

Teeth staining by green propolis extract: in vitro assay

Manchamento de dentes por extrato de própolis verde: ensaio in vitro

Tinción de dientes por extracto de propóleo verde: ensayo in vitro

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Abstract

In dentistry, propolis has aroused interest due to its satisfactory results obtained in several specialties. The aim of this study was to verify whether the green propolis extract applied at concentrations of 17% and 21% on the exposed dentin of healthy bovine teeth were capable of promoting a staining effect on the respective tooth structure. This is an experimental study where 40 crowns of healthy bovine teeth were cut in half, in an axial cut, in the mesiodistal direction, in order to expose the dentin. The specimens were enumerated and randomly divided into four groups (G1 to G4) for acid etching to simulate hypersensitive dentin, and subsequently application of green propolis extract solutions, used as possible obliterating agents in the treatment of dentin hypersensitivity, being: Group 1 (G1): 37% phosphoric acid and 17% propolis extract without brushing, Group 2 (G2): 37% phosphoric acid and 21% propolis extract without brushing, Group 3 (G3): 37% phosphoric acid and 17% propolis extract with brushing and Group 4 (G4): 37% phosphoric acid and 21% propolis extract with brushing. In the week following the application of the propolis extracts, the teeth were submitted to a prophylaxis procedure and it was observed that in all of them the solution remained fixed, conferring a staining effect both on the dentin and on the enamel of the teeth. same. It can be concluded that the green propolis extracts provided a staining effect on the studied bovine teeth.

Keywords: Propolis; Tooth discoloration; Dentin; Dentin permeability; Dental prophylaxis.

Resumo

Na odontologia, a própolis tem despertado interesse devido aos seus resultados satisfatórios obtidos em diversas especialidades. O objetivo deste estudo foi verificar se o extrato de própolis verde aplicado em concentrações de 17% e 21% na dentina exposta de dentes bovinos hígidos foi capaz de promover um efeito de manchamento na respectiva estrutura dentária. Trata-se de um estudo experimental no qual foram utilizadas 40 coroas de dentes bovinos hígidos seccionados ao meio, em corte axial, no sentido méso distal, a fim de expor a dentina. Os espécimes foram enumerados e divididos aleatoriamente em quatro Grupos (G1 a G4) para a realização do condicionamento ácido a fim de simular a dentina hipersensível e posteriormente receberam aplicação das soluções de extrato de própolis verde, utilizado como possível agente obliterador no tratamento da hipersensibilidade dentinária, sendo: Grupo 1 (G1): ácido fosfórico a 37% e extrato de própolis a 17% sem escovação, Grupo 2 (G2): ácido fosfórico a 37% e extrato de própolis a 21% sem escovação, Grupo 3 (G3): ácido fosfórico a 37% e extrato de própolis a 17% com escovação e Grupo 4 (G4): ácido fosfórico a 37% e extrato de própolis a 21% com escovação. Na semana seguinte à aplicação dos extratos de própolis, os dentes foram submetidos a um procedimento de profilaxia e observou-se que em todos eles a solução permaneceu fixada, conferindo um efeito de manchamento tanto na dentina como no esmalte. Pode-se concluir que o extrato de própolis verde proporcionou um efeito de manchamento nos dentes bovinos estudados.

Palavras-chave: Própolis; Descoloração de dente; Dentina; Permeabilidade da dentina; Profilaxia dentária.

Resumen

En odontología, el propóleo ha despertado interés por los resultados satisfactorios obtenidos en varias especialidades. El objetivo de este estudio fue verificar si el extracto de propóleo verde aplicado en concentraciones de 17% y 21% sobre la dentina expuesta de dientes bovinos sanos era capaz de promover un efecto de tinción en la estructura dental respectiva. Este es un estudio experimental en el que 40 coronas de dientes bovinos sanos fueron cortadas por la mitad, en un corte axial, en dirección mesiodistal, con el fin de exponer la dentina. Los especímenes fueron enumerados y divididos aleatoriamente en cuatro grupos (G1 a G4) para grabado ácido para simular dentina hipersensible y posteriormente recibieron aplicación de soluciones de extracto de propóleo verde, utilizado como posible agente obliterante en el tratamiento de la hipersensibilidad dentinaria, siendo: Grupo 1 (G1): 37% ácido fosfórico y 17% extracto de propóleo sin cepillar, Grupo 2 (G2): 37% ácido fosfórico y 21% extracto de propóleo sin cepillar, Grupo 3 (G3): 37% ácido fosfórico y 17% extracto de propóleo con cepillado y Grupo 4 (G4): 37% ácido fosfórico y 21% extracto de propóleo con cepillado. En la semana siguiente a la aplicación de los extractos de propóleos, los dientes fueron sometidos a un procedimiento de profilaxis y se observó que la solución permanecía fijada en todos ellos, confirmando un efecto colorante tanto en dentina como en esmalte. Se puede concluir que el extracto de propóleo verde proporcionó un efecto de tinción en los dientes bovinos estudiados.

Palabras clave: Própolis; Decoloración de dientes; Dentina; Permeabilidad de la dentina; Profilaxis dental.

1. Introduction

Since ancient times, man has had the habit of using natural products for the treatment and prevention of various diseases (Simões et al., 2008). For centuries, propolis has been used in popular medicine and in recent years has been the subject of scientific research (Grégio et al., 2005; Ahuja et al., 2011).

Propolis is an ophoterapeutic, non-toxic and resinous substance. Bees collect it from the mixture of sap, leaves, plant buds, resinous exudates associated with the salivary secretion of these insects (Simões et al., 2008).

Among its constituents are plant resins, beeswax, essential oils, pollen and saliva mixture, as well as other elements such as amino acids, minerals, vitamins and phenolic compounds (phenolic acids and flavonoids) (Almas et al., 2001; Al-Qathami et al., 2003). It has more than 300 components in its chemical composition, being considered one of the most heterogeneous natural mixtures (Pereira et al., 2002; Marcucci et al., 2001, Surek et al., 2021).

Flavonoids are pharmacologically active substances responsible for most of the biological activity of propolis, with effects on bacteria, fungi and viruses (Ahuja et al., 2011; Campos et al., 2021). Its properties are related to its chemical composition that varies according to the bee species, vegetation in the region and season of the year (Pereira et al., 2002; Marcucci et al., 2001, Surek et al., 2021).

Propolis is used to seal the beehive, has a viscous consistency and color that varies from greenish yellow to dark brown (Pensin et al., 2009). At room temperature, it is sticky and becomes rigid and brittle at low temperature (Parolia et al., 2010). Commercially, it can be found in raw form, capsule, tablet, gel, powder and alcoholic, hydro-alcoholic and aqueous extracts (Castaldo et al., 2002).

The importance of this alternative, or even complementary, medicine is extremely relevant in Brazil, mainly because its rural area is quite extensive and very rich in floral varieties with numerous pharmacological properties (Geraldini et al., 2000).

In Dentistry, the action of propolis has been studied in some situations, such as in the treatment of gingivitis, periodontitis, recurrent aphthous ulcerations, prosthetic stomatitis, prevention of dental caries, irrigant and intracanal medicine, pulp capping agent, storage of avulsed teeth and in the treatment of dentinal hypersensitivity (DH) (Almeida et al., 2016; Carvalho et al., 2019).

This product has been gaining prominence due to its therapeutic diversity (Costa et al., 2008). Some studies indicate its application in the health area due to its antibacterial, fungicidal, anti-inflammatory, analgesic, obliterating, healing,

antiviral, anesthetic, antioxidant, immunostimulatory and antitumor properties (Kosalec et al., 2005; Da Silva et al., 2006; Zuhendri et al., 2021).

Research covers several areas such as cariology, stomatology, endodontics, dentistry, periodontics, surgery and add enormous value in terms of assessing the applicability of this product in the treatment and prevention of oral conditions (Bankova, 2005; Feng et al., 2009, Barbosa et al., 2021).

DH is a theme that is present in the daily practice of dentists. New research areas for its treatment have been explored and the use of natural agents such as propolis has been shown to be the basis for further studies (Regiani et al., 2021). Thus, the objective of the present study was to verify whether the green propolis extracts applied at concentrations of 17% and 21% on the exposed dentin of healthy bovine teeth were capable of promoting a staining effect on the respective tooth structure.

2. Methodology

This is an experimental study, for which 40 crowns of healthy bovine teeth donated by a commercial slaughterhouse producing beef cuts, located in the municipality of Pindamonhangaba-SP, were used.

An experimental study is called all research involving some type of experiment, containing experimental models such as animals, cadavers, cell and tissue cultures. The researcher actively participates in conducting the procedure, phenomenon or the fact evaluated, acting on the cause, modifying it, and evaluating changes in the outcome (Hochman et al., 2005).

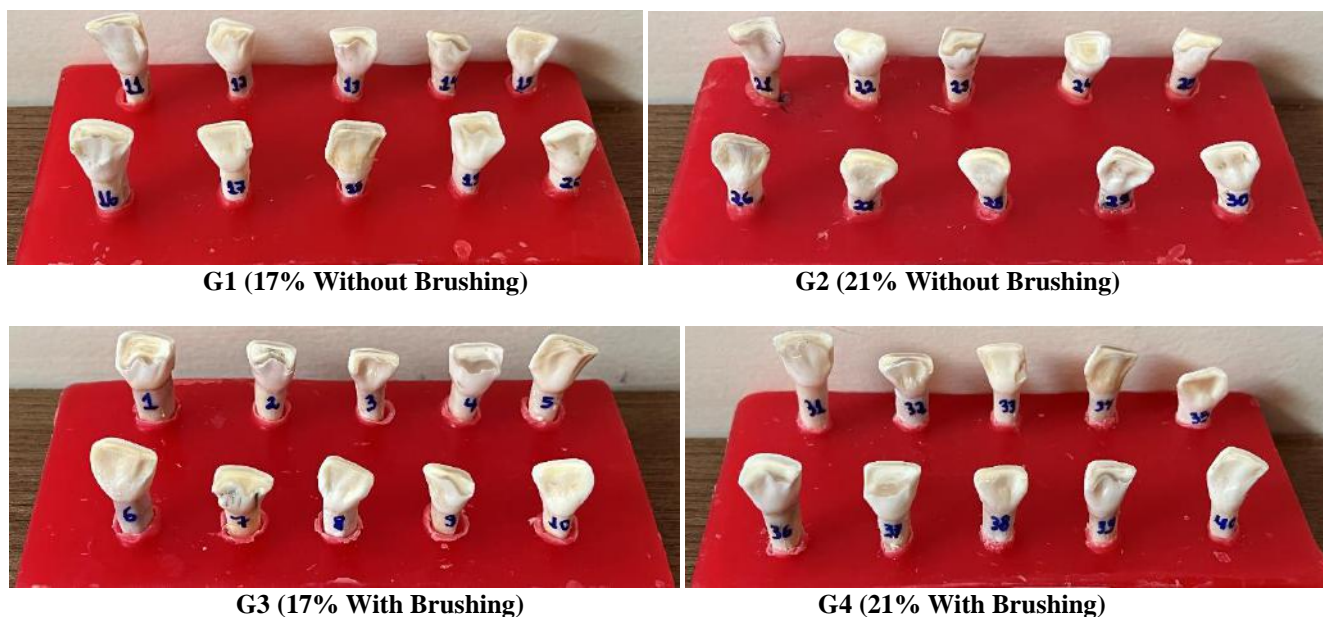
Animal models have been widely used for *in vitro* research with new materials of interest to the dental clinic. Bovine teeth have the same anatomical structures and histological characteristics as human teeth and can be obtained from the jaws of animals commercially slaughtered for meat production in industrial slaughterhouses supervised by a federal agency.

The inclusion criteria of the respective study were the presence of good condition of the dental structure, similar sizes and absence of pulpal exposure of the bovine teeth.

To carry out the experimental procedures, the teeth were sectioned in half, in an axial cut, in the mesiodistal direction, in order to expose the dentin, with a carborundum disc in a handpiece, at the premises of the Multidisciplinary Dental Laboratory of UniFUNVIC, Centro Universitário FUNVIC, Pindamonhangaba-SP.

Then, the specimens were enumerated and randomly divided into four groups (G1 to G4) for acid etching to simulate hypersensitive dentin, and subsequently application of green propolis extract solutions. According to Figure 1, each group consisted of ten teeth arranged, fixed and photographed on a plate made of utility wax, as follows: Group 1 (G1): 37% phosphoric acid and 17% propolis extract without brushing, Group 2 (G2): acid 37% phosphoric acid and 21% propolis extract without brushing, Group 3 (G3): 37% phosphoric acid and 17% propolis extract with brushing and Group 4 (G4): 37% phosphoric acid and propolis extract to 21% with brushing.

Figure 1 – Initial disposition of the specimens divided into four groups (G1, G2, G3 e G4).



Source: Own authorship.

It can be seen that all specimens were clean, with natural color, similar sizes and visible dentin exposure.

The propolis extract used contained green propolis, cereal alcohol and minimal dry extract, at concentrations of 17% and 21%, and both were purchased from the company Apis Brasil®.

In order to simulate hypersensitive dentin the specimens were submitted to acid etching with phosphoric acid at 37% in the form of a gel called *Ataque Gel*® (BDP – Biodynamic Dental Products LTDA, Brazil), for 30 seconds in enamel and 15 seconds in dentin. Then, the teeth were washed with running water for 20 seconds, gently dried with a paper towel free of impurities for 20 seconds. After acid etching on the dentin surfaces of all groups, a regular yellow micro-applicator, *KG Brush*® folding rod (KG SORENSEN) was used to apply the propolis solutions at the respective concentrations mentioned above.

The extracts were applied twice a day, with an interval of six hours, for seven consecutive days. Only in groups G3 and G4 were the specimens brushed for approximately 60 seconds after 30 minutes of each application of propolis extract, with a brush and dentifrice by *Colgate*®. After each treatment, the teeth were stored in a closed and clean container.

In the week following the application of propolis extracts, all teeth in each group (G1, G2, G3 and G4) were submitted to a prophylaxis procedure using *Extra Fine Pumice Stone - SS White*®, *Robson's brush*® and *Micro engine* at low speed, where each tooth was brushed for approximately 60 seconds.

After carrying out the experimental procedures, comparative observations were made between the four groups using photographs of specimens and the results were subsequently analyzed.

3. Results and Discussion

Although propolis has aroused great interest in society in recent times, it has been used since antiquity (Geraldini et al., 2000).

Due to its numerous therapeutic properties, it has stood out and encouraged researchers to analyze its chemical composition and possible relationship with its biological activities (Kumar et al., 2014). In Brazil, there are three different

types of propolis: green, produced from the resin of rosemary of the field, a plant native to the Brazilian cerrado; the red one, in the Northeast, produced from the plant known as “rabo-de-bugio” and the brown one, or traditional propolis, which does not need a specific flower or plant for its production. In addition to the color difference, different propolis may have different properties (França et al., 2020). In the present study, green propolis was used because it is the most widely used and the most studied.

For the use of propolis, it is necessary that it go through a dilution process that can be carried out in alcohol or water, giving rise to the propolis extract. Although both dilution processes have the same functionalities, they can have different results. As propolis has liposoluble phytochemicals in its composition, they are easier extracted in alcohol. Thus, alcoholic extracts ensure greater absorption of the substances present in propolis and, therefore, are considered a better option (Campos et al., 2021). For this reason, in the present study alcoholic extracts of propolis were used.

There are several reports of the use of propolis in the form of ointments, poultices, ointments and sprays to facilitate its application. In solutions, concentrations using alcohol as the main vehicle vary between 5 and 30% (Geraldini et al., 2000). In the present study, products available on the market with concentrations of 17% and 21%, higher than the normally used in the clinical dentistry were used.

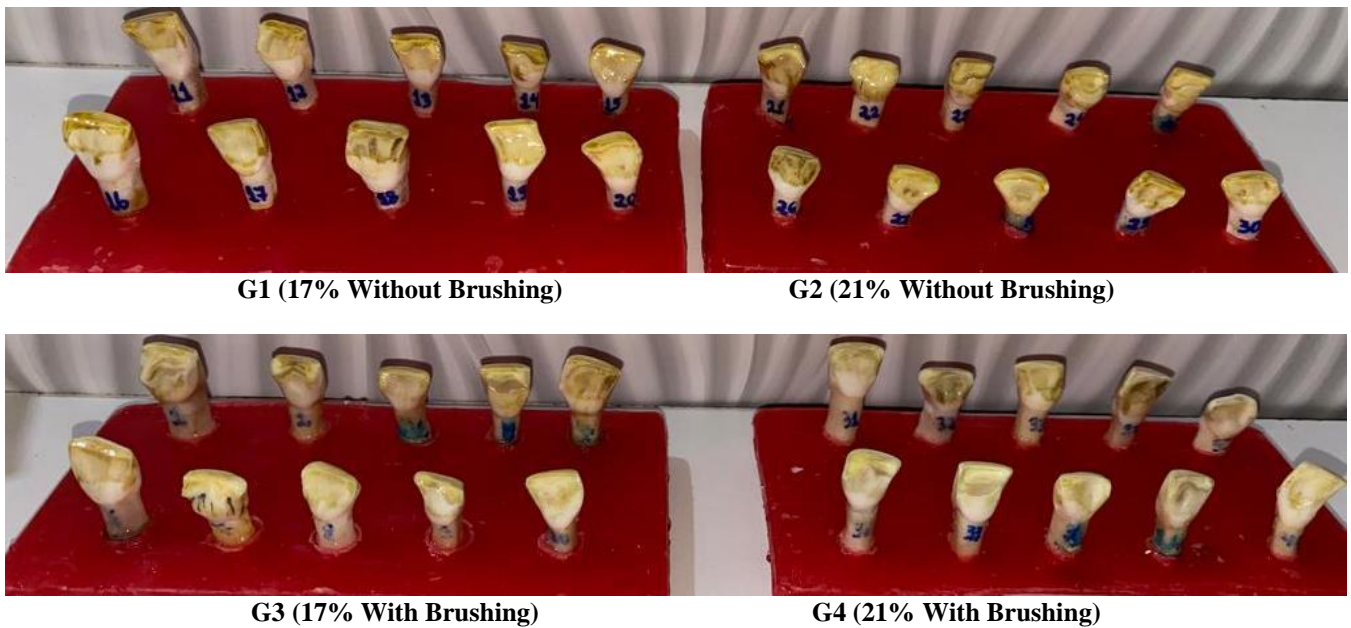
The use of propolis for DH in comparative studies has shown efficiency, however, its mechanism of action is still unknown, however, it is noteworthy that its use in a therapeutic way contributes significantly to the tubular occlusion used both in isolation and in association with other agents (Regiani et al., 2021).

Kripal et al. (2019) also observed the effectiveness of using propolis in their in vitro studies, which evaluated the use of a propolis-based varnish for the treatment of DH and concluded that it proved to be effective. This study showed the promise of propolis varnish as a natural treatment modality for DH.

The present study was directed towards the treatment of the dentin surface with a natural product, in order to follow a worldwide trend of finding alternative products in the treatment of oral health. The study was oriented with the use of propolis on the dental surface, because, as observed in the studies by Geraldini et al. (2016), the formation of a barrier with this substance is possible, suggesting its permanence on the dentin surface, promoting the obliteration of dentinal tubules, thus being able to reduce DH.

Comparative observations were made for the different specimens, through photographs taken on the first, fourth and seventh days and the following week after the prophylaxis procedure. Figure 2 illustrates the first day of application of propolis extracts at concentrations of 17% and 21%, respectively, in the four groups (G1, G2, G3 and G4), and similar penetration and fixation of the solution at both concentrations can be observed, both in the group that underwent brushing and in the group that did not receive brushing.

Figure 2 – First day of application of 17% and 21% green propolis extracts in the four groups of specimens (G1, G2, G3 and G4).

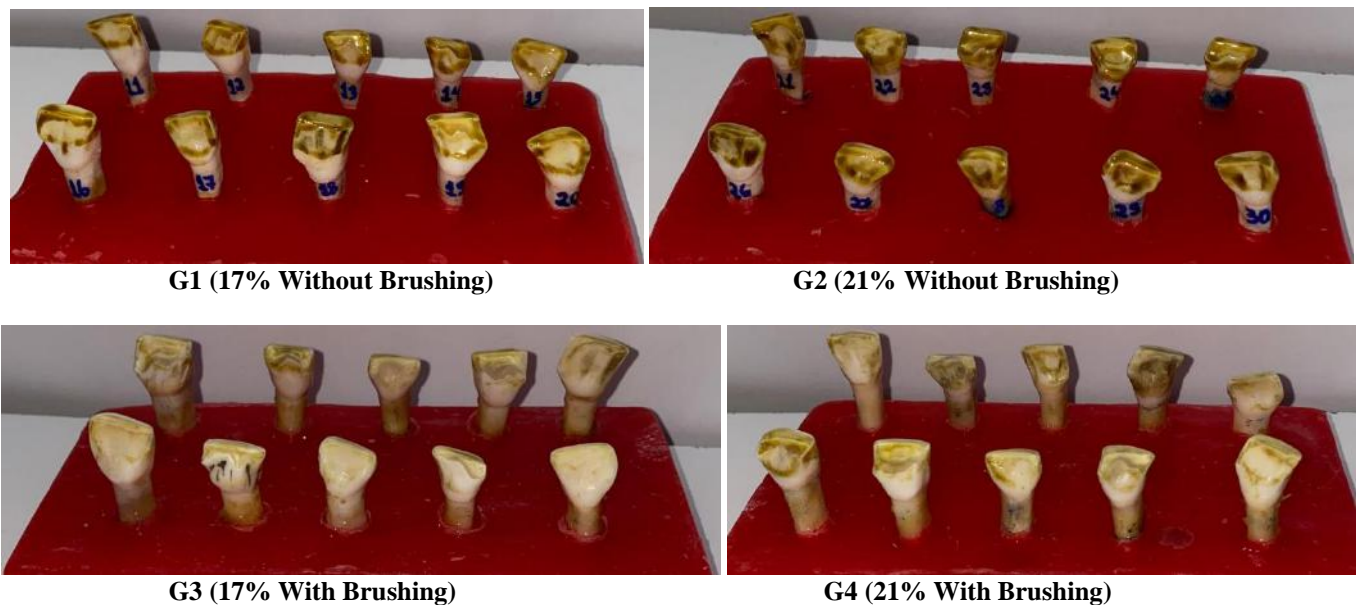


Source: Own authorship.

A slightly more evident staining effect can be observed in groups G1 and G2 compared to groups G3 and G4, which was already expected, due to the fact that the latter two were submitted to the brushing procedure.

Both concentrations of propolis extracts promoted similar pigmentation between the groups, being more evident in the groups that did not receive brushing (G1 and G2), observed on the fourth and seventh days of application of the extracts, as shown in Figures 3 and 4.

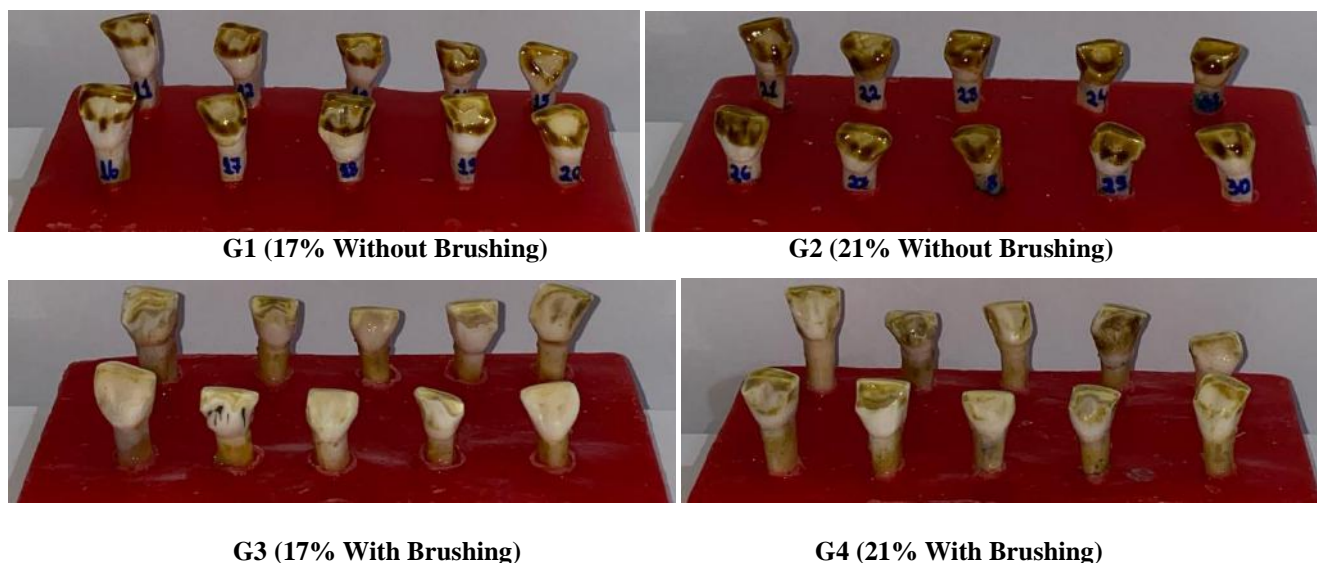
Figure 3 – Fourth day of application of 17% and 21% green propolis extracts in the four groups of specimens (G1, G2, G3 and G4).



Source: Own authorship.

After four consecutive days of application of propolis extracts, greater staining was observed in groups G1 and G2 compared to G3 and G4. In addition, greater pigmentation was also noted in these four groups compared to the first day of extract application, which was already expected due to the cumulative effect of the products on the specimens.

Figure 4 – Seventh day of application of 17% and 21% green propolis extracts in the four groups of specimens (G1, G2, G3 and G4).



Source: Own authorship.

After seven consecutive days of application of the extracts, a very evident staining effect can be observed between groups G1 and G2 with respect to G3 and G4. Compared to the first day of application of the products, the same was observed. Despite groups G3 and G4 having been submitted to the daily brushing procedure, the specimens showed to be more stained in relation to the first day of application of the products, granting the permanence of the staining effect according to the consecutive application of the extracts.

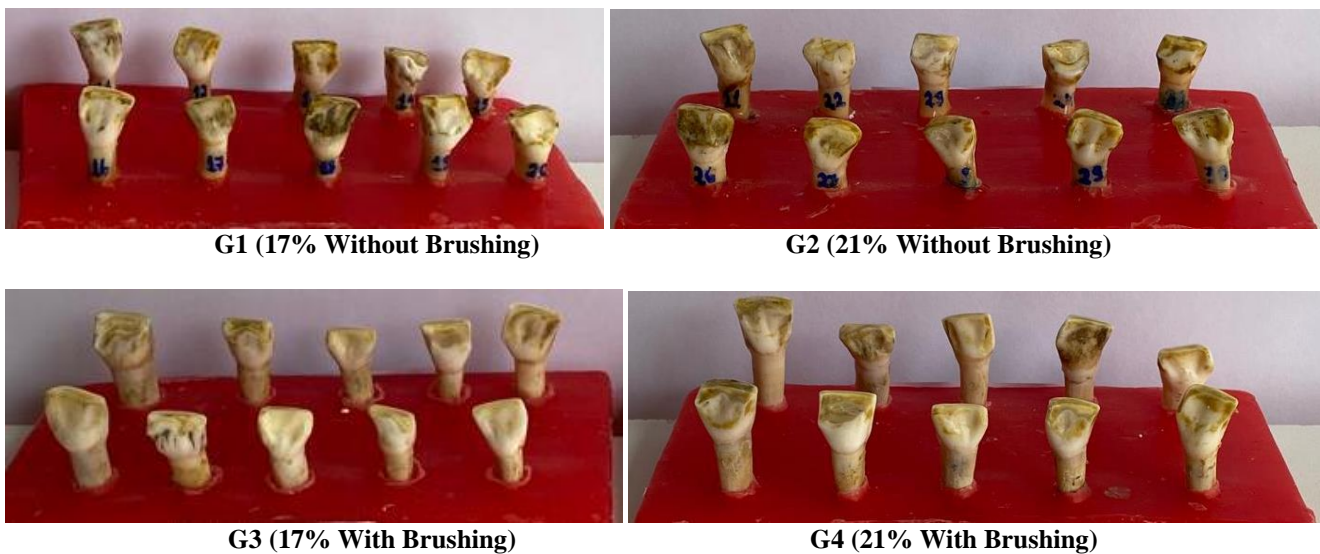
For Cairo et al. (2006), the low cost of propolis makes it more accessible to the population, which would be an advantage for its use, however, Nagai et al. (2003) believe that a challenge is to obtain a formula with a more pleasant aroma and flavor, since most people are resistant to its natural taste and odor.

According to Simões et al. (2008) and Swerts et al. (2005), as propolis is a natural product, it has the advantage of being compatible with the metabolism of mammals in general, as it has greater molecular diversity, with numerous therapeutic substances, which reduces the risk of adverse reactions to oral tissues. compared to industrialized products. On the other hand, some individuals may have adverse reactions to propolis, which would be a disadvantage regarding its use (Wimardhani & Soegyanto, 2014). With the popularization of the use of natural products in order to maintain a more sustainable environment and reduce costs (Teodoro et al., 2022), cases of allergic reaction have been documented more frequently (Belluco et al., 2022).

According to Makabe et al. (2018), the most used active ingredient in mouthwashes is chlorhexidine, however, its prolonged use causes side effects such as temporary loss of taste and the appearance of stains on teeth and tongue. These same researchers stated that these inconveniences do not occur with the use of propolis-based mouthwashes, which is seen as an advantage.

The same was observed in the studies by Kiani et al. (2022), who evaluated the clinical efficacy of a mouthwash containing propolis in the treatment of gingivitis and concluded that it was not capable of causing discoloration and staining of teeth. However, the results presented here do not corroborate the authors' assertion, since in all specimens the solution remained fixed, conferring a staining effect on both dentin and enamel according to Figure 5, and it was not possible to remove the staining even after performing of prophylaxis.

Figure 5 – Presence of staining on the dentin and enamel of specimens from the four groups (G1, G2, G3, G4) after the prophylaxis procedure.



Source: Own authorship.

It can be observed that even after carrying out the prophylaxis procedure in the four groups studied, all specimens remained clearly stained, which showed the staining effect of the propolis extracts at the two concentrations studied, 17 and 21% respectively.

It is important to emphasize that in studies that used propolis-based mouthwashes, a solution with a lower concentration of the product (6%) was used, which could have contributed to less penetration and fixation on the teeth, not causing the appearance of stains on the teeth. same. The same can be suggested in relation to dentifrices and dental gels containing propolis, which are available on the market with a concentration of less than or equal to 7%. In our bibliographic search, no articles were found whose objective was to investigate tooth staining due to propolis-based toothpaste or tooth gel.

Regarding the disadvantages of using propolis in dentistry, Belluco et al. (2022) point out as the most common reactions of hypersensitivity, angular cheilitis and contact dermatitis in the perioral region. But, Tavares et al. (2021), after reviewing 63 articles on the use of propolis extract for the treatment of HD, found six clinical trials in which propolis was more effective in reducing hypersensitivity than placebo and no adverse reactions were reported. These more recent findings encourage the dental use of propolis, showing that it is necessary to adjust the vehicle and concentration of the product.

The scientific literature lacks reports of teeth staining due to the therapeutic use of propolis extract. But, because the product can be purchased freely and often used without proper prescription by a dentist, it may be that its use in inappropriate concentrations is capable of staining teeth, as well as a pre-existing restoration. Given this, Akca et al. (2007) recommend that the therapeutic applicability of propolis be carried out under the supervision of the dentist, however, off-the-shelf, over-the-

counter products are attractive to patients who have painful symptoms, mainly due to their easy access and low cost compared to professional treatment. The policy for distributing, publicizing and selling these products conveys to the patient the idea of a treatment for continuous use, without the need to be guided by a dentist.

Due to the scarcity of experimental tests on the possible staining effects of green propolis extracts on teeth, there is a lack of subsidies to carry out comparisons with the results obtained in this experiment. Further studies are suggested in order to support the observed results.

4. Final Considerations

According to the results, we conclude that the extract of green propolis at concentrations 17% and 21%, when used on dental surfaces that mimic the situation of dentin hypersensitivity, has a staining effect on teeth, both enamel and dentin.

As propolis is a natural product with increasing use in dentistry due to its proven therapeutic properties, new studies must be carried out using lower concentrations to verify this effect and, if not, to be able to safely prescribe propolis in the treatment of DH.

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