BOLETIN LATINOAMERICANO Y DEL CARIBE DE PLANTAS MEDICINALES Y AROMÁTICAS
© / ISSN 0717 7917 / www.blacpma.ms-editions.cl

Articulo Original / Original Article

Effect of *Casearia sylvestris* on the obliteration of dentinal tubules and the control of dental sensitivity

[Efecto de *Casearia sylvestris* sobre la obliteración de los túbulos dentinarios y el control de la sensibilidad dental]

Patrícia Gizeli Brassalli de Melo[1], Laís Kennerly Herrera[2], Patrícia Pinto Saraiva[3], Paulo Henrique Weckwerth[4], Vitor Villas Bôas Weckwerth[2], Rafaela Pignatii de Freitas[2], Guilherme Donadel[5], Mariana Dalmagro[6], Juliana Cogo[7], Emerson Luiz Botelho Lourenço[8], Giuliana Zardeto[1] & Daniela de Cássia Faglioni Boleta-Ceranto[9]

[1]Department of Health Sciences, Universidade Paranaense, Parana, Brazil. [2]Department of Health Sciences, Sagrado Coração University, Sao Paulo, Brazil. [3]Department of Health Sciences, Faculty of Medicine of Jaú, UNOESTE, Sao Paulo, Brasil. [4]Department of Health Sciences, Faculty of Medicine of Botucatu, Sao Paulo, Brazil. [5]Department of Health Sciences, Graduate Program in Animal Science with emphasis on Bioactive Products, Universidade Paranaense, Parana, Brazil. [6]Department of Agricultural Sciences, Graduate Program in Biotechnology Applied to Agriculture, Universidade Paranaense, Parana, Brazil. [7]Department of Health Sciences, Cesumar University of Maringá, Maringá, Brazil. [8]Department of Health Sciences, Stricto Sensu Graduate Programs, Universidade Paranaense, Parana, Brazil. [9]Department of Health Sciences, Professional Master's Degree in Medicinal Plants and Herbal Medicines in Primary Care, Universidade Paranaense, Parana, Brazil

Reviewed by:
Natacha Oyarzo
Pontificia Universidad Católica de Chile
Chile

Siti Farah Binti MD Tohid
Universiti Putra Malaysia
Malaysia

Correspondence:
Giuliana ZARDETO
giulianazardeto@prof.unipar.br

Section Biological activity

Received: 23 August 2021
Accepted: 22 December 2021
Accepted corrected: 10 June 2023
Published: 30 March 2024

Citation:
Melo PGB, Herrera LK, Saraiva PP, Weckwerth PH, Weckwerth VVB, Freitas RP, Donadel G, Dalmagro M, Lourenço ELB, Zardeto G, Boleta-Ceranto DCFB. Effect of *Casearia sylvestris* on the obliteration of dentinal tubules and the control of dental sensitivity **Bol Latinoam Caribe Plant Med Aromat** 23 (2): 229 - 247 (2024). https://doi.org/10.37360/blacpma.24.23.2.16

Abstract: The present study evaluated the efficacy of the mineralizing action of *Casearia sylvestris* ethanolic extract on bovine dentin blocks in its pure form and in dental paste, through scanning electron microscopy. The dentin blocks were immersed in artificial saliva and incubated at 37°C for 7 days. Subsequently, six groups were treated with different test substances and analysed qualitatively and quantitatively at 30 and 60 days. The tests used were Kruskal-Wallis and Dunn's. Shapiro-Wilk and ANOVA. The qualitative analysis at 30 days showed a difference between the groups treated with ethanolic extract and toothpaste. Quantitatively, at 30 days, treatment with ethanolic extract of *Casearia* showed a greater number of open dentinal tubules. At 60 days, the difference persisted only for the blocks treated with toothpaste. The results obtained indicated that there is a positive relationship between the use of *Casearia sylvestris* and obliteration of dentinal tubules.

Keywords: Casearia; Dental desensitizers; Hypersensitivity; Salicaceae family; Formulation.

Resumen: El presente estudio evaluó la eficacia de la acción mineralizante del extracto etanólico de *Casearia sylvestris* sobre bloques de dentina bovina en su forma pura y en pasta dental, mediante microscopía electrónica de barrido. Los bloques de dentina se sumergieron en saliva artificial y se incubaron a 37°C durante 7 días. Posteriormente, se trataron seis grupos con diferentes sustancias de ensayo y se analizaron cualitativa y cuantitativamente a los 30 y 60 días. Las pruebas utilizadas fueron Kruskal-Wallis y Dunn's. Shapiro-Wilk y ANOVA. El análisis cualitativo a los 30 días mostró una diferencia entre los grupos tratados con extracto etanólico y pasta dentífrica. Cuantitativamente, a los 30 días, el tratamiento con extracto etanólico de *Casearia* mostró un mayor número de túbulos dentinarios abiertos. A los 60 días, la diferencia persistió sólo para los bloques tratados con pasta dentífrica. Los resultados obtenidos indicaron que existe una relación positiva entre el uso de *Casearia sylvestris* y la obliteración de los túbulos dentinarios.

Palabras clave: Casearia; Desensibilizadores dentales; Hipersensibilidad; Familia Salicaceae; Formulación

INTRODUCTION

With the improvement in the population's oral health indicators, there has been a reduction in caries disease and consequently an increase in the longevity of teeth. The maintenance of the tooth for longer in the oral cavity is a very satisfactory aspect; however, problems related to dental hypersensitivity may become more frequent (Ritter *et al.*, 2006). The teeth most affected by dental hypersensitivity correspond to premolars, molars, canines, and incisors respectively (Neuhaus *et al.*, 2013; Cavalcante *et al.*, 2015), on their buccal surfaces in the cervical region (Pashley *et al.*, 2008).

The aging process is associated with the physiological gingival recession that, consequently, ends up exposing the cervical dentin (Ritter *et al.*, 2006). Although dentin hypersensitivity also affects the elderly, it is in the age group between 30 and 40 years that it happens most often (Assis *et al.*, 2011; Davari *et al.*, 2013). The justification for this situation is given by the lack of spontaneous sealing of the dentinal tubules when exposed to the oral environment (Sobral, 2003, Moraschini & Barboza, 2016).

Structurally, dentin is composed of dentinal tubules, ranging from the pulp to the amelodentinal junction or dentin cementum (Vongsavan & Matthews, 1991), with a conical shape and an enlarged diameter at the pulp termination (Rebelo *et al.*, 2011). In the crown, the dentin is covered by the enamel and in the root portion, by the cementum. The lack of these protective tissues leads the dentin to respond in a physiological or even pathological way to a given stimulus (Davary *et al.*, 2013), which is known as dentin hypersensitivity.

Dentin hypersensitivity is directly related to exposed dentin and open dentinal tubules to the oral cavity and dental pulp (Trentin & Bervian, 2014). The painful response can occur in the presence of different types of stimuli, such as chemical, thermal, mechanical, and evaporative, which have the ability to act on the dentinal tubules that are exposed to the oral environment (Splieth & Tachou, 2013; Baratieri, 2015).

Different factors can induce dental hypersensitivity, among them: abrasion, abfraction and attrition, erosion, and gingival recession (Bubteina & Garoushi, 2015; Silva & Ginjeira, 2011). For Addy & Urquhart (1992); Wichgers & Emert (1997), other factors also need to be considered, such as chronic traumas during brushing, age, diet, parafunctional habits, excessive intake of beverages with acid pH, bulimia, gum inflammation, and acute trauma resulting from periodontal surgery. Tooth whitening is another factor that also needs to be taken into consideration (Jorsen & Carrol, 2002; Costa & Ruck, 2006; Leite & Dias, 2010). Thus, dental hypersensitivity can deeply interfere in a person's eating habits, with negative (psychosocial) reflexes in their daily life (Splieth & Tachou, 2013; Cartwright, 2014; Baratieri, 2015).

The pain mechanism is still poorly understood. However, the most accepted theory is that of Brännström hydrodynamics, which justifies the pain by the rapid movement of the fluid present in the dentinal tubules, by means of a certain stimulus applied to the dentin, which ends up reaching the nerve fiber of the dental pulp (Brännström & Aström, 1967; Cavalcante *et al.*, 2015, Vano *et al.*, 2018).

Removal of tooth enamel or loss of periodontal lining tissue is considered the cause of dental exposure (Muzzin & Johnson, 1989). Hypersensitive dentin brings changes in its histological conformation7. According to Yoshima (1996) and Absi *et al.* (1987), the person with hypersensitivity has his dentinal tubules increased in number (about eight times) and in diameter (about twice) when compared to the dentine of a patient without hypersensitivity. Thus, large, and numerous dentin tubules, in turn, cause an increase in dentin permeability (Rebelo *et al.*, 2011).

However, it is important to point out that not always an exposed dentin generates hypersensitivity, since its dentinal tubules may be covered by the "smear layer", by residues of dentifrices, or even by saliva minerals (West *et al.*, 2001). The dentin present in the root has little protection, being covered by a thin layer of cementum, which is not very effective against irritant agents, besides being difficult to identify for the clinician (Sneed & Looper, 1985).

Different materials are being used as aids in the treatment of dentin hypersensitivity, some aimed at blocking the spread of neural stimuli from pulp receptors, others based on the impossibility of moving the fluid from the dentinal tubules, or methods that contemplate both forms at the same time (Canadian Advisory Board on Dentin

Hypersensitivity, 2003).

The treatment of dentin hypersensitivity can be done through the closing of the dentin tubules, root recovery, neuronal response agents, and use of substances with anti-inflammatory action (Kerns *et al.,* 1991; Oda *et al.,* 1999; West *et al.,* 2001; Frechoso *et al,* 2003; Arrais *et al.,* 2004; Oberg *et al*., 2006; Mosleh *et al.*, 2018).

The use of fluoride varnish (Shiau, 2012), high and low-intensity laser use (Palazon *et al.,* 2013), as well as oxalate (Mantzourani & Sharma, 2013), represent different treatment options against dental hypersensitivity. Also, restorative materials (resin and glass ionomer cement); casein phosphopeptides; Arginine; Glutaraldehyde; Silver fluoride diamine; Bioactive glasses; Nanometric materials; Potassium salts; Propolis (Ribeiro *et al.,* 2016; Mosleh *et al.,* 2018). However, calcium phosphate represents the most effective alternative to promote the obliteration of dentinal tubules, being bioactive and biocompatible with the dental structure (Shetty *et al.,* 2010).

According to Tian *et al.* (2014), many of these desensitizing substances are present in toothpastes, mouthwash solutions, and for specific use in the dental clinic. The search for the biocompatibility of these substances with oral structures is constant (Lochaiwatana *et al.,* 2015).

The use of medicinal plants, especially phytotherapy, has shown advances in different areas of medicine. Although its use is old, in the last decades there has been a growing resumption of this practice. The great variety of medicinal plants cataloged and those whose biological potential is yet to be discovered have motivated the field of research, public health services, and users, directly influencing the socioeconomic sector (Figueiredo *et al.,* 2014).

Phytotherapics are plant species that have active substances and can be found in different pharmaceutical forms. They represent an alternative measure, mainly in public health, where they prioritize drugs with reduced cost, with the same purposes as industrialized synthetics, and mainly, an attempt to ensure social equity (Di Stasi, 1994; Toledo *et al.,* 2003).

In Brazil, the use of medicinal plants was based on the sum of different cultures (Indigenous, Africans, and Portuguese). Thus, the great diversity of peoples, combined with a large number of plant species, resulted in a vast popular culture (Sousa *et al.,* 2008).

The World Health Organization (WHO) estimates that approximately 80% of the world population, especially developing countries, use this resource, as the main form of treatment in different pathologies, and for many, the only resource too (Rosa *et al.,* 2011; WHO, 2014). The use of plants for therapeutic purposes has been recognized by the WHO since 1978, however, in Brazil, their use was encouraged since 2006, with the creation of the National Policy for Integrative and Complementary Practices (PNPIC) and the National Policy for Medicinal and Phytotherapeutic Plants (PNPMF) for the Unified Health System (Brazil, 2018).

In dentistry, phytotherapy was recognized as an integrative and complementary practice to oral health in 2008, following the resolution of the Conselho Federal de Odontologia 082/2008, Article 7. Scientific studies on herbal medicines in dentistry are necessary to reinforce the importance of the insertion of this practice to dental surgeons (Conselho Federal de Odontologia, 2008).

Although old, the use of plant species for the treatment of different oral pathologies is restricted, either to treat oral diseases or to treat systemic diseases with oral manifestations (Oliveira *et al.*, 2007; Soyama, 2007; Lustosa *et al.,* 2008), with little scientific support (Varoni *et al.,* 2012). Thus, the great therapeutic potential found in different species, associated with the need for new products in the dental field, with biological and antimicrobial properties, has motivated in recent years different segments of research, especially phytotherapy (Bretz *et al*., 1998).

Casearia sylvestris (*C. sylvestris*) is a common species in tropical America, being found in different countries such as Mexico, Uruguay, Argentina, and Brazil (Cavallari *et al*., 2010). It belongs to the Salicaceae family and has several popular names such as guaçatonga, café bravo or erva do bugre (Sassioto *et al*., 2004).

Brazil has about seventy species of the genus *Casearia* (Le Cointe, 1934; Schoenfelder *et al.*, 2008), which are distributed in at least 22 states, as São Paulo, Paraná, Rio de Janeiro, Amazonas, Bahia, and others (Cavallari *et al*., 2010). Due to its therapeutic potential, *C. sylvestris* has become a species of medical and dental interest (Le Cointe,

1934; Schoenfelder *et al.,* 2008). More than 287 components have already been isolated from this plant (Xia *et al.,* 2015), and the antibacterial activity is one of its outstanding characteristics (Schneider *et al.,* 2006; Da Silva *et al.,* 2008; Weckwerth *et al.,* 2011; Falcão *et al.,* 2017). Among the main isolated components of plants are clerodane diterpenes, triterpenes, flavonoids, essential oils, and sesquiterpenes (Csipak, 2011; Prieto *et al.,* 2013a; Prieto *et al.,* 2013b; Bou *et al.,* 2013; Felipe *et al.,* 2014; Ferreira *et al.,* 2014; Xia *et al.,* 2015).

Clerodane diterpenes, known as casearins, are responsible for the different pharmacological properties presented by the species. The healing, antiophidic, antibacterial, antiprotozoal, and anti-inflammatory action is due to the presence of isoprene units, which give rise to active metabolites, present in casearins, and these, found in the leaves of *C. sylvestris* (Mosaddik *et al.,* 2004; Esteves *et al.,* 2005; Mesquita *et al.,* 2007; Cavalcante *et al.,* 2007).

C. sylvestris through its antimicrobial action is used in the treatment of flu, colds, skin ulcerations, and diarrhea (Carvalho *et al.,* 1998; Oberlies *et al.,* 2002; Mosaddik *et al.,* 2004; Silva *et al.,* 2006), as an antiulcerogenic activity (Aboin *et al.,* 1987; Basile *et al.,* 1990; Esteves *et al.,* 2005), presenting effectiveness on tumor cells (Felipe *et al.,* 2014; Pereira *et al.,* 2017) and with anti-hyperalgesic effect (Piovesan *et al.,* 2017).

In Brazil, among the 71 species of medicinal plants published by the Ministry of Health in 2009, through the National List of Medicinal Plants of Interest to the Unified Health System (RENISUS), aimed at the study and production of herbal medicines, *C. sylvestris* is present (Brasil, 2006).

Pinheiro & Andrade (2008), in their studies, concluded that *C. sylvestris* is among the most studied species in the field of dentistry, and these results were presented at meetings of the Brazilian Society of Dental Research (SBPqO).

In the dental area, the studies with *C. sylvestris* are focused on the treatment of herpes labialis (Cury, 2005), thrush (Silva, *et al.,* 2016), the treatment of periapical infections (Da Silva *et al.,* 2004; Duarte *et al.,* 2009) and the use of dentifrices (Arantes, 2002).

Also, in the results described by Duarte *et al.* (2009), it was verified that the association of calcium hydroxide paste with *C. sylvestris*, showed similar results regarding pH and ion release when compared with the association between calcium hydroxide and propylene glycol, as well as in the use of chlorhexidine as well.

Thus, the great challenge of today's dentistry is to find natural products with antimicrobial action, for the treatment of different pathologies that affect the dental element (Roberts, 2002), as well as in the search for substances capable of controlling dental hypersensitivity (Aranha, 2003).

In this way, the objective of the present work was to investigate the effectiveness of the mineralizing action of *C. sylvestris* on dentin block in its pure form and on toothpaste, using scanning electron microscopy.

MATERIAL AND METHODS

Chemicals

Chem 1, chem 2, chem 3 were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Preparation of bovine dentin specimens

To verify the mineralizing action of *C. sylvestris*, bovine teeth (central incisors) were used, obtained through the Faculty of Dentistry of Bauru (FOB - USP).

For the execution of the experimental part, 60 blocks of sterile bovine dentin (central incisors) were prepared. Each block was cut to a thickness of 4 x 0.8 mm using a metallographic cutting machine type ISOMET LowSpeedSaw (Buehler Ltd, Lake Bluff IL, USA), and then sanded from a polishing machine according to the methodology of Oh *et al.* (2015).

Subsequently, to remove organic residues and dentin smear layer, the blocks were treated with 1% sodium hypochlorite for 30 minutes and 17% EDTA for 5 minutes. Then, simulating the oral cavity environment, the dentin blocks were immersed in artificial saliva solution, according to the protocol: 50mM Tris-HCl pH 7.6 buffer solution with 2.58 mM calcium chloride, 1.55 mM phosphates, and 180mM of sodium chloride. They were incubated in closed containers, at a temperature of 37°C for 7 days. After 7 days, the fragments were removed and washed with deionized water for 50 seconds.

Obtaining the test substances

The test substances used in the research were prepared at a compounding pharmacy, according to

the formulation of the Laboratory Panizza, São Paulo, Brasil The paste based on *C. sylvestris* contained in its composition: hydroxyethyl cellulose 2%, methylparaben 0.18%, propylparaben 0.05%, Sorbitol 5%, calcium carbonate 5%, sodium lauryl sulfate 0.1%, aroma of strawberry 0.2%, tincture of guaçatonga 10%, sucralose 0.05%, menthol 0.2%, dye and purified water. The extracts were added with Carbopol (polymer) to obtain the gels.

Obtaining and fractionating the ethanolic extract of C. sylvestris leaves

The collection of the leaves of *C. sylvestris* was carried out in the Garden of Medicinal and Toxic Plants at the Faculty of Pharmaceutical Sciences of UNESP-Araraquara. The C. sylvestris exsiccata, under the specimen number AGS 102 (21º81'46", 6 South, 48º20'21", 5 West) is stored at the State Scientific Herbarium "Maria Eneyda P. Kauffman Fidalgo", at the Botanical Institute of the Government of the State of São Paulo.

The Chemistry Institute of Universidade Estadual Paulista de Araraquara, in partnership with the pharmacognosy laboratory of the Faculty of Pharmaceutical Sciences - UNESP, performs the isolation, identification, and characterization of the purity of the Casearia species.

According to Oda *et al.* (2019), the casearin J (cas J) and casearin O (cas O) were isolated from the ethanolic extract of the leaves of *C. sylvestris*. The dried and powdered leaves (1.5 kg) were extracted by maceration with ethanol (1:15 w/v, 120 h) at 40°C with occasional stirring. The organic solvent was evaporated by an IKA® DEST KV 05S3 (Labcontrol, São Paulo, Brazil) evaporator to yield the dry extract (163.9 g, 10.9%, m/m).

The extract fractionation was conducted as described by Sposito *et al.* (2019). Casearin J was obtained from fractions SF 17–19 and casearin O from SF 15–16 after semi-preparative purification (tR=27.8 min) using an Agilent® Eclipse XDB C18 column (250×21.20 mm×7 μm) (Santa Clara, EUA) with a mobile phase employing 77% isocratic methanol for 90 min, a flow rate of 15.0 mL/min and an injection volume of 2.000 μL.

Methodology for mineralization analysis of dentinal tubules

The analysis of the samples was performed in two distinct periods, with 30 and 60 days. After being removed from the artificial saliva, the dentin blocks went through a washing process (three times) with sterile buffer solution, in the sequence, dried with absorbent paper, and distributed in a number of six on sterile Petri dishes.

Six test groups were prepared with the aid of a sterile microbrush (Figures No. 1A, No. 1B and No. 1C), and distributed, according to the material of choice, into 24-well cell culture plates, like methodologies describe by Oda *et al.,* (2019) and Spósito *et al.*, (2019):

- Group 1: prepared with an ethanolic extract from *C. sylvestris*.
- Group 2: composed of fractions of diterpenes, rich in casearin, from *C. sylvestris*.
- Group 3: composed of fractions of diterpenes, rich in casearin, from *C. sylvestris* added of an aqueous solution of Iron III Chloride (0.5g/L).
- Group 4: containing *C. sylvestris* based paste, commercially available.
- Group 5: containing calcium hydroxide paste with propylene glycol vehicle.
- Group 6: negative control with sterile deionized water.

The applications of the respective substances were performed every 24 hours, totaling four applications for each group. The artificial saliva, in which the dentin blocks were immersed, was replaced once a week, every 7 days, at room temperature.

After the determined time (30 and 60 days), the samples were removed for later analysis of the mineralization capacity of the dentinal tubules, with the aid of scanning electron microscopy (SEM).

Preparation of samples for the scanning electron microscopy procedure

For the analysis of the mineralization capacity of the dentinal tubules, using a scanning electron microscope, the samples underwent a dehydration process in ethyl alcohol in different concentrations, 25%, 50%, 70%, 90%, and absolute alcohol (99.5%). The specimens were immersed in alcohol for 10 minutes, each concentration. In the sequence, the samples were stored in an oven (37ºC) for a period of 24 hours and then placed in a vacuum desiccator for 48 hours.

Figure No. 1A
Application of the tested substances in the dentin blocks with the aid of a sterile microbrush in sterile Petri dishes

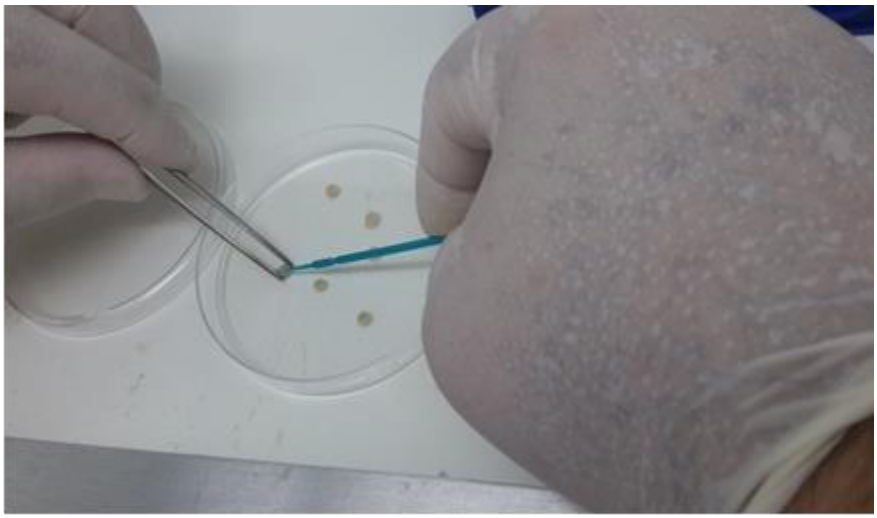


Figure No. 1B
Dentine blocks being packed in cell culture plates of 24 wells and covered by artificial saliva, after the application of the test substances

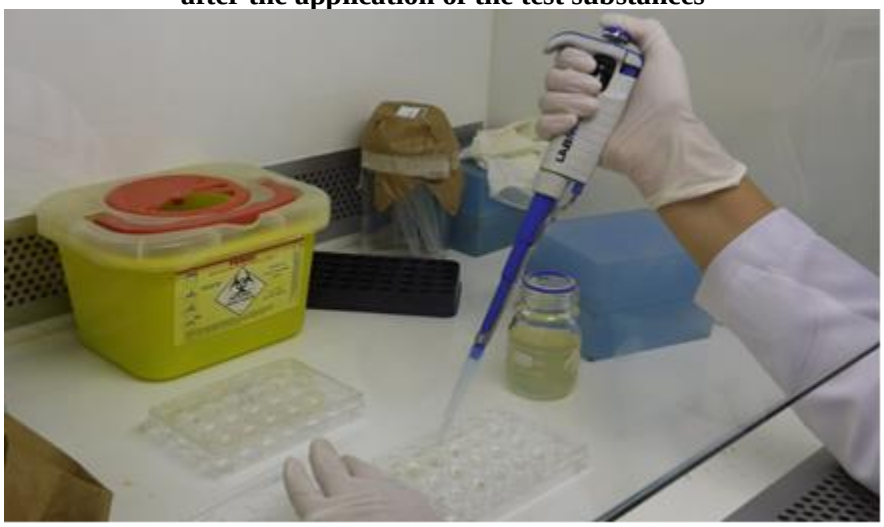
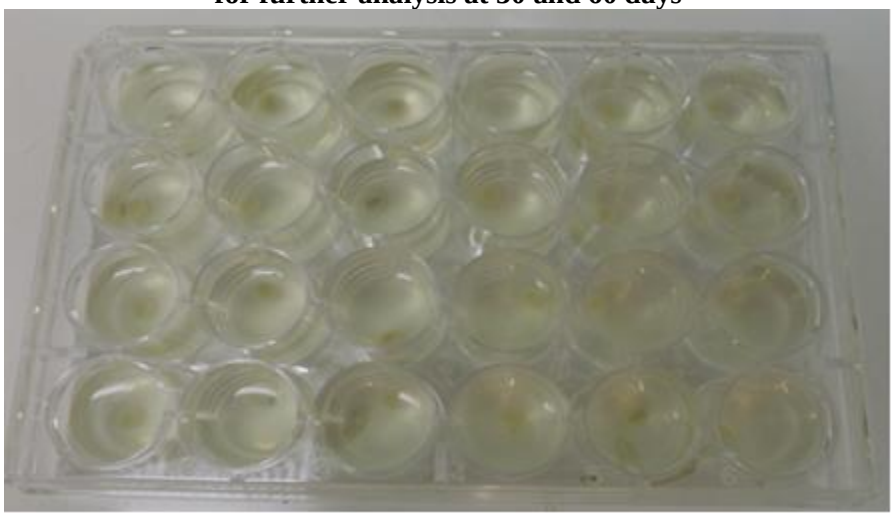


Figure No. 1C
Cell culture plate of 24 wells containing the dentin blocks with the respective test groups for further analysis at 30 and 60 days



The device used to metalize the samples was the Shimadzu C-50 (Shimadzu do Brasil Comércio Ltda, São Paulo, SP, Brazil) for 10 minutes. Then, a SEM Shimadzu SSX-550 Superscan (Shimadzu do Brasil Comércio Ltda, São Paulo, SP, Brazil) was used, with a power of 20 kV and increases of 500 and 1000 times, where 9 images of each block were obtained.

In the quantitative analysis of the photomicrographs, the number of open, partially closed, and closed tubules was observed with the help of a computer program (Image Pro Plus®), and in the qualitative analysis, criteria such as the characteristic of the dentin surface, obliterated dentin tubules and film deposit or precipitated were considered. The analysis of the photomicrographs was performed by a single observer, establishing a score from 1 to 4 according to the observed results (Al-Saud & Nahedh, 2012).

The Kruskal-Wallis and Dunn tests helped to calculate the comparative analysis of the dentin surface, established from the mean scores. To check the degree of dentin tubule obliteration, the Shapiro-Wilk normality test was used, followed by the one-way analysis of variance (ANOVA) and Tukey's test for multiple comparisons. Finally, to check the significance level ($p<0.05$), the software Prisma 6.0 (GraphPad Software Inc., La Jolla, CA, USA) was used.

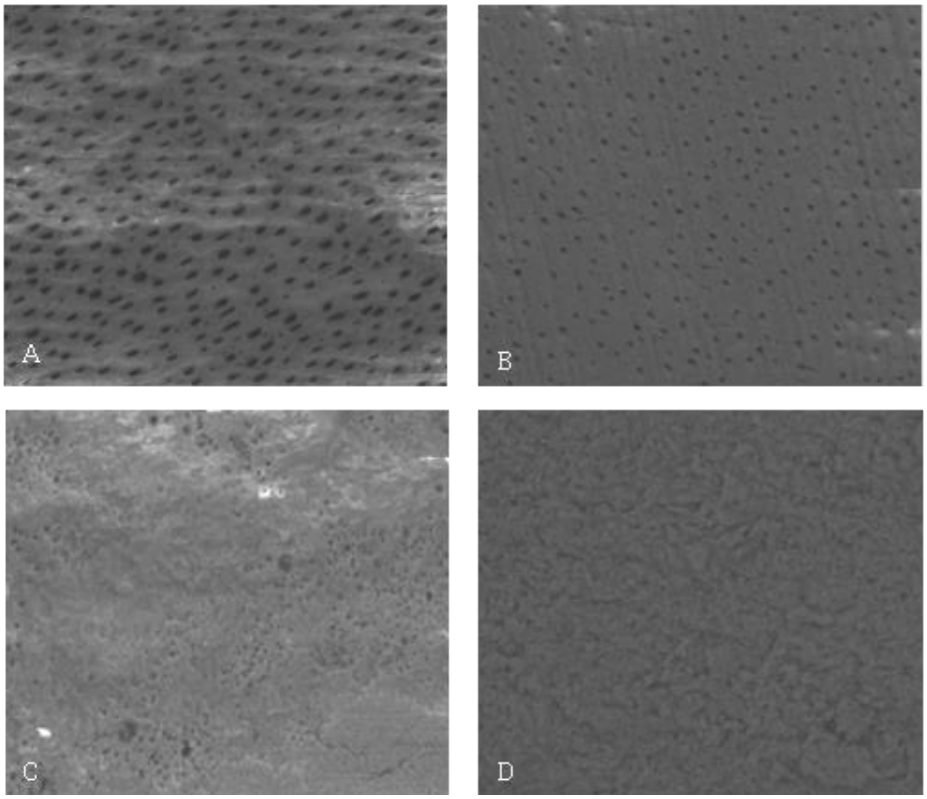
RESULTS

Mineralizing action of C. sylvestris (descriptive analysis of dentin surface)

The qualitative analysis of the obliteration of the dentinal tubules was established by means of scores 1 to 4, considering characteristics such as dentin surface and deposits in the dentinal tubules (Figure No. 2).

- Score 1: Dentinal tubules partially occluded, surface without precipitation.
- Score 2: Most occluded dentin tubules, surface without precipitation.
- Score 3: Most occluded dentin tubules, surface partially covered with film or precipitated.
- Score 4: All dentin tubules fully occluded and surface fully covered with film or precipitated.

Figure No. 2
Representative photomicrographs of the qualitative analysis of the dentinal tubules' obliteration (A: Score 1; B: Score 2: C: Score 3; D: Score 4)



In the comparative analysis between the mean and standard deviation of the scores in relation to the obliteration of the dentinal tubules, the results obtained revealed a significant difference ($p<0.05$) in the evaluation between groups 1 and 4 at 30 days (Figure No. 3). At 60 days no statistically significant differences were observed between the groups (Figure No. 4).

Figure No. 3
Average scores of dentin tubule obliteration obtained at 30 days in the different groups analyzed (G1: ethanolic extract; G2: diterpene fractions, rich in casearin; G3: diterpene fractions, rich in casearin + aqueous solution of iron III chloride (0.5 g/L); G4: paste based on *C. sylvestris*; G5: calcium hydroxide paste with propylene glycol vehicle; G6: negative control with sterile deionized water)

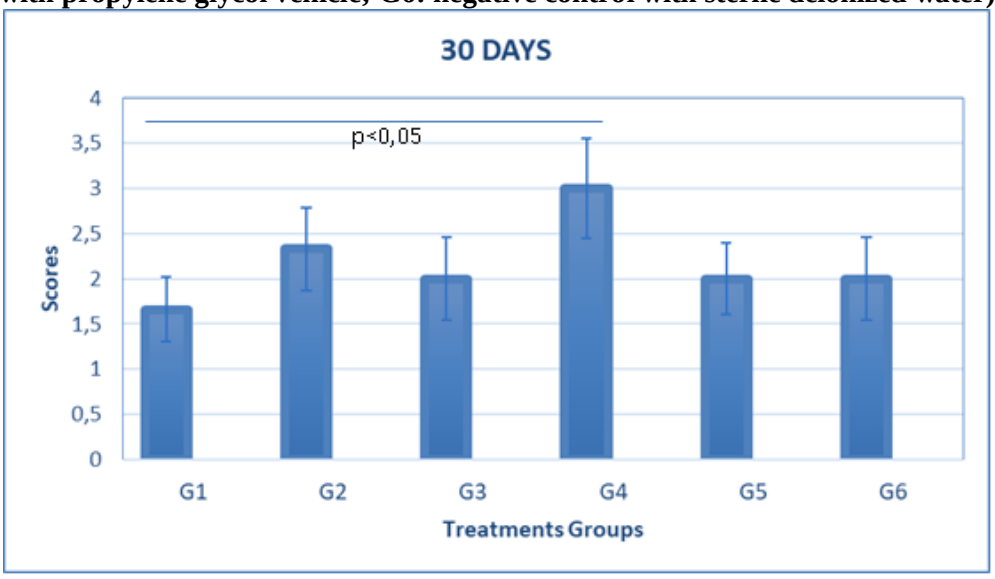
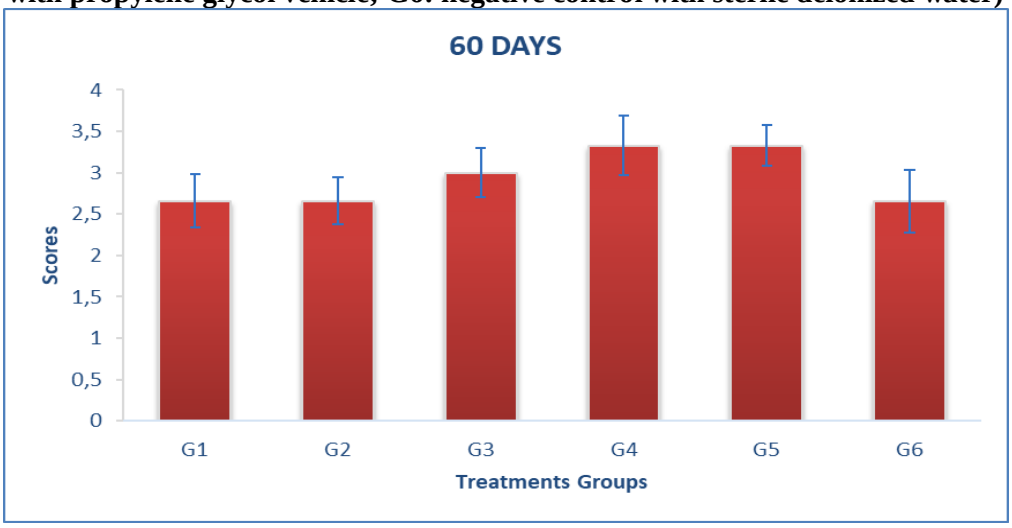


Figure No. 4
Average scores of dentinal tubule obliteration obtained at 60 days in the different groups analyzed (G1: ethanolic extract; G2: diterpene fractions, rich in casearin; G3: diterpene fractions, rich in casearin + aqueous solution of iron III chloride (0.5 g/L); G4: paste based on *C. sylvestris*; G5: calcium hydroxide paste with propylene glycol vehicle; G6: negative control with sterile deionized water)



Tables No. 1, No. 2, and No. 3 represent the mean and standard deviation from the counting of open, partially closed, and closed dentinal tubules of the different groups and treatment time. Figures No. 5, Figure No, 6, and Figure No. 7 show the relationship between the groups at different periods (30 and 60 days).

The comparison of the number of open dentin tubules between the same groups (Table No. 1), taking into account the analyzed periods, showed no difference in any of the proposed treatments (p>0.05).

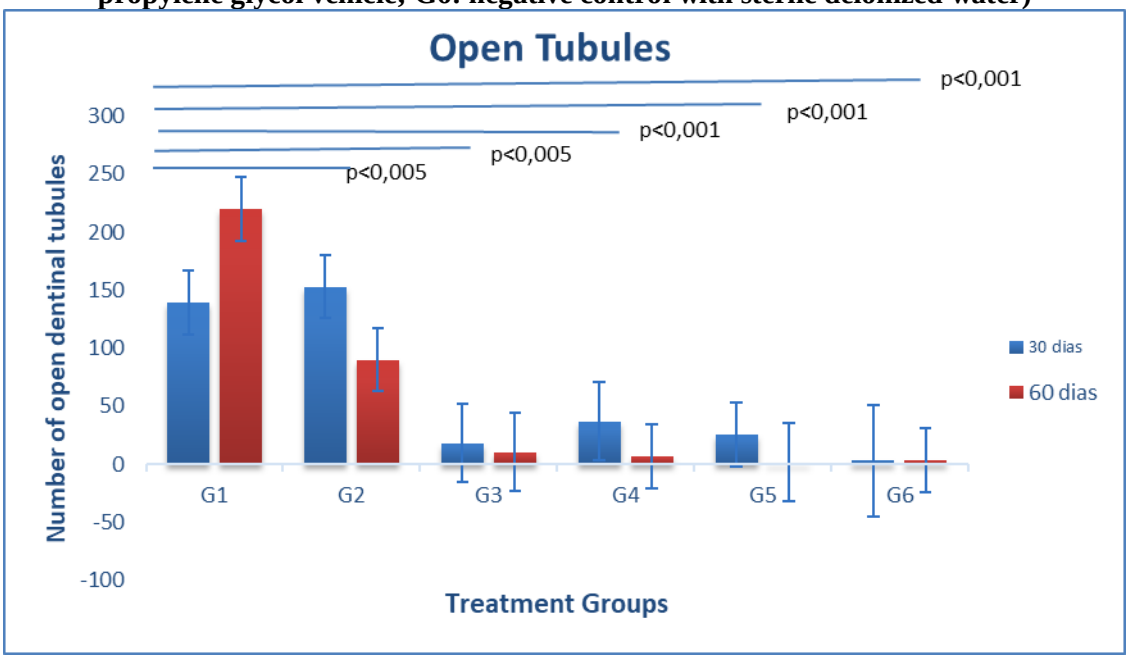
The open dentin tubules showed no difference between the groups at 30 days. The difference between treatments was found at 60 days (p<0.005) (Figure No. 5).

Table No. 1
Comparison between the mean and standard deviation of the number of open dentin tubules, at 30 and 60 days, in the different groups analyzed

	Dentinal Tubules Open					
	G1	**G2**	**G3**	**G4**	**G5**	**G6**
30 days	139,16 ± 27,47	153 ± 27,47	18 ± 33,64	37 ± 33,64	25,33 ± 27,47	3 ± 47,58
60 days	220 ± 27,47	90 ± 27,47	10,5 ± 33,64	7 ± 27,47	1,5 ± 33,64	3,66 ± 27,47
	p > 0,05	p > 0,05	p > 0,05	p > 0,05	p > 0,05	p > 0,05

G1: ethanolic extract; G2: diterpene fractions, rich in casearin; G3: diterpene fractions, rich in casearin + aqueous solution of Iron III Chloride (0,5 g/L); G4: paste based on *C. sylvestris;* G5: calcium hydroxide paste with propylene glycol vehicle; G6: negative control with sterile deionized water

Figure No. 5
Open dentinal tubules. Comparison between treatment groups, in periods of 30 and 60 days (G1: ethanolic extract; G2: fractions of diterpenes, rich in casearin; G3: fractions of diterpenes, rich in casearin + aqueous solution of Iron III Chloride (0.5 g/L); G4: paste based on *C. sylvestris*; G5: calcium hydroxide paste with propylene glycol vehicle; G6: negative control with sterile deionized water)



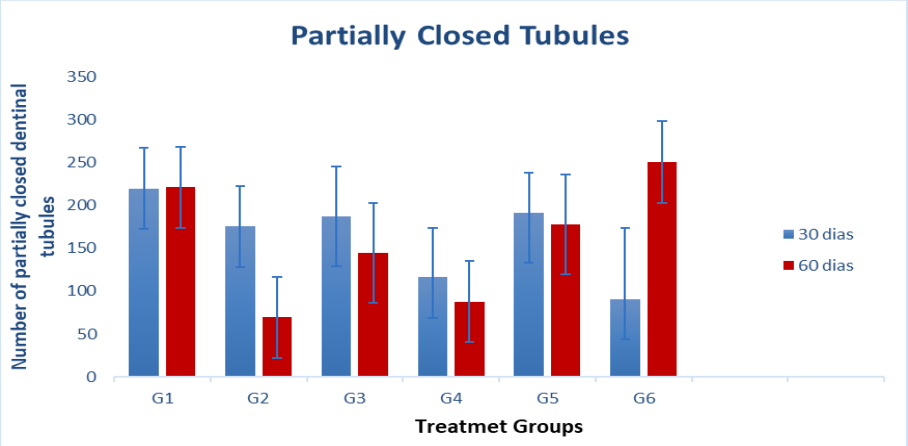
The number of partially closed dentin tubules (Table No. 2) showed no difference between treatment times within the same group or between different treatments at 30 and 60 days (p>0.05) (Figure No. 6).

Table No. 2
Comparison between the mean and standard deviation of the number of partially dentinal tubules closed, at 30 and 60 days, in the different groups analyzed

Partially Closed Dentinal Tubules						
	G1	**G2**	**G3**	**G4**	**G5**	**G6**
30 days	219,5 ± 47,41	175,33 ± 47,41	187 ± 58,07	116,5 ± 58,07	191 ± 47,41	91 ± 82,13
60 days	221 ± 47,41	69,33 ± 47,41	144,5 ± 58,0	87,66 ± 47,41	177,5 ± 58,07	250,33 ± 47,41
	p > 0,05	p > 0,05	p > 0,05	p > 0,05	p > 0,05	p > 0,05

G1: ethanolic extract; G2: diterpene fractions, rich in casearin; G3: diterpene fractions, rich in casearin + aqueous solution of Iron III Chloride (0,5 g/L); G4: paste based on *C. sylvestris;* G5: calcium hydroxide paste with propylene glycol vehicle; G6: negative control with sterile deionized water

Figure No. 6
Partially closed dentinal tubules. Comparison between treatment groups, in periods of 30 and 60 days (G1: ethanolic extract; G2: fractions of diterpenes, rich in casearin; G3: fractions of diterpenes, rich in casearin + aqueous solution of Iron III Chloride (0.5 g/L); G4: paste based on *C. sylvestris*; G5: calcium hydroxide paste with propylene glycol vehicle; G6: negative control with sterile deionized water)



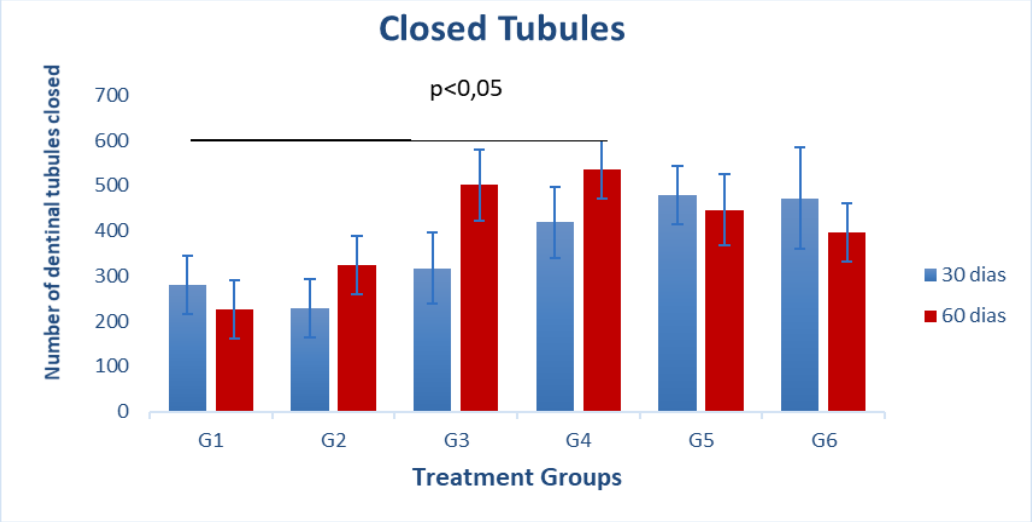
In the analysis between the same group (Table No. 3), regarding the closed dentin tubules, it was found that there was no difference between the periods (p>0.05). When the comparison between groups took place (Figure No. 7), a significant difference was noted only between groups 1 and 4, at 60 days (p<0.05).

Table No. 3
Comparison between the mean and standard deviation of the number of closed dentin tubules at 30 and 60 days in the different groups analyzed

Closed Dentinal Tubules						
	G1	**G2**	**G3**	**G4**	**G5**	**G6**
30 days	279,83 ± 64,4	230 ± 64,41	317,5 ± 78,8	419,5 ± 78,8	480 ± 64,41	472 ± 111,5
60 days	226 ± 64,41	324,66 ± 64,41	502 ± 78,8	536 ± 64,41	446,5 ± 78,8	397,33 ± 64,41
	$p > 0,05$	$p > 0,05$	$p > 0,05$	$p > 0,05$	$p > 0,05$	$p > 0,05$

G1: ethanolic extract; G2: diterpene fractions, rich in casearin; G3: diterpene fractions, rich in casearin + aqueous solution of Iron III Chloride (0,5 g/L); G4: paste based on *C. sylvestris;* G5: calcium hydroxide paste with propylene glycol vehicle; G6: negative control with sterile deionized water

Figure No. 7
Closed dentinal tubules. Comparison between treatment groups, in periods of 30 and 60 days (G1: ethanolic extract; G2: fractions of diterpenes, rich in casearin; G3: fractions of diterpenes, rich in casearin + aqueous solution of Iron III Chloride (0.5 g/L); G4: paste based on *C. sylvestris*; G5: calcium hydroxide paste with propylene glycol vehicle; G6: negative control with sterile deionized water)



DISCUSSION

As an aid to clinical studies, "*in vitro*" research with desensitizing agents has become frequent, mainly involving the use of plant species (Toledo *et al.*, 2003). However, it is important to consider that "*in vitro*" *research* has certain limitations, mainly because it does not fully reproduce the oral cavity environment, microorganisms, saliva, food patterns, as well as psychic determinants (Martineli *et al.*, 2001).

Demystifying the true mechanism of action by which dental hypersensitivity occurs, as well as the search for effective desensitizing agents, remains a great challenge in dentistry (Gillam *et al.*, 2002) since it represents a common cause among people, ranging from 4% to 57% of the population (Orchardson & Gillam, 2000).

Among the components of desensitizing toothpastes, several plant species have been tested, such as propolis (Ribeiro *et al.*, 2016); cashew nutshell liquid (Moreira, 2018), and *C. sylvestris*, proposed in this study.

The aqueous solution of iron chloride, associated with casearin and calcium hydroxide paste with propylene glycol vehicle, was used to establish the degree of effectiveness of *C. sylvestris* in the

obliteration of dentinal tubules (Ejima *et al*., 2013).

In the qualitative analysis of the dentin surface after 30 days, it was possible to observe differences in the scores, between the ethanolic extract and the paste based on *C. sylvestris*. The ethanolic extract showed no deposition of material on the surface, resulting in a large number of open dentin tubules. The treatment with *C. sylvestris*-based paste made it possible to verify the presence of surface material, and almost all the obliterated tubules.

It was also verified that at 30 days there was the proximity of results between groups 5 and 6, which may be justified by the fact that it is a closed system, *in vitro*, and could interfere with the mechanism of action of calcium hydroxide.

Dias *et al*. (2014), in their studies, evaluated the different dentin drying protocols and found that isopropyl alcohol maintained the wettability of the dentinal tubules by the lower water removal from the dentinal tubules. However, in our studies, even not using Isopropyl alcohol but 100% ethanol added to *C. sylvestris* extract, the results obtained were similar to those described by Dias *et al*. (2014), resulting in less deposition of precipitated and less amount of obliterated dentinal tubules.

The satisfactory results when the dentin surface was treated with *C. sylvestris*-based paste are in line with Cummins (2011), studies since the *C. sylvestris*-based paste contained calcium carbonate in its formulation. Thus, this association promotes the formation of a layer of calcium and phosphate on the dentin surface, leading to a reduction in dentin permeability by increasing the rate of tubular obliteration, culminating in a reduction in dentin sensitivity. All these characteristics were observed in the quantitative analysis at 60 days, where the group treated with *C. sylvestris* based paste showed an expressive amount of dentin tubules closed by deposition of material on the dentin surface.

Also, when establishing comparison patterns of the associations made between calcium carbonate and *C. sylvestris* extract, and calcium hydroxide having propylene glycol as the vehicle, it was verified that the first association presented similar results to the second one, being the last one considered the gold standard in the mineralization of dentinal tubules. However, the satisfactory results obtained from this association in the present study may represent a viable measure of home treatment for patients with dentin hypersensitivity (Godonfini *et al*., 2008), since the choice of treatment, in most cases, falls on the indication of desensitizing toothpastes (Pinto *et al*., 2012), which should be used regularly (Davies *et al*., 2011; Wang *et al*., 2011). It is important to emphasize the need for continuity of these studies since most of the tubular obliteration occurred at 60 days.

The conventional treatment for hypersensitivity is done through the use of anti-inflammatory drugs. Considering that *C. sylvestris* is rich in terpenes, which are a class of secondary metabolites formed by isoprenic units, classified according to the number of carbons: monoterpenes, sesquiterpenes, diterpenes and triterpenes, being that the triterpenes, of the oleanane type demonstrate anti-inflammatory and hepatoprotective effect, being this effect dependent on the inhibition of the enzymes lipooxygenase-5, nitric oxide synthetase, cyclooxygenase-2 and nuclear activation factor-κB (Zhang *et al*., 2013) , which may justify the effect of the extract in reducing hypersensitivity through the anti-inflammatory activity of this active, in the experimental protocol used.

It is important to emphasize that it is difficult to dissociate the effect of calcium carbonate isolated from *C. sylvestris* in an *in vitro* study, since in the present study obliteration of dentinal tubules was observed. Therefore, a clinical study is suggested, to evaluate to what extent the obliteration observed in the in vitro study was able to decrease pain sensitivity, which could be caused by the anti-inflammatory action of *C. sylvestris*.

CONCLUSION

The results obtained indicated a positive relationship in the use of *C. sylvestris* in the formulation of a toothpaste, for the purpose of obliteration of the dentinal tubules.

The satisfactory indicators, resulting from the association of *C. sylvestris* with calcium carbonate, by the obliteration capacity of the dentinal tubules, require new studies, including clinical trials, to better prove its efficacy in the treatment of dentinal hypersensitivity.

ACKNOWLEDGMENTS

To Universidade Paranaense, Universidade do

Sagrado Coração de Jesus, Faculty of Dentistry of Bauru (FOB - USP) and to the Chemistry Institute of Universidade Estadual Paulista de Araraquara in partnership with the pharmacognosy laboratory of the Faculty of Pharmaceutical Sciences - UNESP for supporting and encouraging research. I would also like to point out that all plant material as well as obtaining the extract of *C. sylvestris* and its fractions were kindly provided by student Fernando Bombarda Oda, from UNESP's Pharmacognosy Laboratory, under the guidance of Professor Dr. André Gonzaga dos Santos.

REFERENCES

Aboin E, Arquero P, Balboa V. 1987. Prostaglandina PGE1, análogo sintético (misoprostol) no manuseio de lesões de mucosa gástrica induzidas por etanol: trabalho experimental em ratos. **Arq Bras Med Vet Zoot** 61: 277 - 283.

Absi EG, Addy M, Adams D. 1987. Dentin hypersensitivity. A study of the patency of dentinal tubules in sensitive and non-sensitive cervical dentin. **J Clin Periodontol** 14: 280 - 228. **https://doi.org/10.1111/j.1600-051x.1987.tb01533.x**

Addy M, Urquhat E. 1992. Dentine hypersensitivity: Its prevalence, etiology, and clinical management. **Dent Update** 19: 10 - 12.

Al-Saud LMS, Al-Nahedh HNA. 2012. Occluding effect of Nd: YAG laser and different dentin desensitizing agents on human dentinal tubules *in vitro*: A scanning electron microscopy investigation. **Operative Dentistry** 37: 340 - 355. **https://doi.org/10.2341/10-188-L**

Aranha ACC. 2003. **Estudo *in vivo* da efetividade de diferentes métodos de dessensibilização dentinaria em lesões cervicais não cariosas**. Tesis, Universidade Estadual de Campinas, Brasil.

Arantes AB. 2002. **Desenvolvimento de dentifrícios com extratos fluidos de *Calendula officinalis* L. (Asteraceae) e *Casearia sylvestris* SW. (Flacoutiaceae), destinada ao tratamento de periodontias.** Tesis, Universidade Federal do Paraná, Brasil.

Arrais CAG, Chan DCN, Giannini M. 2004. Effects of desensitizing agents on dentinal tubule occlusion. **J Appl Oral Sci** 12: 144 - 148. **https://doi.org/10.1590/S1678-77572004000200012**

Assis JS, Rodrigues LKA, Fonteles CSR, Colares RCR, Souza AMBD, Santiago SL. 2011. Dentin hypersensitivity after treatment with desensitizing agents: a randomized, double-blind, split-mouth clinical trial. **Braz Dent J** 22: 157 - 161. **https://doi.org/10.1590/S0103-64402011000200012**

Baratieri LN, Monteiro S. 2015. **Odontologia restauradora**: fundamentos e possibilidades. Artes Médicas, São Paulo, Brasil.

Basile AC, Sertié JA, Panizza S, Oshiro TT, Azzolini CA. 1990. Pharmacological assay of *Casearia sylvestris*. I: Preventive anti-ulcer activity and toxicity of the leaf crude extract. **J Ethnopharmacol** 30: 185 - 197. **https://doi.org/10.1016/0378-8741(90)90007-g**

Bou DD, Lago JHG, Figueiredo CR, Matsuo AL, Guadagnin RC, Soares MG, Sartorelli P. 2013. Chemical composition and cytotoxicity evaluation of essential oil from leaves of *Casearia sylvestris*, Its main 142 compound α-zingiberene and derivatives. **Molecules** 18: 9477 - 9487. **https://doi.org/10.3390/molecules18089477**

Brännström M, Lindén LA, Aström A. 1967. The hydrodynamics of the dental tubule and of pulp fluid. A discussion of its significance in relation to dentinal sensitivity. **Caries Res** 1: 310 - 317. **https://doi.org/10.1159/000259530**

Brasil. Ministério da Saúde. 2018. Secretaria de Atenção à Saúde. Departamento de Atenção Básica. **Política nacional de práticas integrativas e complementares no SUS: atitude de ampliação de acesso** / Ministério da Saúde. Secretaria de Atenção à Saúde. Departamento de Atenção Básica. Ministério da Saúde, Brasília, Brasil.

Brasil. Ministério da Saúde. Secretaria de Ciência, Tecnologia e Insumos Estratégicos 2006. Departamento de Assistência Farmacêutica. **Política nacional de plantas medicinais e fitoterápicos / Ministério da Saúde, Secretaria de Ciência, Tecnologia e Insumos Estratégicos, Departamento de Assistência Farmacêutica.** Ministério da Saúde, Brasília, Brasil.

Bretz WA, Chiego DJ, Marcucci MC, Cunha I, Custódio A, Schneider LG. 1998. Preliminary report on the effects of propolis on wound healing in the pulp. **Z Naturforsch C J Biosci** 53: 1045 - 1048. **https://doi.org/10.1515/znc-1998-11-1217**

Bubteina N, Garoushi S. 2015. Dentine hypersensitivity: A review. **Dentistry** 5: 136 - 145. **https://doi.org/10.4172/2161-1122.1000330**

Canadian Advisory Board on Dentin Hypersensitivity. 2003. Consensus-based recommendations for the diagnosis and management of dentin hypersensitivity. **J Can Dent Assoc** 69: 221 - 226.

Cartwright RB. 2014. Dentinal hypersensitivity: a narrative review. **Comm Dent Health** 31: 15 - 20. **https://doi.org/10.1922/CDH_3287Cartwright06**

Carvalho PRF, Furlan M, Young MCM, Kingston DGI, Bolzani VS. 1998. Acetylated DNA-damaging clerodane diterpenes from Casearia sylvestris. **Phytochemistry** 49: 1659 - 1662. **https://doi.org/10.1016/s0031-9422(98)00249-0**

Cavalcante MS, Pereira TB, Tenório Neto JF, Santos NBD, Ribeiro CMB, Batista LHC. 2015. Improvement of cervical dentin hypersensitivity after two different treatments. **Rev Dor** 19: 259 - 262. **https://doi.org/10.5935/1806-0013.20150052**

Cavalcante WL, Campos TO, Dal Pai-Silva M, Pereira PS, Oliveira CZ, Soares AM, Gallacci M. 2007. Neutralization of snake venom phospholipase A2 toxins by aqueous extract of *Casearia sylvestris* (Flacourtiaceae) in mouse neuromuscular preparation. **J Ethnopharmacol** 112: 490 - 497. **https://doi.org/10.1016/j.jep.2007.04.002**

Cavallari MM, Gimenes MA, Billot C, Torres RB, Zucchi MI, Cavalheiro AJ, Bouvet JM. 2010. Population genetic relationships between *Casearia sylvestris* (Salicaceae) varieties occurring sympatrically and allopatrically in different ecosystems in south-east Brazil. **Ann Bot** 106: 627 - 636. **https://doi.org/10.1093/aob/mcq151**

Conselho Federal de Odontologia. 2008. **Resolução CFO-82/2008.** Reconhece e regulamenta o uso pelo cirurgião-dentista de práticas integrativas e complementares à saúde bucal. Rio de Janeiro, Brasil.

Costa CAS, Huck C. 2006. Efeitos citotóxicos e biocompatibilidade de agentes clareadores usados na odontologia: Uma revisão de literatura. **Robrac** 15: 3 - 14. **https://doi.org/10.36065/robrac.v15i39.79**

Csipak AR. 2011. **Potencial antimutagênico e antigenotóxico de compostos isolados da planta *Casearia sylvestris* em camundongos tratados com compostos extraídos da biomassa de queima de cana-de-açúcar.** Tesis, Universidade Estadual Paulista, Araraquara, Brasil.

Cummins D. 2011. Advances in the clinical management of dentin hypersensitivity: a review of recent evidence for the efficacy of dentifrices in providing instant and lasting relief. **J Clin Dent** 22: 100 - 107.

Cury VG. 2005. **Eficácia terapêutica de *Casearia sylvestris* sobre herpes labial e perspectiva de uso em saúde coletiva**. Tesis, Universidade Estadual de Campinas, Piracicaba, Brasil.

Da Silva FB, Almeida JM, Sousa SMG. 2004. Natural medicaments in endodontics a comparative study of the anti-inflammatory action. **Braz Oral Res** 18: 174 - 179. **https://doi.org/10.1590/S1806-83242004000200015**

Da Silva SL, Chaar JDS, Damico DC, Figueiredo PDM, Yano T. 2008. Antimicrobial activity of ethanol extract from leaves of *Casearia sylvestris*. **Pharmaceut Biol** 46: 347 - 351. **https://doi.org/10.1080/13880200801887963**

Davari AR, Ataei E, Assarzadeh H. 2013. Dentin hypersensitivity: etiology, diagnosis and treatment: A literature review. **J Dent Shiraz Univ Med Sci** 14: 136 - 145.

Davies M, Paice EM, Jones SB, Leary S, Curtis AR, West NX. 2011. Efficacy of desensitizing dentifrices to occlude dentinal tubules. **Eur J Oral Sci** 119: 497 - 503. **https://doi.org/10.1111/j.1600-0722.2011.00872.x**

Di Stasi LC. 1994. **Plantas medicinais:** arte e ciência. Um guia de estudo interdisciplinar. UNESP, São Paulo, Brasil.

Dias KC, Soares CJ, Steier L, Versiani MA, Abi Rached-Júnior FJ, Pécora JD, Silva-Sousa YTC, de Sousa-Neto MD. 2014. Influence of drying protocol with isopropyl alcohol on the bond strength of resin-based sealers to the root dentin. **J Endodontics** 40: 1454 - 1458. **https://doi.org/10.1016/j.joen.2014.02.021**

Duarte MA, Midena RZ, Zeferino MA, Vivan RR, Weckwerth PH, Dos Santos F, Guerreiro-Tanomaru JM, Tanomaru-Filho M. 2009. Evaluation of pH and calcium ion release of calcium hydroxide pastes containing different substances. **J Endodontics** 35: 1274 - 1277. **https://doi.org/10.1016/j.joen.2009.05.009**

Ejima H, Richardson JJ, Liang K, Best JP, van Koeverden MP, Such GK, Cui J, Caruso F. 2013. One-step assembly of coordination complexes for versatile film and particle engineering. **Science** 341: 154 - 157. **https://doi.org/10.1126/science.1237265**

Esteves I, Souza IR, Rodrigues M, Cardoso LGV, Santos LS, Sertie JAA, Perazzo FF, Lima LM, Schneedorf JM, Bastos JK, Carvalho JCT. 2005. Gastric antiulcer and anti-inflammatory activities of the essential oil from *Casearia sylvestris* Sw. **J Ethnopharmacol** 101: 191 - 196. **https://doi.org/10.1016/j.jep.2005.04.020**

Falcão L, Roman SS, Zakrzevski SB, Pereira A, Paroul N, Cansian RL. 2017. Ação antimicrobiana do óleo essencial de folhas de *Casearia sylvestris* Swartz. **Perspectiva** 41: 115 - 123.

Felipe KB, Kviecinskia MR, Silva FO, Bücker NF, Farias MS, Castro LSEPW, Grinevicius VMAS, Motta NS, Correia JFG, Rossi MH, Pedrosa RC. 2014. Inhibition of tumor proliferation associated with cell cycle arrest caused extract and fraction from *Casearia sylvestris* (Salicaceae). **J Ethnopharmacol** 155: 1492 - 1499.

Ferreira PMP, Militão GCG, Lima DJB, Costa NDJ, Machado KC, Santos AG, Cavalheiro AJ, Bolzani VS, Silva DHS, Pessoa C. 2014. Morphological and biochemical alterations activated by antitumor clerodane diterpenes. **Chem-Biol Interact** 222: 112 - 125. **https://doi.org/10.1016/j.cbi.2014.10.015**

Figueiredo CA, Gurgel IGD, Junior GDG. 2014. Política Nacional de Plantas Medicinais e Fitoterápicos: construção, perspectivas e desafios. **Physis** 24: 2. **https://doi.org/10.1590/S0103-73312014000200004**

Frankenberger L. 2017. **Terpenos de oleoresina de *Cola nitida* (Vent.) Schott & Endl. (Malvaceae): caracterização, semissíntese, avaliação antiprotozoária e anti-inflamatória *in vitro*.** Tesis, Universidade Federal de Santa Catarina, Brasil.

Frechoso SC, Menéndez M, Guisasola C, Arregui I, Tejerina JM, Sicilia A. 2003. Evaluation of the efficacy of two potassium nitrate bioadhesive gels (5% and 10%) in the treatment of dentine hypersensitivity. A randomized clinical trial. **J Clin Periodontol** 30: 315 - 320. **https://doi.org/10.1034/j.1600-051x.2003.20077.x**

Gillam GD, Tang JY, Mordan NJ, Newman HN. 2002. The effects of a novel Bioglass dentifrice on dentine sensitivity: a scanning electron microscopy investigation. **J Oral Rehab** 29: 305 - 313. **https://doi.org/10.1046/j.1365-2842.2002.00824.x**

Godolfini MG, Silvia FHPD, Gasparotto G, Carlo P. 2008. Calcium silicate coating derived from Portland cement as treatment for hypersensitive dentine. **J Dent** 36: 565 - 578. **https://doi.org/10.1016/j.jdent.2008.03.012**

Jorgensen MG, Carrol WB. 2002. Incidence of tooth sensitivity after home whitening treatment. **J Am Dent Assoc** 133: 1076 - 1082. **https://doi.org/10.14219/jada.archive.2002.0332**

Kerns DG, Scheidt MJ, Pashley DH, Horner JA, Strong SL, Van Dyke TE. 1991. Dentinal tubule occlusion and root hypersensitivity. **J Periodontol** 62: 421 - 428. **https://doi.org/10.1902/jop.1991.62.7.421**

Le Cointe P. 1934. **Amazônia Brasileira**. Árvores e plantas úteis. São Paulo, Brasil.

Leite TC, Dias KRHC. 2010. Efeitos dos agentes clareadores sobre a polpa dental: revisão de literatura. **Rev Bras Odont** 67: 203 - 208.

Lochaiwatana Y, Poolthong S, Hirata I, Okazaki M, Swasdison S, Vongsavan N. 2015. The synthesis and characterization of a novel potassium chloride-fluoridated hydroxyapatite varnish for treating dentin hypersensitivity. **Dent Material J** 2014: 102. **https://doi.org/10.4012/dmj.2014-102**

Lustosa LJ, Mesquita MA, Oliveira RJ. 2008. **Planejamento e controle da produção**. Elsevier, Brasil.

Mantzourani M, Sharma D. 2013. Dentine sensitivity: past, present, and future. **J Dent** 41: 3 - 17. **https://doi.org/10.1016/S0300-5712(13)70002-2**

Martineli ACBF, Santiago SL, Pereira JC. 2001. Avaliação da eficácia de agentes anti-hiperestésicos: métodos clínicos e laboratoriais. **Rev Fac Odont Porto Alegre** 9: 157 - 166.

Mesquita ML, Grellie P, Mambu, L, De Paula JE, Espíndola LS. 2007. *In vitro* antiplasmodial activity of Brazilian Cerrado plants used as traditional remedies. **J Ethnopharmacol** 110: 165.

https://doi.org/10.1016/j.jep.2006.09.015

Moraschini V, Barboza ESP. 2016. Use of platelet-rich fibrin membrane in the treatment of gingival recession: a systematic review and meta-analysis. **J Periodontol** 87: 281 - 290. **https://doi.org/10.1902/jop.2015.150420**

Moreira MM. 2018. **Síntese e caracterização de novo monômero derivado do líquido da casca da castanha de caju (LCC) e seu efeito na oclusão dos túbulos dentinários.** Tesis, Universidade Federal do Ceará, Brasil.

Mosaddik MA, Banbury L, Forster P, Booth R, Markham J, Leach D, Waterman PG. 2004. Screening of some Australian Flacourtiaceae species for *in vitro* antioxidant, cytotoxic and antimicrobial activity. **Phytomedicine** 11: 461 - 466. **https://doi.org/10.1016/j.phymed.2003.12.001**

Mosleh AA, Niazy M, El-yassaky M. 2018. Clinical and laboratory evaluation of the efficacy of three different treatment modalities in management of dentin hypersensitivity, **AL-AZHAR Dent J Girls** 5: 129 - 134.

Muzzin KB, Johnson R. 1989. Effects of potassium oxalate on dentine hipersensitivity in vivo. **J Periodontol** 60: 151 - 158. **https://doi.org/10.1902/jop.1989.60.3.151**

Neuhaus KW, Milleman JL, Milleman KR, Mongiello KA, Simonton TC, Clark CE, Proskin HM, Seemann R. 2013. Effectiveness of a calcium sodium phosphosilicate-containing prophylaxis paste in reducing dentine hypersensitivity immediately and 4 weeks after a single application: a double-blind randomized controlled trial. **J Clin Periodontol** 40: 349 - 357. **https://doi.org/10.1111/jcpe.12057**

Oberg C, Coutinho PG, Pochapski MT, Pilatti GL, Santos FA. 2006. Análise do potencial de substâncias dessensibilizantes na obliteração de túbulo dentinários – Estudo *in vitro*. **Rev Periodontia** 16: 71 - 77.

Oberlies NH, Burgess JP, Navarro HA, Pinos RE, Fairchild CR, Peterson RW, Soejarto DD, Farnsworth, NR, Kinghorn AD, Wani MC, Wall ME. 2002. Novel bioactive clerodane diterpenoids from the leaves and twigs of *Casearia sylvestris*. **J Nat Prod** 65: 95 - 99.

Oda FB, Crevelin EJ, Crotti AEM, Orlando AB, Medeiros AI, Nogueira FAR, Santos AG. 2019. Acidic and hepatic derivatives of bioactive clerodane diterpenes casearins J and O. **Fitoterapia** 137: 104197. **https://doi.org/10.1016/j.fitote.2019.104197**

Oda M, Matos AB, Liberti EA. 1999. Morfologia da dentina tratada com substâncias dessensibilizantes: avaliação através da microscopia eletrônica de varredura. **Rev Odont Univ Cidade de São Paulo** 13: 337 - 342.

Oh D, Prajatelistia E, Ju SW, Kim HJ, Baek SJ, Cha HJ, Jun SH, Ahn JS, Hwang DS. 2015. A rapid, efficient, and facile solution for dental hypersensitivity: The tannin-iron complex. **Sci Rep** 5: 10884. **https://doi.org/10.1038/srep10884**

Oliveira FQ, Gobira B, Guimarães C, Batista J, Barreto M, Souza M. 2007. Espécies vegetais indicadas na odontologia. **Braz J Pharmacogn** 17: 466 - 476.

OMS Estrategia de la OMS sobre medicina tradicional - 2014-2023. **http://apps.who.int/iris/bitstream/10665/95008/1/9789243506098spa.pdf**

Orchardson R, Gillam DG. 2006. The efficacy of potassium salts as agents for treating dentin hypersensitivity. **J Oral Facial Pain Headache** 14: 9 - 19.

Palazon MT, Scaramucci T, Aranha ACC, Prates RA, Lachowski KM, Hanashiro FS, Youssef MN. 2013. Immediate and short-term effects of in-office desensitizing treatments for dentinal tubule occlusion. **Photomed Laser Surg** 31: 274 - 282. **https://doi.org/10.1089/pho.2012.3405**

Pashley D, Tay F, Haywood V, Collins M, Drisko C. 2008. Dentin hypersensitivity and gingival recession. **Inside Dentistry** 4: 19 - 24.

Pereira FG, Marquete R, Oliveira-Cruz L, Quintanilha-Falcão D, Mansur E, de Lima Moreira D. 2017. Cytotoxic effects of the essential oil from leaves of *Casearia sylvestris* Sw. (Salicaceae) and its nanoemulsion on A549 tumor cell line. **Bol Latinoam Caribe Plant Med Aromat** 16: 506 - 512.

Pinheiro ML, Andrade, ED. 2008. Fitoterápicos como alternativa ao uso de medicamentos convencionais em odontologia. **Revista ABO Nacional** 16: 107 - 110.

Pinto SCS, Silveira CMM, Pochapski MT, Pillatti GL, Santos FA. 2012. Effect of desensitizing toothpastes on dentin. **Braz Oral Res** 26: 410 - 417. **https://doi.org/10.1590/s1806-83242012000500006**

Piovezan AP, Batisti AP, Benevides ML, Turnes BL, Martins DF, Kanis L, Duarte ECW, Cavalheiro AJ, Bueno PCP, Seed MP, Norling LV, Headland DCS, Souza PRPS, Perretti, M. 2017. Hydroalcoholic crude extract of *Casearia sylvestris* Sw. reduces chronic post-ischemic pain by activation of pro-resolving pathways. **J Ethnopharmacol** 204: 179 - 188. **https://doi.org/10.1016/j.jep.2017.03.059**

Prieto JA, Patiño OJ, Plazas EA, Pabón LC, Ávila MC, Guzmán JD, Delgado WA, Cuca LE. 2013a. Natural products from plants as potential source agents for controlling Fusarium. Fungicides-showcases of integrated plant disease management from around the world. Intech, Croacia. **https://doi.org/10.5772/52338**

Prieto AM, Santos AG, Oliveira APS, Cavalheiro AJ, Silva DHS, Bolzani VS, Varanda EA, Soares CP. 2013b. Assessment of the chemopreventive effect of casearin B, a clerodane diterpene extracted from *Casearia sylvestris* (Salicaceae). **Food Chem Toxicol** 53: 153 - 159. **https://doi.org/10.1016/j.fct.2012.11.029**

Rebelo D, Loureiro M, Ferreira P, Paula A. 2011. Carrilho E. Tratamento médico dentário da hipersensibilidade dentinária: o estado da arte. **Rev Portuguesa Estomatol Med Dent Cirurgia Maxilofacial** 52: 98 - 106.

Ribeiro PJT, Araújo AMPD, Mafra RP, Vasconcelos MG, Vasconcelos RG. 2016. Mecanismos de ação dos recursos terapêuticos disponíveis para o tratamento da hipersensibilidade dentinária cervical. **Rev Odontol Clín-Científ Scientif** 15: 83 – 90.

Ritter AV, Dias WL, Miguez P, Caplan DJ, Swift Jr EJ. 2006. Treating cervical dentin hypersensitivity with fluoride varnish: a randomized clinical study. **J Am Dent Assoc** 137: 1013 - 1020. **https://doi.org/10.14219/jada.archive.2006.0324**

Roberts MC. 2002. Antibiotic toxicity, interactions, and resistance development. **J Periodontol** 28: 280 - 297. **https://doi.org/10.1034/j.1600-0757.2002.280112.x**

Rosa C, Câmara SG, Béria JU. 2011. Representações e intenção de uso da fitoterapia na atenção básica à saúde. **Ciênc Saúde Coletiva** 16: 311 - 318. **https://doi.org/10.1590/S1413-81232011000100033**

Sassioto MCP; Filho NC, Facco GG, Sodré ST, Neves N, Purisco SU, Farias AG. 2004. Efeito da *Casearia sylvestris* no reparo ósseo com matriz óssea bovina desvitalizada em ratos. **Acta Cirúrgica Bras** 19: 637 - 641. **https://doi.org/10.1590/S0102-86502004000600010**

Schneider NFZ, Moura NF, Colpo T, Flach A. 2006. Composição química e atividade antimicrobiana do óleo volátil de *Casearia sylvestris* Swart. **Rev Bras Farm** 87: 112 - 114.

Schoenfelder T, Pich CT, Geremias R, Ávila S, Daminelli EN, Pedrosa RC, Bettiol J. 2008. Antihyperlipidemic effect of *Casearia sylvestris* methanolic extract. **Fitoterapia** 79: 465 - 467. **https://doi.org/10.1016/j.fitote.2008.03.008**

Shetty S, Kohad R, Yeltiwar R. 2010. Hydroxyapatite as an in-office agent for tooth hypersensitivity: a clinical and scanning electron microscopic study. **J Periodontol** 81: 1781 - 1789. **https://doi.org/10.1902/jop.2010.100172**

Shiau HJ. 2012. Dentin hypersensitivity. **J Evid Based Dent Pract** 12: 220 - 228. **https://doi.org/10.1016/S1532-3382(12)70043-X**

Silva AC, Balz D, De Souza JBD, Morsc V, Corrêa MC, Zanetti GD, Manfron MP Schetinger MRC. 2006. Inhibition of NTPDase, 50-nucleotidase, Na+/K+-ATPase and acetylcholinesterase activities by subchronic treatment with *Casearia sylvestris*. **Phytomedicine** 13: 509 - 514. **https://doi.org/10.1016/j.phymed.2005.01.011**

Silva M, Ginjeira A. 2011. Hipersensibilidade dentinária: etiologia e prevenção. **Rev Portuguesa Estomatol Med Dent Cirurgia Maxilofacial** 52: 217 - 224. **https://doi.org/10.1016/j.rpemd.2011.09.002**

Silva TK, Marchi A, Soares M, Miranda E, Junior E, Friedrich J, Boleta-Ceranto D. 2016. Avaliação do efeito de pomada a base de *Casearia sylvestris* para o tratamento de ulcerações aftosas recorrentes. **Rev Científ** 15: 126.

Sneed WD, Looper SW. 1985. Shear bond strength of a composite resin to an etched glass ionomer. **Dent Material J** 1: 127 - 128. **https://doi.org/10.1016/S0109-5641(85)80003-8**

Sobral MAP. 2003. **Lesões cervicais não cariosas e hipersensibilidade dentinária cervical**. In: Garone Netto N, Carvalho RCR, Russo EMA, Luz MAA, Sobral MA. Introdução a Dentística Restauradora. Santos, São

Paulo, Brasil.
Sousa FCF, Melo CTV, Citó MCO, Félix FHC, Vasconcelos SMM, Fonteles MMF, Filho JMB, Viana GSB. 2008. Plantas medicinais e seus constituintes bioativos: uma revisão da bioatividade e potenciais benefícios nos distúrbios da ansiedade em modelos animais. **Braz J Pharmacogn** 18: 642 - 654. **https://doi.org/10.1590/S0102-695X2008000400023**
Soyama P. 2007. Plantas medicinais são pouco exploradas pelos dentistas. **Rev Ciênc Cultura** 59: 12 - 13.
Splieth CH, Tachou A. 2013. Epidemiology of dentin hypersensitivity. **J Clin Oral Inv** 17: 3 - 8. **https://doi.org/10.1007/s00784-012-0889-8**
Spósito L, Oda FB, Vieira JH, Carvalho FA, Ramos MAS, Castro RC, Crevelin EJ, Crotti AEM, Santos AG, Silva PB, Chorilli M, Bauab TM. 2019. *In vitro* and *in vivo* anti-helicobacter pylori activity of *Casearia sylvestris* leaf derivatives. **J Ethnopharmacol** 233: 1 - 12. **https://doi.org/10.1016/j.jep.2018.12.032**
Tian L, Peng C, Shi Y, Guo X, Zhong B, Qi J, Wang G, Cai Q, Cui F. 2014. Effect of mesoporous silica nanoparticles on dentinal tubule occlusion: An *in vitro* study using SEM and image analysis. **Dent Mater J** 33: 125 - 132. **https://doi.org/10.4012/dmj.2013-215**
Toledo ACO, Hirata LL, Buffon MDCM, Miguel MD, Miguel OG. 2003. Fitoterápicos: uma abordagem farmacotécnica. **Rev Farm Biol Univ São Francisco (Lecta)** 21: 7 - 13.
Trentin MS, Bervian J. 2014. Hipersensibilidade dentinária cervical: uma revisão da literatura. **Rev Fac Odontol UPF** 19: 252 - 257. **https://doi.org/10.5335/rfo.v19i2.3572**
Vano M, Derchi G, Barone A, Pinna R, Usai P, Covani U. 2018. Reducing dentine hypersensitivity with nano-hydroxyapatite toothpaste: a double-blind randomized controlled trial. **J Clin Oral Inv** 22: 313 - 320. **https://doi.org/10.1007/s00784-017-2113-3**
Varoni EM, Lodi G, Sardella A, Carrassi A, Iriti M. 2012. Plant polyphenols and oral health: old phytochemicals for new fields. **Curr Med Chem** 19: 1706 - 1720. **https://doi.org/10.2174/092986712799945012**
Vongsavan, N., Matthews, B. 1991. The permeability of cat dentine *in vivo* and *in vitro*. **Arch Oral Biol** 36: 641. **https://doi.org/10.1016/0003-9969(91)90016-n**
Wang Z, Jiang T, Sauro S, Pashley DH, Toledano M, Osorio R, Liang S, Xing W, Sa Y, Wang Y. 2011. The dentine remineralization activity of a desensitizing bioactive glass-containing toothpaste: an *in vitro* study. **Aust Dent J** 56: 372 - 381. **https://doi.org/10.1111/j.1834-7819.2011.01361.x**
Weckwerth PH, Siquinelli NB, Weckwerth ACVB, Vivan RR, Duarte MAH. 2011. Determinação *in vitro* do efeito antimicrobiano direto do hidróxido de cálcio associado a diferentes substâncias frente a cepas de *Enterococcus faecalis*. **Dental Press Endodontics** 1: 46 - 51.
West NX, Hughes JA, Addy M. 2001. The effect of pH on the erosion of dentine and enamel by dietary acids *in vitro*. **J Oral Rehabil** 28: 860 - 864. **https://doi.org/10.1046/j.1365-2842.2001.00778.x**
Wichgers TG, Emert RL. 1997. Dentin hypersensitivity**. Oral Health** 87: 51 - 59.
Xia L, Guo Q, Tu P, Chai X. 2015. The genus *Casearia*: a phytochemical and pharmacological overview. **J Phytochem Rev** 14: 99 - 135.
Yoshiyma AM, Suge T, Kawasaki A, Ebisu S. 1996. Morphological characterization of tube-like structures hypersensitive human radicular dentin. **J Dent** 24: 57 - 63. **https://doi.org/10.1016/0300-5712(95)00016-x**
Zhang Y, Ning Z, Lu C, Zhao S, Wang J, Liu B, Xu X, Liu Y. 2013. Triterpenoid resinous metabolites from the genus *Boswellia*: pharmacological activities and potencial species-identifying properties. **Chem Central J** 7: 1 - 16. **https://doi.org/10.1186/1752-153X-7-153**