at 5%. The graphics were performed using the software GraphPad Prism® 4.00, GraphPad Software Inc., San Diego, CA, USA.

RESULTS

All samples the toothpastes tested were classified in dental gels following pharmacotechnical principles [Figure 1]. ANOVA indicated that the bacteria responded differently to the dental gels ($P < 0.0001$). A *post-hoc* Tukey's test showed that *P. aeruginosa* had a different response compared to *S. mutans* ($P < 0.0001$) and to *E. faecalis* ($P < 0.0001$). The Bonferroni's *post-hoc* test showed variable responses to the dental gels ($P < 0.0001$) in *S. mutans*

Table 1: Toothpastes tested for antimicrobial potential

Number identification	Experimental group	Name	Ingredients as listed on packages with chemistry/IUPAC/name/ description	Manufacturer
I	Sorbitol	Colgate Gel®	Sorbitol, dental type silica, demineralized water, PEG-12, sodium lauryl sulfate, sodium carboxymethyl cellulose, tetrasodium pyrophosphate, sodium saccharin, sodium fluoride, FD 7 C Blue number 1 CI 42090 (dihydrogen (ethyl) [4-[4-[ethyl (3-sulphonatobenzyl)]amino]-2'-sulphonatobenzhydrylidene] cyclohexa-2,5-dien-1-ylidene] (3-sulphonatobenzyl) ammonium, disodium salt)	Colgate-Palmolive, Brazil
II	Tocopherol	Gel com Vitamin E	Sorbitol, hydrated silica, water, PEG-12, sodium lauryl sulfate, sodium carboxymethyl cellulose, tetrasodium pyrophosphate, sodium saccharin, sodium fluoride, Vitamin E, FD 7 C Blue number 1 CI 42090 (dihydrogen (ethyl)[4-[4-[ethyl (3-sulphonatobenzyl)] amino]-2'-sulphonatobenzhydrylidene] cyclohexa-2,5-dien-1-ylidene] (3-sulphonatobenzyl) ammonium, disodium salt)	Fleming Manipulações, Brazil
III	Mint	Sensodyne® Extra fresh	Sodium fluoride, potassium nitrate 5%, sorbitol, acqua hydrated silica, glycerin, titanium dioxide, cocamidopropyl betaine, flavor, xanthan gum, sodium hydroxide, sodium saccharin, *Mentha piperita* oil, sucralose, aroma	GlaxoSmithKline, Brazil
IV	Cinnamon/mint	Sorriso® Whitening explosion - gel canela/mint	Sorbitol, acqua, hydrated silica, PEG-12 (PEG 600), sodium lauryl sulfate, cocamidopropyl betaine, aroma, cellulose gum, tetrasodium pyrophosphate, sodium fluoride (1450 ppm), sodium saccharin, polyethylene, CI 77891 (titanium dioxide), CI 16035 (disodium 6-hydroxy-5-' (2-methoxy-4-sulphonato-m- tolyl) azo[naphthalene-2-sulphonate)	*Colgate*-Palmolive, Brazil
V	Propolis/ melaleuca	Natural propolis toothpaste with tea tree oil	Calcium carbonate, glycerin, acqua, propolis extract, *Melaleuca alternifolia* (tea tree) leaf oil, xanthan gum, maltodextrin, CI 75810 (trisodium (2S-trans)-[18-carboxy-20-(carboxymethyl)-13-ethyl-2,3-dihydro-3,7,12,17-tetramethyl-8-vinyl-21H,23H-porphine-2-propionato (5-)-N21, N22, N23, N24]cuprate (3-)/chlorophyllin-copper complex)	Comvita, New Zealand
VI	Mint/açaí	Sorriso® Fresh menta + açaí	Sorbitol, acqua, hydrated silica, PEG-12 (PEG 600), sodium lauryl sulfate, cocamidopropyl betaine, aroma, cellulose gum, tetrasodium pyrophosphate, sodium fluoride (1450 ppm), sodium saccharin, polyethylene, CI 77891 (titanium dioxide)	*Colgate*-Palmolive, Brazil
VII	Mint/guaraná	Sorriso® Fresh Menta + Guaraná	Sorbitol, acqua, hydrated silica, PEG-12 (PEG 600), sodium lauryl sulfate, cocamidopropyl betaine, aroma, cellulose gum, tetrasodium pyrophosphate, sodium fluoride (1450 ppm), sodium saccharin, polyethylene, CI 77891 (titanium dioxide)	*Colgate*-Palmolive, Brazil
VIII	Propolis	Sorriso® Fresh menta + própolis	Sorbitol, acqua, hydrated silica, PEG-12 (PEG 600), sodium lauryl sulfate, cocamidopropyl betaine, aroma, cellulose gum, tetrasodium pyrophosphate, sodium fluoride (1450 ppm), sodium saccharin, polyethylene, CI 77891 (titanium dioxide)	*Colgate*-Palmolive, Brazil
IX	Negative control	Negative gel control	1% hydroxyethylcellulose gel (Natrosol)	Fleming Manipulações, Brazil
X	Positive control	Close-up® Fresh whitening	Sodium fluoride (1500 ppm fluoride Ion), water, sorbitol, silica, perlite, PEG-32 (PEG 1540), sodium lauryl sulfate, flavor, cellulose gum, sodium saccharin, titanium dioxide, triclosan, formaldehyde, CI 74160 (29H, 31H-phthalocyaninato (2-)-N29, N30, N31, N32) copper)	Unilever, Brazil

PEG: Polyethylene glycol, IUPAC: International Union of Pure and Applied Chemistry

culture. The diameters of the inhibition halos against *S. mutans* were, in decreasing order of efficacy: Propolis/melaleuca > mint/guarana > mint/açai > sorbitol > tocopherol > cinnamon/mint > propolis > mint > negative control [Figure 2]. *E. faecalis* showed variable responses to the dental gels ($P < 0.0001$) in the following order of decreasing efficacy (diameter of inhibition halo): Mint/guarana > propolis > sorbitol > mint/açai > tocopherol > cinnamon/mint > mint = propolis/melaleuca = negative control [Figure 3]. The product with the highest antimicrobial activity was mint/guarana, which was significantly different than propolis/melaleuca ($P < 0.001$), mint ($P < 0.001$), cinnamon/mint ($P < 0.001$), tocopherol ($P < 0.001$), and negative control ($P < 0.001$). The statistical analysis indicated that propolis, sorbitol, and mint/açai did not show any differences compared to mint/

Figure 1: Classification of toothpastes for experimental analysis

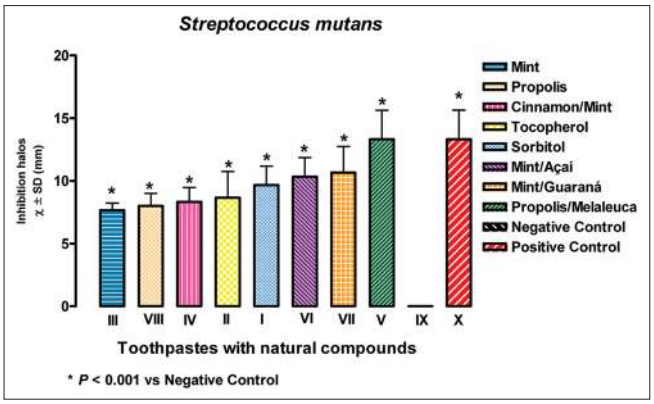

Figure 2: The means and standard deviations of inhibition halos (X ± standard deviation; millimeter) to toothpaste's antimicrobial activity tested in *Streptococcus mutans* culture

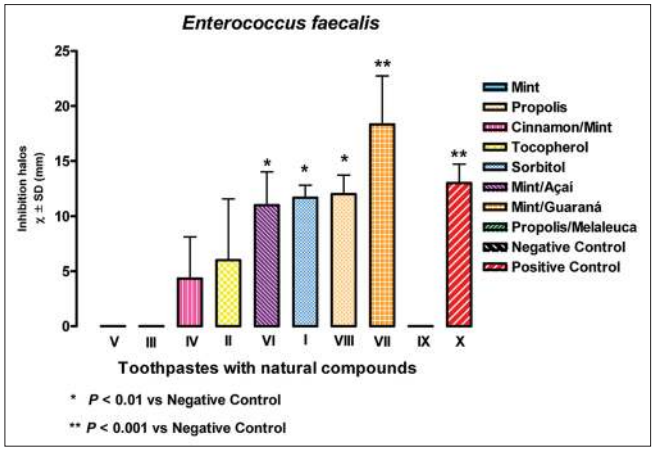

Figure 3: The means and standard deviations of inhibition halos (X ± standard deviation; millimeter) to toothpaste's antimicrobial activity tested in *Enterococcus faecalis* culture

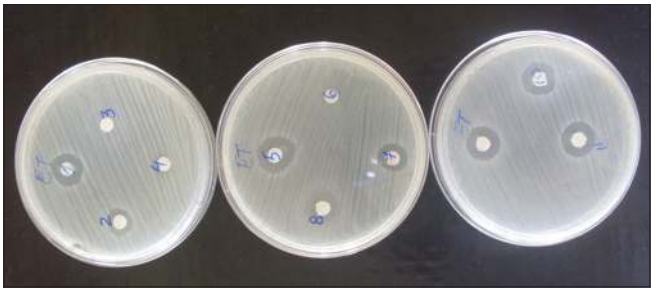

Figure 4: The inhibition halos to toothpastes tested to *Enterococcus faecalis* culture in Mueller–Hinton agar

guarana (P > 0.05) and positive control (P > 0.05) but showed statistically significant differences to mint and propolis/melaleuca (P < 0.01) [Figure 4]. *P. aeruginosa* was resistant to all dental gels tested including positive control [Figure 5].

DISCUSSION

The microorganisms tested in this study are intimately related to oral health. *S. mutans* is one of the most common bacteria found in a dental biofilm, *P. aeruginosa,* and *E. faecalis* are well-known for their roles in periodontal diseases and endodontic infections.[19] *E. faecalis* and *S. mutans* were also evaluated in Mistry's study[20] to compare the antimicrobial activity of common plants in India, and the results showed differences between the microorganisms tested.

In our study, it was observed that there is little information about the concentration of natural compounds on the labels of toothpastes. It is necessary that the qualitative and quantitative composition must be listed on the packages of products for dental hygiene, cosmetics, and perfumes. According to Ganavadiya *et al*.,[21] the most accepted method of oral health maintenance is brushing of teeth, and an adjunct safe toothpaste to help maintaining this condition. Normally, the manufacturer's advertising refers to cosmetic claims and not the safety and therapeutic application of the product, and the consumer trust the publisher who ensures that the product does not cause health damage. This problem could be solved if the manufacturers include in the product labels the qualitative and quantitative compositions of each compound, along with their function and instructions for use, should be included in an explanatory note that accompanies the product. All the products tested not had the information of the concentration of the natural product. Seven marks specified natural products such as flavors and one mark specified natural product such as antimicrobial.

The medicinal use of plants for the treatment, cure, and prevention of diseases is one of the oldest forms of medicinal practice.[22] In a review of the therapeutic use of popular plants for oral diseases, Vieira *et al*.[19]

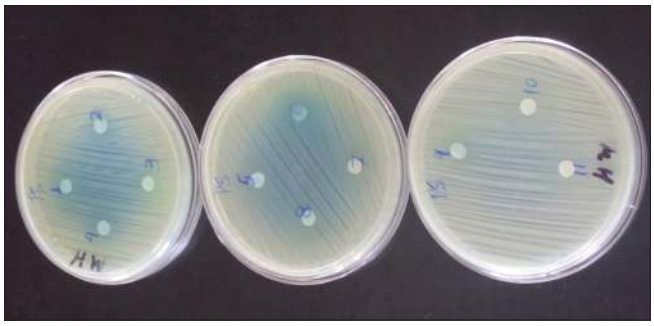

Figure 5: The inhibition halos to toothpastes tested to *Pseudomonas aeruginosa* culture in Mueller–Hinton agar

identified several studies that evaluated the *in vitro* antimicrobial activities of plants.

In the present study, different dental gels showed different responses to the tested microorganisms. Mint is often cited as an artificial flavoring component; however, this study showed that mint led to a mean inhibition halo of 7.66 mm against *S. mutans*. Nogueira *et al*.[23] observed similar results, with mean inhibition halos of 7 mm and 9 mm against *S. mutans* using *Mentha piperita* and *Mentha pulegium* extracts, respectively. The results show that mint has antibacterial activity in the dental gels tested; however, based on this and other studies in the literature, further studies should be conducted on the applicability of *Mentha sp*. extract as coadjuvant in oral health in the future.

Our results indicated that *P. aeruginosa* was resistant to all gels. However, another study found this species to be sensitive to toothpastes with natural compounds such as *M. piperita*, myrrh, ratanhia, salvia, and chamomile.[24] A possible explanation for this could be some difference in the product concentration.

Another natural compound that deserves attention is propolis. Almeida *et al*.[25] demonstrated that the chemical composition of propolis, especially total flavonoids, is dependent on a variety of factors. A characteristic of propolis is that its concentration of the natural compounds varies depending on the vegetation throughout the region where it originates. Nogueira *et al*.[23] observed that the samples of propolis extract from three different states in Brazil showed different antibacterial activities against *S. mutans*. The sample from the state of Parana did not show any antibacterial activity; however, those from the states of São Paulo and Minas Gerais had antimicrobial activities though with different minimum inhibitory concentrations (MIC). This variation was confirmed by Siqueira *et al*.[13] too. These researchers evaluated different concentrations of propolis from the Northeast Brazil and reported that propolis showed important antimicrobial activity on *E. faecalis* with an inhibition halo of 16 mm by a 7.5% solution of red propolis (150 mg). The origin red propolis can be from Cuba, Venezuela, and Brazil. Furthermore, Ercan *et al*.[26] found a decrease in the plaque index and gingival inflammation in patients who used oral products with propolis. We observed in our data that the dentifrice VIII (propolis) exhibited antimicrobial activity against *S. mutans* and *E. faecalis* similar the positive controls.

The performances of products VIII (propolis) and V (propolis/melaleuca) were not similar, even though both gels contain propolis. *E. faecalis* exhibited resistance to product V (propolis/melaleuca) and sensitivity to product VIII (propolis). A conflicting result can be justified that the propolis in these two products did not have the same origin and concentration.

It was estimated that there would be sensibility to *E. faecalis* to both toothpastes regardless of the presence of *M. alternifolia*. Thosar *et al*.[27] found that the *E. faecalis* is sensitive to essential oil the *M. alternifolia* in contrast to the present study. *S. mutans*, although sensitive, also showed significantly different responses toward products V and VIII. *S. mutans* was highly sensitive to product V (propolis/melaleuca = 13.3 mm), and the less responsive to the product VIII (propolis = 8.0 mm) compared to the other gels. These results demonstrated the proven antimicrobic activity of *M. alternifolia*,[28] corroborated by Nogueira *et al*.,[12] who compared antiseptic solutions and reported that *M. alternifolia* product had the strongest antimicrobial activity against *S. mutans* and other microorganisms. These researchers, however, observed that the residual effect was inferior to those of other solutions tested.

It should be noted that the manufacturer of gel V (propolis/melaleuca) indicated the presence of *M. alternifolia*, while the manufacturer of gel VII (mint/guarana) indicated the presence of *P. cupana* (guarana). In the present study, *P. cupana* was the most effective agent against *E. faecalis* and the second most effective for *S. mutans*. This antimicrobial activity against *S. mutans* had been previously reported by Yamaguti-Sasaki *et al*.[11]

Freire *et al*.[29] observed that *S. mutans* is sensitive to *C. cassia* (cinnamon) with low MIC values. The product IV (mint/cinnamon) exhibited antimicrobial activity against *S. mutans* and *E. faecalis* but not against

P. aeruginosa, which was resistant to all toothpastes tested. These researchers observed that cinnamon and citronella showed strong antimicrobial activity against *S. mutans* compared to the control.

Products I (sorbitol) and II (tocopherol) also showed antimicrobial activity against *S. mutans* and *E. faecalis*. The mixed tocopherols are found in several plants and has antioxidant activity.[30] Therefore, it is impossible to relate the antimicrobial activity only to the presence of tocopherol, and it would be necessary to test alpha, beta, gamma, and delta-tocopherol individually.[31] Especially, because Green *et al*.[32] reported a significant delivery of Vitamin E acetate to the surface of the gingival tissues from the test toothpaste (mean level = 50.7 ppm) compared to a control dentifrice (=1.4 ppm) and Scott *et al*.[33] showed that buccal and gingival epitheliums are able to metabolize Vitamin E acetate, to the free form, Vitamin E.

Sorbitol ferments considerably more slowly than saccharose and glucose, and its environmental pH is often higher than the environmental pH values of the other two sugars, which prevents demineralization and the consequent formation of dental caries.[34] Besides, different studies have demonstrated a consistent decrease in dental caries, among subjects using sorbitol or xylitol in toothpastes by decreased lactic acid production, enhanced clearance of sugars from the mouth and reduction the levels of *S. mutans*,[35] what it is coherent with the microbiology data obtained. Petersson *et al*.[36] evaluated four different toothpastes in a 3-year clinical and microbiology study, and these researchers found no significant differences between the experimental groups to caries lesions and number of mutans streptococci and lactobacilli in saliva. However, the patients who used toothpaste with the xylitol-sorbitol mixture showed a lower caries increment as compared with children who used the toothpaste with sorbitol alone.

CONCLUSIONS

It is suggested that, according to this study and the literature, the toothpastes with natural compounds have therapeutic potential and need more detailed searches for the correct clinic therapeutic application. The results from this study revealed differences in the antimicrobial activities of commercial toothpastes with natural compounds. Further studies on the pharmacological activities such as antifungal, antiviral, and anti-inflammatory effects of the natural compounds are necessary to improve the understanding of the applicability of such products in toothpastes. Furthermore, the clinical, toxicological, and safety tests must be conducted, monitoring the side and idiosyncratic effects, aimed at increasing the implementation of new pharmaceutical formulations using natural products.

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Conflicts of interest
There are no conflicts of interest.

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