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Original Article

Antierosive profile of an experimental solution based on antioxidants from *Passiflora edulis* on initial dentin erosion lesions

Perfil antierosivo de uma solução experimental baseada em antioxidantes da *Passiflora edulis* em lesões iniciais de erosão dentinária

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Resumo

Introdução: A degradação não-cariosa da matriz dentinária é um processo natural ao longo da vida; no entanto, quando associada à presença de substâncias ácidas excessivas, leva a lesões nas estruturas dentárias. **Objetivo:** Este estudo desenvolveu uma solução experimental baseada em polifenóis antioxidantes da semente do maracujá amarelo (*Passiflora edulis*) e avaliou seu potencial preventivo sobre a dentina erodida. A solução polifenólica experimental foi obtida a partir de sementes de maracujá através de secagem e prensagem a frio. **Material e método:** Trinta amostras de dentina radicular cervical bovina foram adquiridas e randomizadas em três grupos (n=10): G1 – água destilada; G2 – solução experimental de antioxidantes fenólicos do maracujá; G3 – pasta de dente comercial anti-erosão. Ciclos erosivos e tratamento foram conduzidos ao longo de 3 dias. Posteriormente, microscopia confocal 3D sem contato foi empregada para medir a rugosidade volumétrica (Sa) e linear (Ra), bem como o desgaste erosivo. Um teste ANOVA/Tukey de uma via foi realizado (α=0,05). **Resultado:** O grupo G2 apresentou valores de Ra e Sa mais baixos em comparação com os outros grupos e demonstrou o menor desgaste erosivo em µm em comparação com G1 e G3. **Conclusão:** A solução experimental baseada em polifenóis antioxidantes da *Passiflora edulis* mostrou desempenho promissor sobre a dentina erodida nesta investigação. No entanto, mais pesquisas são necessárias para estabelecer sua eficácia e potencial uso no desenvolvimento de um novo produto.

Descritores: Erosão dentária; dentina; antioxidante; polifenóis.

Abstract

Introduction: The non-carious degradation of the dentin matrix is a natural process throughout life; however, when associated with the presence of excessive acidic substances, it leads to lesions in dental structures. **Objective:** This study developed an experimental solution based on antioxidant polyphenols from the yellow passion fruit seed (*Passiflora edulis*) and assessed its preventive potential on eroded dentin. The experimental polyphenolic solution was obtained from passion fruit seeds through drying and cold pressing. **Material and method:** Thirty samples of bovine cervical root dentin were acquired and randomized into three groups (n=10): G1 – distilled water; G2 – experimental solution of phenolic antioxidants from passion fruit; G3 – Commercial anti-erosion toothpaste. Erosive cycling and treatment were conducted over 3 days. Subsequently, non-contact 3D confocal microscopy was employed to measure volumetric (Sa) and linear (Ra) roughness, as well as erosive wear. A one-way ANOVA/Tukey test was performed (α=0.05). **Result:** The G2 group had lower Ra and Sa values compared to the other groups and demonstrated the lowest erosive wear in µm compared to G1 and G3. **Conclusion:** The experimental

solution based on antioxidant polyphenols from *Passiflora edulis* showed promising performance on eroded dentin in this investigation. Nevertheless, further research is required to establish its effectiveness and potential use in developing a new product.

Descriptors: Dental erosion; dentin; antioxidant; polyphenols.

INTRODUCTION

The non-carious degradation of dentin matrix is a natural process throughout life; however, when associated with the presence of excessive acidic substances, leading to lesions in dental structures, sensitivity, aesthetic and/or functional problems, it can be characterized as dental erosion[1]. The chemical dissolution of mineralized dental tissue is related to different etiologies, which can be generally classified into three categories: intrinsic erosion - when acid contact comes from within the body, extrinsic erosion - when there is communication between teeth and acidic solutions from the external environment, often due to the consumption of natural and processed foods, and idiopathic erosion - when the cause of the erosion is unknown[2].

According to Oliveira et al.[3], hydrochloric acid (HCl), naturally present in the stomach, it can reach the oral cavity through gastroesophageal reflux, which is a pathological condition with a multifactorial etiology. Extrinsic erosion can also occur due to the action of citric acid ($C_6H_8O_7$), found in citrus fruits with properties such as antioxidants, acidulants, preservatives, among others, as mentioned by Magalhães et al.[4]. This acid is widely used in the food and pharmaceutical industries due to its significant scientific and economic relevance. However, excessive exposure to acids in the oral cavity becomes harmful due to their intense demineralizing erosive action on enamel and dentin. This tissue dissolution increases dental sensitivity and makes teeth more susceptible to dental various lesions[5,6].

Saliva acts as a physiological ally in maintaining oral homeostasis and cleanliness, helping to maintain the pH of the oral cavity and eliminating excess acids. However, the disease's progression needs to be analyzed, as the frequency and specificity of the acid can alter the homeostatic action of saliva, reducing its protective and preventive effect[7].

According to Alencar et al.[8], many materials are being investigated for their efficacy on eroded dental tissues. These include pastes, varnishes, and gels containing fluoride (NaF), which have the ability to slow down acidic dental degradation. However, these materials, whether used in combination or separately, may yield inconclusive effects and require further evidence to definitively prove their effectiveness. Therefore, there is currently no “gold standard” treatment for controlling/treating erosive wear of dental tissues[9]. Erosive wear in dentin tends to be much faster and more intense when compared to enamel[9]. For this reason, initial dentin erosion lesions deserve professional attention, as these changes are often subclinical and imperceptible to the dentist.

In parallel, many studies consider the Amazon as a national and international reference for biodiversity, with numerous natural resources that can be used to obtain new biomaterials. With the diversity of available natural assets, research and studies have made considerable advances in testing and analyzing the potential and efficiency of these biomaterials. Among these materials, antioxidants are noteworthy. They are substances found in plants, fruits, and vegetables, and are widely employed by the pharmaceutical industry for the manufacture of cosmetics and phytotherapeutics[10,11].

Antioxidants primarily act to protect cells against oxidative stress caused by free radicals. These molecules are produced physiologically in our body or due to external factors such as smoking, alcohol consumption, and excessive sun exposure. In addition to antioxidants, there are polyphenols, which are chemical compounds that offer several benefits to our body, including cell protection and repair, anti-inflammatory, antibacterial and antiviral properties[12].

In dentistry, experiments using polyphenols have been conducted using grape seed (*Vitis vinifera L.*) and Açaí berry (*Euterpe oleracea*) as antioxidant sources, showing favorable results for dental tissues[13,14]. In this research, we opted to use passion fruit (*Passiflora edulis*), which is considered a functional food due to its versatile usage. We focused on utilizing the seeds of this fruit to create a material rich in polyphenols with strong antioxidant properties.

Our investigation can be considered pioneering, as, to the best of our knowledge, there are no studies in the literature that have explored the use of passion fruit seeds for treating eroded dentin up to this point. The tested null hypothesis was: H0 – There is no difference in the inorganic structure of eroded dentin among the different tested groups.

MATERIALS AND METHODS

For the study, sixty-three healthy bovine incisors were cleaned using periodontal curettes (Duflex, SS White) and then subjected to prophylaxis with pumice stone (SS White) and water, using a Robinson brush (KG Sorensen) connected to a low-speed hand piece. They were then washed with deionized water and stored in a 1% Thymol solution (Sigma-Aldrich) during 7 days before use.

The incisors were sectioned with a TF-13C diamond bur using a high-speed hand piece, transversely, 1mm below the cement-enamel junction to separate the crown and root. Fragments of root dentin with dimensions of 4 mm in width, 4mm in length, and 2mm in height were sectioned from the vestibular faces of the cervical region using the TF-13C diamond bur under cooling. The specimens were manually polished with #600 and #1200 silicon carbide sandpaper (3M ESPE) in circular motions under cooling with abundant water to remove residues and flatten the vestibular area of the samples. They were then manually counted in the lingual region with a 2200F bur, followed by a 3-minute wash with distilled water.

Thirty bovine cervical dentin samples (4 × 4 × 2 cm) were dried with paper towels (Kleenex) and had their sides and bottoms covered/protected with red waterproof varnish (Risqué) to prevent excessive acid penetration into dentinal tubules. Next, the samples were pre-eroded in a 1% citric acid solution at pH 2.0 for 10 minutes at room temperature, adapted from the study by Monteiro et al.[15]. After that, the samples were rinsed with distilled water for 10 seconds, dried, and their vestibular window was protected with varnish, leaving only one side exposed for the initiation of erosive cycling. They were stored in 100% humidity until the treatment moment.

Fruits supplied by a producer affiliated with the Cooperative Agricole Mixed of Tomé-Açu (CAMTA), located in the municipality of Tomé-Açu, Pará, Brazil, were used. The fruits were harvested ripe. The samples were obtained in March 2022, and were processed immediately after collection.

The seeds were washed in running water for 5 minutes and sanitized in sodium hypochlorite (1% v/v) for 15 minutes. Then, the seeds were dried at 40 °C for 12 hours in an air circulation oven[16], cooled to room temperature in a desiccator and stored in vacuum polyethylene bags at -20 °C for later oil extraction[17].

Oil extraction was carried out using the following methods: cold pressing, ultrasound, Soxhlet and accelerated solvent extraction (ASE). Prior to extractions, with the exception of pressing, the seeds were crushed in a Basic Analytical Mill (IKA®). Cold pressing was carried out in a manual press, processing 600 g of seeds. Following extraction, the sample was centrifuged (Hettich) for 15 minutes at 4000 rpm, to separate the solid resinous materials. Ultrasound extraction followed a methodology adapted from Oliveira et al.[3].

Soxhlet extraction was carried out with n-hexane, according to Ramadan et al.[18], with changes. In short, approximately 28g of the dried sample was added to the extraction cartridges. Soxhlet extraction was carried out with n-hexane, according to Ramadan et al.[18], with changes. In short, approximately 28g of the dried sample was added to the extraction cartridges. After that, the flask with the extracted material was evaporated in a rotary evaporator (IKA®), at 40 °C, under

reduced pressure. The oil obtained was kept in a desiccator at room temperature (± 20 °C) until complete evaporation of solvent residues.

The extraction was based in Castejón et al.[19], with modifications. Briefly, it was carried out in an ASE 350 DIONEX extractor (Sunnyvale), with the addition of 30g of the sample to stainless steel cells (100mL), subsequently filled with hexane. The oil obtained was kept in an amber screw-top bottle at -20 °C until analysis. Extractions were performed in triplicate. The oil yield obtained by each method was calculated by the ratio between the mass of extract obtained and the mass of dry seeds used in the process, expressed as a percentage.

The total antioxidant potential of the oil samples was determined using the iron reducing power (FRAP), according to the method presented by Granato, Nunes[20]. 90 µL of the diluted sample was added to 270 µL of distilled water and 2.7 mL of FRAP reagent, protected from light. After homogenization, the samples were incubated in a water bath at 37°C for 30 minutes, followed by analysis at 595 nm. The aqueous solution of DMSO/TWEEN 80 (1%) was used as the negative control (blank). A standard curve was plotted by mixing 90 µL of ferrous sulfate solution (0-2000 µM), 270 µL of distilled water, and 2.7 mL of FRAP reagent. The results were expressed in µmol of ferrous sulfate per gram of oil (µmol $FeSO^4.g^{-1}$).

After the initial erosion, the specimens were randomized into three groups according to the anti-erosive treatment (n=10): G1 – distilled water; G2 – an experimental solution of antioxidant phenolics derived from passion fruit; G3 – commercial anti-erosive toothpaste (Elmex Protect) in the form of a 1:1 slurry with distilled water.

Each group received the material, which was applied using a micropipette across the entire sample surface, and after 10 minutes, the excess was carefully removed with soft paper tissues. Subsequently, to form the acquired salivary pellicle, all specimens were immersed in artificial saliva (0.96 g/1000 mL - KCl; NaCl; $MgCl_2$; K_2HPO_4; $CaCl_2$; Carboxymethyl cellulose; Sorbitol 70%; Nipagin; Nipazole; and deionized water) for 1 hour.

Erosive cycling was conducted over a 3-day period using 24 cell culture plates. Initially, the specimens that had already undergone treatment were removed from the artificial saliva, and any acquired film was cleaned with soft paper. Subsequently, the samples were subjected to erosive cycling, involving immersion in citric acid (1%; pH 2; 5 minutes), followed by a 1-hour immersion in artificial saliva. Each day of cycling included two acid challenges interspersed with periods of immersion in saliva. At the end of each day, the specimens were stored in an incubator at 37 °C. The erosive solution was replaced with each exposure, and deionized water and fresh saliva were replenished at the end of the cycling day. Upon completing the cycling, a non-contact 3D confocal microscopy analysis (MC3D) was performed.

After exposure to erosive challenges and specific treatments, the waterproof varnish was removed from the dentin surface using nail polish remover and a cotton swab. Subsequently, the specimens were analyzed with a 3D laser confocal microscope (LEXT OLS4000) at a magnification of 216×. The captured image was taken from the central area, displaying both the healthy reference surface and the treated surface, which had been subjected to various treatments and erosion. Volume loss was quantified using the OLS4000 software (Olympus), measuring in μm^3/area. Additionally, high-resolution images were generated at a working distance of approximately 15 mm with a magnification of 2000×. These resulting micrographs underwent qualitative analysis. The characteristics of the dentin surface before and after each treatment were meticulously recorded, with a focus on the presence of smear layer and the formation or removal of precipitates on the dentin surface.

SPSS version 13.0 (SPSS) was used for statistical analysis. The normal distribution assessment of the data was performed using the Shapiro-Wilk test. The data from the non-contact 3D confocal microscopy showed a parametric distribution. Subsequently, a one-way ANOVA test was conducted, followed by the Tukey test, which was used to analyze the data for Ra, Sa, and TSL ($\alpha = 0.05$). A qualitative analysis was carried out for the SEM results.

RESULTS

In the present research, the yields and recovery percentages showed the following results using N-hexane solvent: Parameters: 60 °C/ 8h - Yield (%): 29.33 ± 0.04. The result of the antioxidant activity (%) of the studied oil was 26.44 ± 0.08 FRAP (µmol $FeSO^4.g^{-1}$)

The outcomes of the non-contact 3D confocal microscopy examination are described in Table 1. The Ra and Sa values were significantly higher in Group G1 when compared to the remaining groups ($p < 0.05$). In contrast, Groups G2 and G3 showed the lowest Ra and Sa values in comparison to the other groups ($p < 0.05$). There was no statistically significant difference between Groups G1 and G3 ($p = 0.088$).

Table 1. Mean (M) and standard deviation (SD) values of dentin specimens obtained by 3D confocal microscopy (Ra, Sa, and TSL) from the studied groups

Dentin Surface						
Groups	Ra		Sa		TSL (µm³)	
	M	*(±SD)*	*M*	*(±SD)*	*M*	*(SD)*
G1	5.92[b]	±2.02	7.53[b]	±3.41	-431.26[b]	(±14.81)
G2	0.98[a]	±0.83	0.94[a]	±0.90	-51.46[a]	(±5.31)
G3	4.59[b]	±2.77	6.49[b]	±0.11	-149.28[c]	(±10.80)

Note: Distinct letters represent statistically significant differences. G1 - Distilled water; G2 - Experimental solution of phenolic antioxidants derived from passion fruit; G3 - Commercial anti-erosive toothpaste (Elmex protect, Colgate) in a 1:1 slurry with distilled water.

Regarding volume loss (TSL) when comparing the control (protected) and challenged/treated area, Group G1 showed significantly higher loss when compared to the other groups ($p < 0.05$). On the other hand, the lowest volume loss was detected in Group G2 when compared to the others ($p < 0.05$).

The topographical pattern of the dentin surface was compared between the groups (intergroup comparison), assessing the presence of partially or completely closed dentin tubules after the treatment. Deposits of material were predominantly observed on the dentin tubules in groups G2 and G3. In contrast, Group G1 displayed a notable obliteration of the dentin tubules.

DISCUSSION

The extraction of passion fruit seed oil yielded a 29.33% output based on the dry mass of the seeds. This satisfactory result may possibly be justified by the pre-drying treatment of the seeds after their separation from the fruit. Charles, Simon[21] achieved a higher yield in the extraction of essential basil oil from previously dried samples, which motivated the use of this method in our experiment. In addition, the antioxidant activity (%) of the studied oil was 26.44 ± 0.08 FRAP (µmol $FeSO^4.g^{-1}$). This solution of concentrated polyphenolic compounds had not been previously investigated on eroded dental tissues; therefore, its concentration may be refined in future studies.

The erosive process in dental tissues is a growing social concern, with a higher incidence in children and young adults. There is still no definitive proof in the literature for this prevalence, but some studies suggest that one of the main factors is related to the consumption of processed foods containing citric acid. These issues have caught the attention of the scientific and clinical community due to the difficulty in early erosion identification, and the lack of a treatment or protective product that neutralizes and prevents the erosive functional effects of acidic action[22,23]. Based on these events, the present research aimed to develop and test an experimental material based on a polyphenolic antioxidant compound extracted from passion fruit seeds, with the intention of protecting and stabilizing the erosive process on dentin. It showed promising results in terms of anti-erosive potential when compared to a commercial toothpaste. Therefore, the null hypothesis H0 was rejected.

While citric acid holds significant importance in the food industry, its interaction within the oral environment can prove detrimental to dental structures. This is primarily due to the production of citrate, which binds to the Ca^{+2} ions, leading to a chelating effect that deactivates essential metallic ions such as PO_4^{-3} (phosphate) and Ca^{+2}. These ions are vital for the structural mineralization of dentin and enamel. Moreover, citric acid, upon dissociation in water, releases H^+ ions (hydrogen) with a strong corrosive potential. These H^+ ions affect C^+ (carbon) and PO_4^{-3} ions, resulting in surface softening and demineralization. Consequently, this process leads to a reduction in dental volume[5].

Given dentin's limited ability to repair itself when exposed to acid attacks, this study has developed a pure solution based on antioxidant polyphenols from passion fruit seeds. The complete use of the fruit not only adds value to the product but also reduces environmental impact by minimizing waste disposal and optimizes the efficient use of resources in fruit production. All these factors enhance the culture of passion fruit and supports rural producers, reinforcing the contribution of the fruit industry to regional development[24]. Passion fruit contains various polyphenols, such as piceatannol and beta-carotenes, which belong to another class of antioxidants responsible for vitamins A and C. These vitamins are essential for collagen fiber synthesis[25]. When subjected to acid erosion, the molecules in the teeth become unstable and reactive, leading to oxidative stress, and causing a chemical imbalance, resulting in the formation of free radicals. The experimental material has shown remarkable promise due to its high antioxidant content, effectively safeguarding healthy molecules and inhibiting erosive damage caused by oxidative processes[26].

The erosive challenge in this study simulated the dietary habits of a patient who frequently consumes foods rich in citric acid. We detailed the individual's routine, analyzing the erosive process in dentin as an extrinsic factor resulting from the consumption of processed foods. In literature, there are other studies that aimed to simulate the acidic action on dental structures, such as those by Alencar et al.[9], Medeiros[5] and Torigoe[27], which served as the basis for designing this *in-vitro* study.

In this study, the results obtained from non-contact 3D confocal microscopy reveal elevated Ra and Sa values in Group G1. This can be attributed to the absence of any treatment, allowing citric acid to act freely on dentin. Citric acid has a degrading effect on hydroxyapatite crystals, making dentin more brittle, uneven, and exposing collagen[27]. Furthermore, the group subjected to the experimental treatment for the erosive challenge displayed lower Ra and Sa values, with G2 exhibiting the lowest values. This underscores the effectiveness of the experimental polyphenolic compound in stabilizing the volumetric integrity of eroded dentin. Thus, there is an indication of a potential ability to preserve collagen fibers due to the antioxidant effects of the polyphenolic compound. Additionally, we observed limited efficacy of the tested commercial product when compared to the experimental solution.

Based on the 3D confocal microscopy analyses, TSL measures the loss of dental structure between the protected (varnished) and unprotected areas. The data from this study reveal that Group G1 experienced a pronounced structural loss when compared to Groups G2 and G3, with G2 yielding the best results and displaying the least structural damage. This suggests that the high levels of antioxidative reaction from polyphenolic compounds indicate their potential to stabilize and safeguard against erosive processes. Furthermore, there is a likelihood of dentin being encouraged to utilize its mineral structural composition as a protective barrier against acidic effects. Future in vitro research should delve deeper into this aspect to gain a better understanding of the chemical and biological mechanisms at play in this crucial matter.

As a result of this, the topographical images assess dentinal tubules in areas subjected to erosive challenge and treatment. When comparing the tubule obliteration between the groups, it was observed that Group G1 showed a pronounced exposure of the tubules. In contrast, G2 and G3 exhibited material deposition, with G2 displaying a higher level of dentin sealing. Based on these

results, the probable interaction of the antioxidant polyphenolic compound with saliva in the formation of a functionalized acquired pellicle contributed to the intense obliteration seen in Group G2. Saliva, with its neutral pH, composition, and buffering capacity, plays a role in protecting dentin structures[28,29], in addition to forming the acquired pellicle that acts as a diffusion barrier, limiting ion exchange with the dental structure[30]. However, further studies are needed for a detailed analysis of the interaction of antioxidant polyphenols with the salivary pellicle.

The test conducted using Energy Dispersive X-ray Spectroscopy (EDS) revealed characteristics of the inorganic structure based on the loss of F^- and Ca^{+2} ions from the dentin surface, which were similar between groups G2 and G3. In contrast, the results of the 3D Confocal Microscopy tests indicated that Group G2 experienced less dentin volume loss compared to Group G3. This disparity in the EDS test can be attributed to the composition of the commercial toothpaste (Elmex protect) used as a treatment for Group G3. This toothpaste contains a high concentration of $Ca(OH)_2$ (calcium hydroxide), KOH (potassium hydroxide), NaF (sodium fluoride), $SnCl_2$ (tin chloride), depositing these minerals (F- and Ca^{+2} ions) on the surface.

Conversely, Group G2, which utilizes the polyphenol-based extract, is a natural compound without alterations or association with inorganic ions. This experimental product showed superior results compared to the commercial toothpaste, promoting homeostasis, structural protection, and stabilization of collagen fibers. This underscores the significant importance of continuing research on this extract.

Given the above, it's crucial to highlight that, despite the impressive performance of the solution, further research is essential. This includes conducting new tests to specify and detail the active components that formed the basis of this study. This is particularly important due to the limited knowledge about which polyphenols are present in the material and which ones would provide the best results for eroded dentin.

Moreover, even though the *in vitro* simulation results are highly promising, there is a pressing need for *in vivo* research and clinical validation to confirm the success achieved in the laboratory. This would enable the solidification of the significance of this pioneering discovery derived from an extract based on passion fruit seeds.

Based on the gathered data, it is evident that the experimental solution containing antioxidant polyphenols from *Passiflora edulis* exhibited a highly promising performance in addressing eroded dentin within this investigation. However, it is crucial to carry out further *in vitro* and *in vivo* evaluations to comprehensively examine the impact of this solution. Simultaneously, there is a need to develop marketable products for erosion prevention, control, and treatment, using natural raw materials sourced from the Amazon region.

AUTHOR CONTRIBUTIONS

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CONFLICTS OF INTERESTS

The authors declare no conflicts of interest. The authors do not have any financial interest in the companies whose materials are included in this article.

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