Chapter

Biopharmaceutical Products and Biomaterials of the Amazon Region Used in Dentistry

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Abstract

In dentistry, biomaterials are used in restorative procedures, such as dental restorations; in dentures; dental implants; surgical procedures; and endodontic materials. Most dental biomaterials are classified as devices, including filling materials, diagnostic aids, cements, bonding agents, and implants, in addition to mouthwashes. In the field of health, the use of natural products for dental biomaterials and curing diseases has always emphasized, rather than depending on the conventional allopathic medicine. Brazil has an advantage in this market, because it has the greatest biodiversity in the world, especially in the Amazonian Region, and a genetic heritage of great potential for the development of new herbal products, especially in dentistry. Given the growth of products derived from medicinal plants in Brazil, it was necessary to implement a statute that covered the requirements for all medicines and biomaterials to ensure the quality, efficacy, and safety of these products. Thus, researches in dentistry have been developed with the aim of searching for new bioactive principles for the formulation of drugs with different types of applications, capable of acting in both preventive strategies and curative treatments. This has encouraged the use of phytotherapic agents such as *Copaifera multijuga, Apis mellifera* (propolis), and *Libidibia ferrea*.

Keywords: medicinal plants, *Copaifera multijuga*, *Apis mellifera*, *Libidibia ferrea*, dentin surface, orabase, mouthwash

1. Introduction

According to the American National Institute of Health (NIH), biomaterial is defined as "any substance or combination of substances, other than drugs, synthetic or natural in origin, which can be used for any period of time, which augments or replaces partially or totally any tissue, organ or function of the body, in order to maintain or improve the quality of life of the individual" [1]. The advances led to a pronounced increase in the range of use and efficacy of biomaterials over time. Thus, biomaterials have become critical components used in many industries,

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including medical devices, dental restoratives, and drug delivery, and are increasingly being used in technological applications such as in vitro diagnostics [2, 3].

These materials must be biocompatible at the material-tissue interface: "ability of a biomaterial to perform its desired function with respect to a medical therapy, without eliciting any undesirable local or systemic effects in the recipient or beneficiary of that therapy, but generating the most appropriate beneficial cellular or tissue response to that specific situation" [1]. In dentistry, biomaterials are used in restorative procedures, such as dental restorations; dentures; dental implants; surgical procedures; and endodontic materials. Most dental biomaterials are classified as devices, including filling materials, diagnostic aids, cements, bonding agents, and implants [1, 4, 5]. In addition, commercial mouthwashes are used as antiseptics for better oral hygiene. Therapeutic mouthwashes reduce bacterial counts, have antiplaque effects, work as an astringent, and help to reduce gingivitis and carious lesions [6, 7].

Dental materials should not be toxic, irritant, or corrosive and should be easy to use. The biomaterials used in dentistry can be metals (amalgam of silver, titanium, and gold), ceramics (feldspar, alumina, zirconia, porcelain reinforced with silica), composites [1], materials that can optimize dentin bonding, and mouthwashes. However, in the field of health, the use of natural products for dental biomaterials and curing of diseases has always emphasized, rather than depending on the conventional allopathic medicine [7].

Following this trend, in addition to the devices and materials themselves, biological advances have revolutionized the methods used in the chemical and material industries to produce and transform raw materials. Living plants can be processed in large quantities to produce a much larger variety of liquids and materials, without the cost of energy or effluent streams—typical by-products of the chemical industry. Nature is not only capable of allowing the synthesis of new chemical substances but also significantly reducing the costs and environmental impacts associated with the manufacture of current chemicals and drugs [3].

In 1978, the World Health Organization (WHO) recognized medicinal plants as a therapeutic resource [8]. The Ordinance No. 971 dated May 03, 2006, approved the National Policy on Integrative and Complementary Practices (PNPIC) in the Unified Health System (SUS) in Brazil, including the use of phytotherapy [9]. At present, phytotherapy is defined as a science-based practice for the treatment of diseases, which uses medicinal plants, plant drugs, and preparations, not including substances from another source [10]. Therefore, biomaterials made today are routinely information rich and incorporate biologically active components derived from nature [2, 3, 5, 7]. Today, the variety of natural products used in the biomaterials for dental and oral health care may include natural silk [11], propolis [12–15], chitosan [16], herbal tea [17], and miswak [18], as well as natural products for bone repair such as dolomite [4].

Since phytotherapy is a feasible method for the control and prevention of the development of oral pathologies, with the additional possibility of incorporating phyto-derived compounds into biomaterials, the discovery of new phytotherapic compounds has been of high relevance to dentistry [5, 14, 19–22]. Brazil has an advantage in this market because it has the greatest biodiversity in the world, especially in the Amazo nian Region, and a genetic heritage of great potential for the development of new herbal products [23], especially in dentistry. Given the growth of products derived from medicinal plants in Brazil, it was necessary to implement a statute that covered the requirements for all medicines and/or biomaterials to ensure the quality, efficacy, and safety of these products. In this sense, the Brazilian National Health Surveillance Agency (ANVISA) establishes product quality control requirements, involving stages ranging from obtaining of raw materials through to the qualitative and quantitative characterization of their active principles [24, 25].

The official recognition of phytotherapy in dentistry in Brazil was accompanied by several gaps in scientific research on medicinal plants, specifically for plant species with applications in diseases of the oral cavity. The state of Amazonas, specifically the city of Manaus, did not have a diagnosis of the applicability of medicinal plants in dental services. In this sense, these researchers conducted an ethnobotanical study to identify the main plants used for pathogenesis of the oral cavity, with the aim of reducing their empirical use and favoring the use of medicinal plants based on scientific evidence [10].

The search for the biomaterials, their development, and pharmaceutical forms comprises products derived from medicinal plants with compounds that are safe and have proven quality. Thus, researches in dentistry have been developed with the objective of searching for new bioactive principles for the formulation of drugs with different types of applications, capable of acting in both preventive strategies and curative treatments, thus encouraging the use of phytotherapic agents such as *Copaifera multijuga*, *Apis mellifera* (propolis), and *Libidibia ferrea*.

2. *Copaifera multijuga*—copaiba oil

According to the growing interest in antimicrobial agents derived from medicinal plants, natural products are considered an excellent alternative to synthetic chemicals. Amazonian biodiversity products that have been used for years in folk medicine have emerged as feasible and promising alternatives for inhibiting microorganisms in dental biofilm. Copaiba oil—as it is popularly called—a phytotherapeutic agent widely used by the Amazonian population, is known for its antibacterial, anti-inflammatory, anesthetic healing and antitumoral medicinal properties.

The studies developed with copaiba oil have complied with all the norms required. The *Copaifera multijuga* Hayne species were collected from official research institutions (National Research Institute of Amazonian—INPA) to guarantee the legitimacy of the species. The exsiccata was stored in the INPA Herbarium under No. 270709.

2.1 Antibacterial activity of copaiba oil formulations

The first reports demonstrated the use of copaiba oil as an effective agent against the etiological agents of caries disease, as seen in Ref. [26]. This research demonstrated the antibacterial activity of calcium hydroxide and zinc oxide pastes associated with essential oil and *C. multijuga* resin against *Streptococcus mutans*. The bacteriostatic and bactericidal activities of the oil *in natura* against the same microorganism were also reported, giving rise to a line of research in dentistry in the search of scientific evidence.

C. multijuga Hayne has presented promising antibacterial activity against *S. mutans, S. mitis*, *S. salivarius*, *S. constellatus*, and *S. sanguinis* from the copaiba gel production for the dental biofilm control [27]. In addition, evidence was shown of antibacterial activity against *Enterococcus faecalis* and *Candida albicans* present in endodontic flora, biological compatibility test in the teeth of rats and dogs using the copaiba oil as vehicle for calcium hydroxide [26], biological compatibility test in gingival tissue and a physical-chemical study and antibacterial activity dental cement to suit the conditions of the oral environment [28], and antibacterial activity in copaiba oil emulsions, as shown in **Table 1** [22].

The results of the chromatographic analysis of *C. multijuga* oil revealed that the structures of its components are made up of various sesquiterpenes, primarily being constituted of β-caryophyllene and its oxide, forming its biological activity [26–28].

Tested copaiba emulsions	MIC of the copaiba emulsions (μL/mL) against the bacteria			
	S. mutans (μL/mL)	*S. oralis* (μL/mL)	*S. salivarius* (μL/mL)	*L. casei* (μL/mL)
Emulsion 10%	12.5	12.5	12.5	12.5
Emulsion 10% + PB 1%				
Emulsion 30%	37.5	37.5	37.5	37.5
Emulsion 30% + PB 1%				

Table 1.
Minimum inhibitory concentration (MIC) of copaiba emulsions against microorganisms.

In [29], a high proportion of sesquiterpenes (88.55–98.05%) was revealed in the copaiba oils analyzed, with β-caryophyllene being the main type.

2.2 Copaiba oil emulsion as dentin biomodifier

Different approaches have been proposed to improve the restorative material bond to the dental structure by optimizing the infiltration of resinous monomers into the demineralized dentin and reduce the rate of water absorption and collagen degradation, by means of such as the application of an additional layer or multiple layers of a hydrophobic adhesive agent [30], vigorous solvent evaporation [31], polymerization, and the use of electric current to improve impregnation of the monomers [32].

Metalloproteinases (MMPs) trapped in the extracellular matrix are calcium-dependent and zinc-activated enzymes that mediate the denaturation of the extracellular matrix through collagenase (MMP-8 is the major collagenase in human dentin) and gelatinase (MMP-2 and MMP-9), as well as the enamelysin MMP-20 and the stromelysin MMP-3, which are naturally entrapped in the mineralized dentin during odontogenesis [33]. Since the bonding process occurs as a result of encapsulation of the collagen by the adhesive system, it is necessary to inhibit these enzymes to preserve the adhesive interface from proteolytic and hydrolytic degradation, by forming the hybrid layer [34].

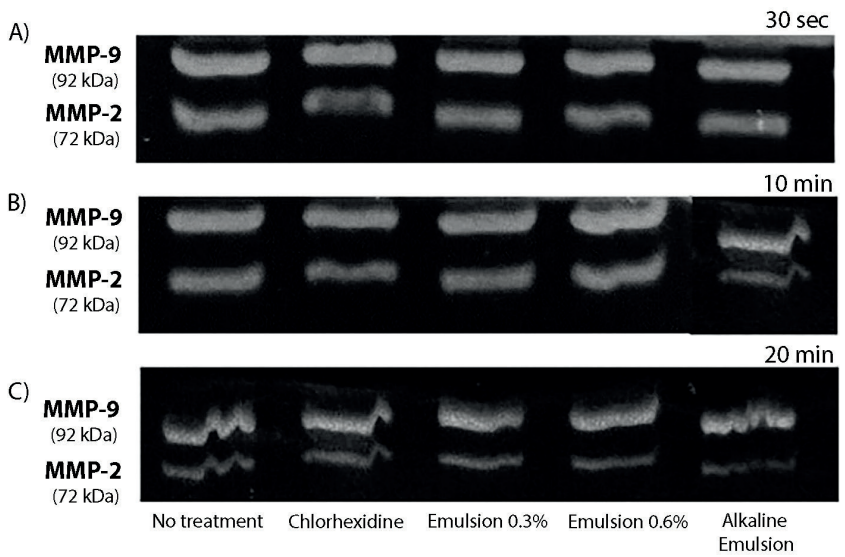


Figure 1.
Effect of copaiba oil on metalloproteinase (MMP) activity (gelatin zymography).

In recent studies, a zymography assay was performed with HT1080 cells. This demonstrated that the copaiba oil emulsion (CO) as dentin biomodifier showed the potential to inhibit matrix metalloproteinases –2 and –9 (**Figure 1**).

In the time of 30 seconds, statistical difference in the decrease of the MMP-9 activity was observed among all solutions tested when compared with the control group without treatment. In the time of 10 minutes, there was a statistical difference only between chlorhexidine (CLX), 10% CO + 0.3% CV, and 10% alkaline CO. At 20 minutes, the only solution that presented a statistical difference in the decrease of MMP-9 activity was the 10% CO + 0.3% CV. The best result obtained was with 10% alkaline CO in a time of 10 minutes, with a 35% decrease in MMP-9 activity (**Figure 2A**).

A decrease in the MMP-2 enzymatic activity was also observed. At 30 seconds, there was a statistical difference between the CLX solution and the copaiba oil emulsion at 0, when compared to the control group. At 10 minutes, a statistical difference was observed in the CLX solution and the alkaline CO, each presenting a 44 and 67% reduction in enzyme activity, respectively. Finally, at 20 minutes, there was a statistical difference in the CLX solution (21%), in the COs 0.3% (42%) and 0.6% (53%), and in the alkaline CO (**Figure 2B**).

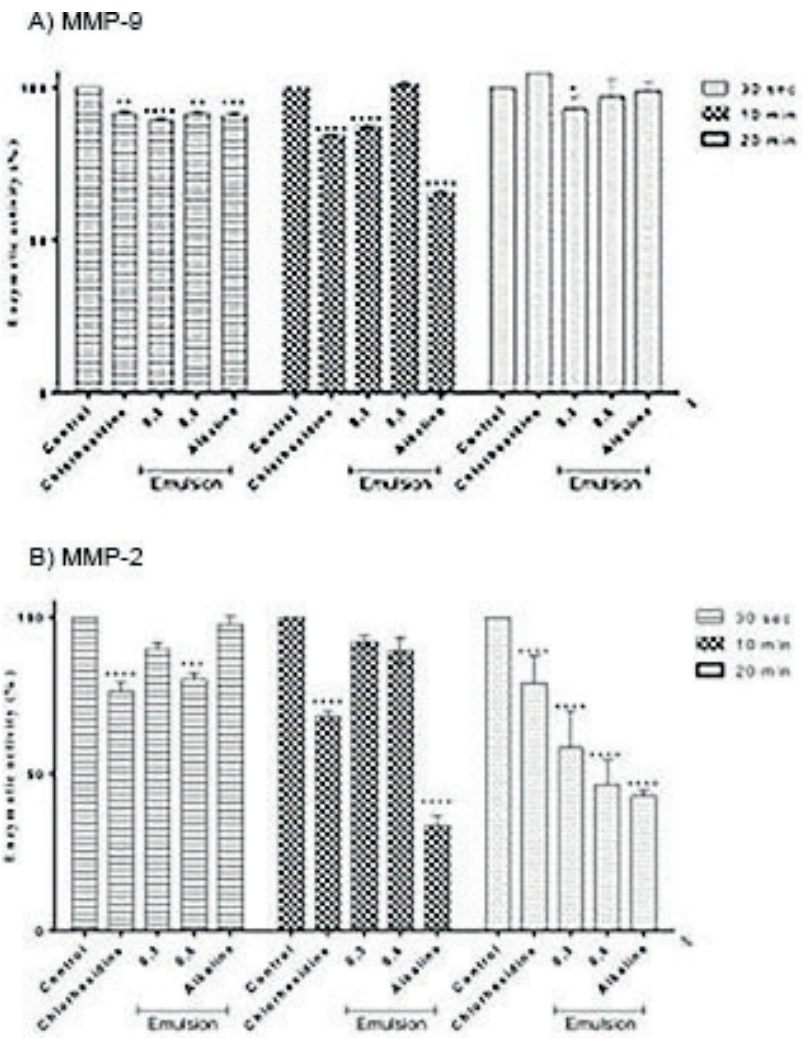


Figure 2.
Anti-proteolytic activity of the copaiba oil emulsions on (A) MMP-9 and (B) MMP-2.

2.3 Influence of copaiba oil emulsions as dentin surface biomodifiers

Bandeira et al. [5] used scanning electron microscopy (SEM) to investigate the morphology of the dentin surface, cut and treated with CO and ethanolic extract of propolis, with the aim of using them as bioactive agents for cleaning teeth. For the formulations of 10% CO, 10% CO + PB, 30% CO, and 30% CO + PA emulsions, the same cleaning pattern was obtained as that obtained with 2% chlorhexidine, which is considered the gold standard, because it had substantivity and showed bacteriostatic and bactericidal activities (**Figure 3**).

The bond of polymer-based materials to dentin is still considered a significant challenge because the latter is a complex substrate, predominantly tubular, and intrinsically moist. The use of disinfectant and anti-proteolytic solutions may be an alternative for reducing these effects. In a histological evaluation, 10% CO was used on the exposed collagen of the dentin matrix, with the purpose of verifying whether there was interference in the adhesive system. Thus, 80 specimens (CPs) were prepared from healthy third molars, and after the induction of caries lesions, the specimens were treated with test materials for 3 months of aging. The CPs treated with the copaiba emulsions presented higher exposed and hybridized collagen thickness values than the groups treated with CLX 2% and AD. Relative to caries-affected dentin, the group treated with CLX 2% showed a higher proportion of CPs with hybridized collagen. The emulsion presented 100% specimens with hybridized collagen and improved hybrid layer homogeneity (**Figure 4**).

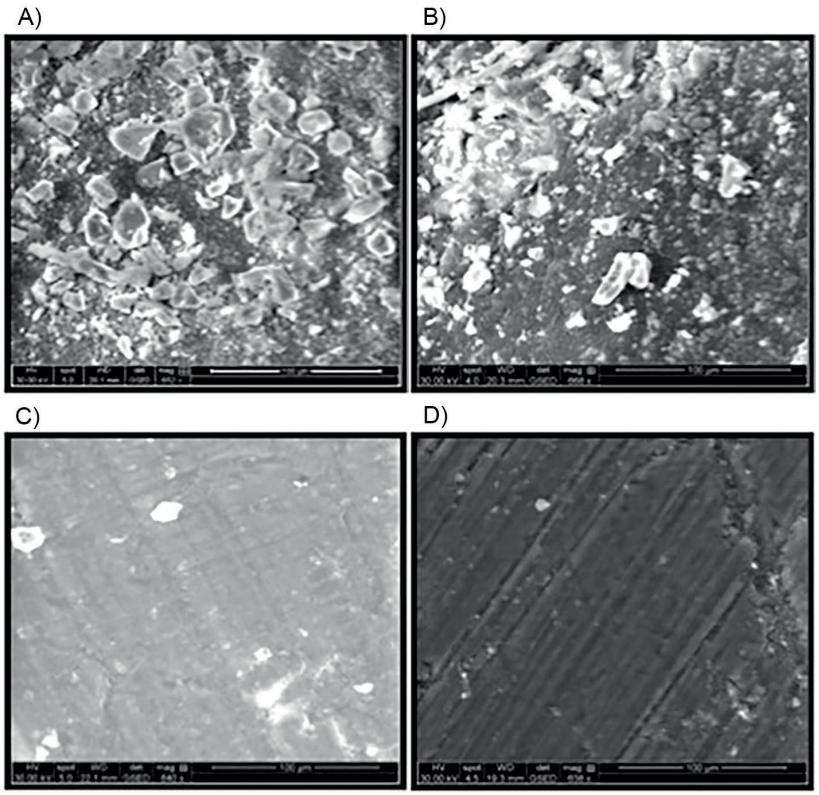


Figure 3.
Photomicrograph of the group (A) without dentin surface treatment, (B) air/water spray treatment, (C) chlorhexidine, and (D) copaiba oil emulsion treatments.

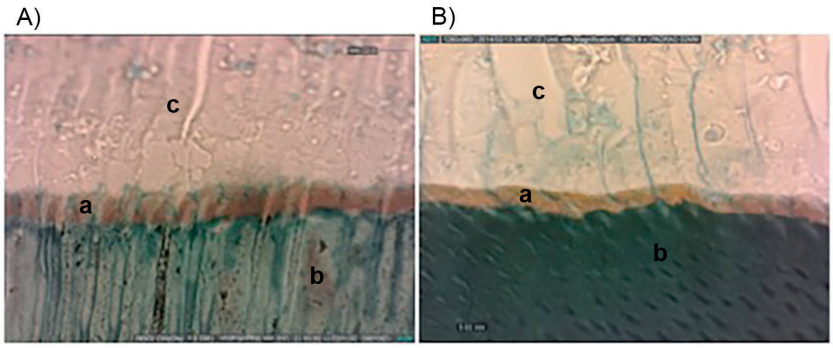


Figure 4.
Hybrid dentin specimen stained with Goldner's Trichrome. (A) Chlorhexidine treatment and (B) copaiba oil emulsion treatment; (a) hybridized collagen, (b) mineralized dentin, (c) adhesive.

Given the biological properties of *C. multijuga* Hayne, a copaiba oil emulsion was formulated for use before applying the adhesive to improve the quality of dentin bonding. The morphological characteristics of the dentin surface and the hybrid layer formed with etch-and-rinse and self-etching adhesive systems in healthy and caries-affected dentin after using the 10% copaiba oil emulsion were analyzed. A total of 96 third molars from Biobank of the School of Dentistry, Federal University of Amazonas (FAO-UFAM), were used. Half of the teeth underwent artificial induction of dental caries and the other half formed the group of healthy teeth. The roots of all the teeth were removed, yielding dentine disks that were divided into groups according to test substances (CLX 2%, copaiba oil emulsion, calcium hydroxide solution, and distilled water), the dentin (sound or caries-affected), and the adhesive system (Adper Single Bond 2® and Clearfil SE Bond®).

SEM was used to analyze the dentin surface and hybrid layer of the specimens obtained, according to the experimental groups. The dentin surface treatment with copaiba oil emulsion showed no physical barrier to adhesive penetration. The dentin surface treated with 2% chlorhexidine showed phosphate salts in two types of dentin. Dentin surface treatment with calcium hydroxide solution resulted in the deposition of mineral precipitate obstructing the lumen of the tubules in sound dentin. The result of calcium hydroxide solution applied on the conditioned sound dentin differed from those of the other substances ($p < 0.05$). On the smear layer surface, the result of distilled water on sound dentin showed a significant difference from the results of all experimental groups ($p < 0.05$). There was no statistical difference between the hybrid layer formed with the Single Bond® adhesive in otherwise healthy dentin specimens and those of caries-affected dentin treated with the test substances.

In the dentin-rich and caries-affected dentin treated with CLX 2%, the hybrid layer formed with Adper Single Bond® was thick, regular, and homogeneous, with long resin tags, but in smaller quantity than those in the distilled water group. The hybrid layer formed with Clearfil SE Bond® on the dentin treated with CLX showed few irregular resin tags with little adhesive infiltration into the dentin (**Figure 5**).

The hybrid layer formed with the Clearfil SE Bond® adhesive on the caries-affected dentin and caries-affected dentin treated with the copaiba oil-based emulsion presented regular and homogeneous hybrid layer with a large number of resin tags (**Figure 6**).

The CO application showed no morphological change in sound and caries-affected dentin, irrespective of phosphoric acid etching, and presented a uniform hybrid layer, regular, and extensive monomer infiltration into sound and caries-affected dentin, irrespective of the adhesive system.

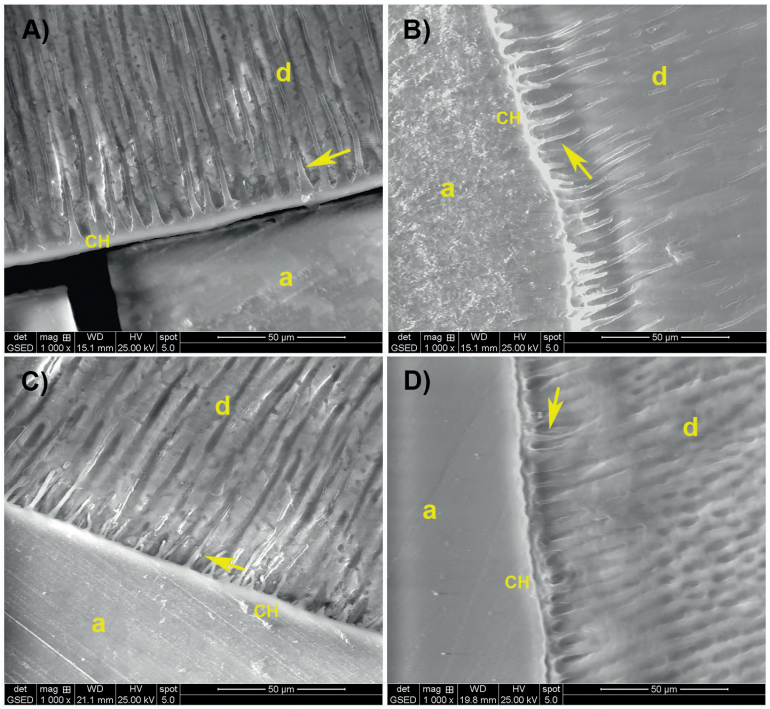


Figure 5.
Hybrid layer formed with Single Bond in the dentin treated with chlorhexidine: (A) Healthy dentin and (B) caries-affected dentin. Hybrid layer formed with Clearfil on the dentin treated with chlorhexidine: (C) Healthy dentin and (D) caries-affected dentin. (a) Adhesive, (d) Dentin, (CH) Hybrid layer. The arrows point to the resin tags.

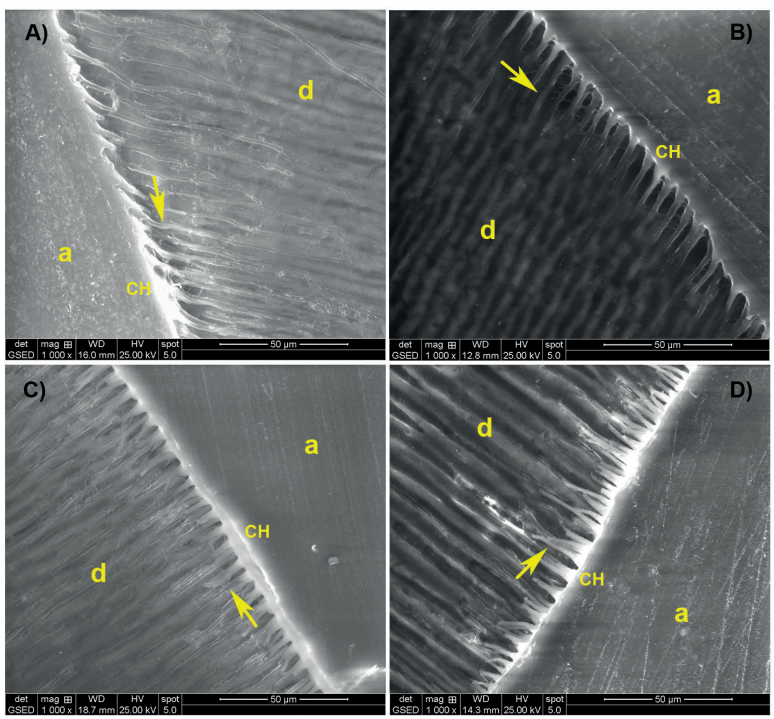


Figure 6.
Hybrid layer formed with Single Bond in dentin treated with copaiba oil: (A) healthy dentin and (B) caries-affected dentin. Hybrid layer formed with Clearfil in dentin treated with copaiba oil: (C) healthy dentin and (B) caries-affected dentin. (a) adhesive, (d) dentin, and (CH) hybrid layer. The arrows point to the resin tags.

The hybrid layer formed with Adper Single Bond® in the carious-affected and caries-affected dentin treated with copaiba oil-based emulsion was thick, regular, and homogeneous, with long resin tags, but in smaller quantity than those in the distilled water group (**Figure 6**).

Thus, the copaiba oil emulsion as dentin biomodifiers with their antibacterial activity and property of inhibiting MMPs may contribute to stability of the hybrid layer, perhaps because they prevent the enzymatic hydrolysis of collagen due to their oily nature similar to that of mineral oil. Further studies must be conducted to show the mechanism of action of the oil on the MMPs and the formation of the hybrid layer. In the present work, it was shown that the MMPs were resistant to the time-dependent destruction of the hybrid layer and that the use of inhibitors could improve the durability of the composite resin-dentin bond [35].

3. *Apis mellifera*—Propolis

Propolis is a resinous substance, collected and transformed by the *Apis mellifera* bees, used in the hives as sealant. The vegetation, climate, and other factors influence the characteristics and composition of the propolis of each region. Studies have shown that propolis is composed of more than 300 (three hundred) substances, among them the most important are flavonoids and their phenolic compounds. Its biological properties include anticancer, antioxidant, anti-inflammatory, antibiotic, and antifungal activities [12, 13].

Worker bees of the *A. mellifera* species remove propolis from the shoots and resinous secretions present in the trees. By using their jaws and their legs, they mix these secretions with the wax synthesized by them. The mandibular glands of the workers secrete 10-hydroxydecanoic acid, which enables the finishing of propolis. Factors such as plant ecology of the region where the propolis was collected and even the genetic variability of the queens also influence the chemical composition of propolis. According to CONAPIS (information body of CONAP, National Council of Apiculture Ltda.), the ideal propolis is the type produced in regions where there is as little environmental pollution as possible, away from urban centers and factories that emit pollutants [14].

Almeida et al. [15] reported that propolis is a complex mixture of resinous, gummy, and balmy substances collected by *A. mellifera* bees. It has a bactericide, antimicrobial, antioxidant, anti-inflammatory, immunomodulatory, hypotensive, anesthetic, anti-cancerous, anti-HIV, and anti-carious biologic function. These biological activities are related to their chemical components present in the propolis of the Amazon region.

Ishida et al. [14] analyzed the ethanolic propolis extracts from four propolis samples (E1–E4) from Manaus (Brazilian Amazon) by HPLC/DAD/ESI–MS/MS and GC/EIMS. The main components of E2 and E4 were polyprenylated benzophenones: 7-epi-nemorosone, 7-epi-clusianone (major E4 constituents), xanthochymol, and gambogenone (major E2 constituents), making up a chemical profile so far unreported for Brazilian propolis. Aristophenone, methyl insigninone, 18-ethyloxy-17-hydroxy-17, 18-dihydroscrobiculatone B, and derivatives of dimethyl weddellianone A and B, propolones, and a scrobiculatone derivative were detected as minor constituents. Triterpenoids (b-amyrins, b-amyrenone, lupeol, and lupenone) were ubiquitous and predominant in E1 and E3. The extracts E2 and E4 were highly active against the cariogenic bacteria *S. mitis*, *S. mutans*, and *S. salivarius*. E2 was more active than E4, probably due to a higher content of 2-epi-nemorosone, while the latter was more abundant in dihydroxylated compounds.

By histological analysis of the extracts in subcutaneous connective tissue in rats, a propolis solution for cavity cleansing and its toxicity was investigated through hemolytic and *Artemia franciscana* tests. Fifteen male rats were selected and randomly distributed into three experimental time intervals (07, 30, and 45 days), in which each animal received the four groups of treatment in rounds: Group I, Propolis I; Group II, Propolis II; Group III, calcium hydroxide water; and Group IV, 2% CLX; the sides of the tube were the control group. As regards biocompatibility, the results showed that all materials presented a significant reduction in inflammatory infiltrate and an increase in collagen fiber thickness values (**Figure** 7). In decreasing order of biocompatibility, the use of the following materials may be suggested: calcium hydroxide-water, 2% chlorhexidine®, Propolis I, and Propolis II. In the cytotoxicity test using *Artemia franciscana*, the propolis extract showed high toxicity in the tested concentrations, and in the hemolytic activity test, the Propolis I extract showed more activity than Propolis II. Therefore, the present study suggested the use of propolis as a cavity cleansing solution for shallow and medium cavities similarly to the use of 2% chlorhexidine® [15].

In continuing studies of the application of biomaterials in dentistry, the response of inflammatory periodontal disease (PD) induced in rat periodontal tissue was histologically evaluated after the use of 0.1, 1, and 10% aqueous suspensions of propolis (SAP) for subgingival irrigation. A total of 84 Wistar rats (*Rattus norvegicus*) were distributed into the following experimental groups: Group I (n = 14, 0.1% SAP), Group II (n = 14, 1% SAP), Group III (n = 14, 10% SAP), Group IV (n = 14, 5% Tween 80 solution), Group V (positive control, n = 14, with induced and untreated PD), Group VII (n = 14, 2% CLX), and Group VI (n = 84, negative control, non-induced and unprocessed contralateral teeth of Groups I, II, III, IV, V, and VII). After induction of PD by the ligation technique in the cervical portion of the mandibular left first molar for 15 days, the periodontal pocket was irrigated three times (first, fourth, and seventh days). The animals were sacrificed at 15 and 30 days after the treatment. The results suggested that SAP demonstrated an effective inflammatory response in the short term (15 days) and in the concentration of 0.1% was associated with the presence of dense gingival fibers, blood vessels

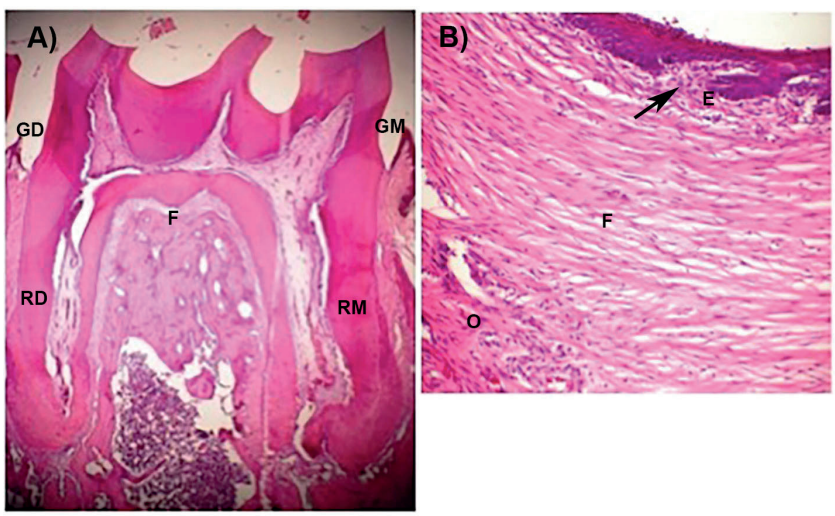


Figure 7.
(A) Anatomical shape of rat molar (H.E. 40x): (RM) mesial root, (RD) distal root, (F) furca, (GM) mesial gingiva, and (GD) interproximal gingiva. (B) Furcation region of the tooth treated with aqueous suspension of 0.1% propolis at 30 days (H.E. 200x): (E) epithelium of the exocytosis purse-string, (F) moderate collagen fibers, (O) bone tissue, and (arrow) moderate, chronic, and focal inflammatory infiltrate.

without congestion, absence of dental resorption and bone loss, and may be an alternative treatment of PD. However, future studies are required to demonstrate the biological feasibility of the use of propolis as an adjuvant to periodontal therapy.

4. *Libidibia ferrea*

4.1 Plant species and its applicability

Among the vast biodiversity of medicinal plants, *Libidibia ferrea*—popularly referred to as Jucá or ironwood—is another plant from the Brazilian biome noted for several therapeutic properties [20]. Characterized as belonging to the Leguminosae family tree, its scientific name is *Libidibia ferrea* (Mart. ex Tul.) L.P.Queiroz *var. ferrea*, ex *Caesalpinia ferrea* Mart. ex Tul. (International Plant Names Index, 2009) [36].

The fruits (pods) of this species are used for the treatment of diabetes and cancer prevention, in addition to wound healing [37]; the roots have been documented as having antipyretic effects, being used in the treatment of diarrhea, and having anticancer properties [38, 39]; and the bark has been used for treatment of enterocolitis and rheumatism [40]. Therefore, these parts of this species have shown anti-inflammatory [36], antifungal [19], antihistaminic, antiallergic, anticoagulant [41], antiproliferative, cytoprotective, and antimutagenic effects [42]. Furthermore, *L. ferrea* has been used for biosynthesis of silver nanoparticles (AgNPs), thereby preserving its antimicrobial activity, reducing its toxic effects on human cell lines and increasing its practical use without the impact on the environment. AgNPs are one of the most important nanomaterials among several metallic nanoparticles that are involved in biomedical applications [23].

In dentistry, *L. ferrea* has demonstrated potential antibacterial activity against oral microorganisms [20, 21], in addition to anti-inflammatory and analgesic properties [41, 43]. Nevertheless, several tests are still necessary to improve new dental products and prove their efficacy when used in the oral cavity, with the aim of using them in the dental clinic.

4.2 Preliminary studies—standardization of the extract

Initially, taking into consideration the vast biodiversity of medicinal plants of the Amazon region (Brazil), which have been used empirically due to their antibacterial action, these species have been screened with regard to their antimicrobial activity against microorganisms isolated from dental biofilm [44].

Since the research began, there has been constant concern to ensure the legitimacy of plant species used to obtain the study extracts. The species were collected from official research institutions (Brazilian Agricultural Research Corporation, EMBRAPA; Federal University of Amazonas, UFAM; and Brazilian Institute of Environment and Renewable Natural Resources, IBAMA) and were stored at the Lauro Pires Xavier Herbarium in the Systematic and Ecology Department/Federal University of Paraíba, according to the Genetic Heritage Component Sample Access and Delivery Authorization (No. 044/2004–IBAMA/MMA). From this initial screening, both *L. ferrea* and *C. multijuga* extracts exhibited antimicrobial activity against *S. sobrinus, S. mutans, S. mitis, S. sanguis*, and *Lactobacillus casei* strains and inhibited microbial adherence of these tested strains (**Table 2**) [44].

Sequentially, studies have shown *L. ferrea* fruit extract inhibited the in vitro growth of the following oral pathogens (*C. albicans*, *S. mutans, S. salivarius, S. oralis*, and *L. casei*) on planktonic cells and multispecies biofilm models, supporting the use of this extract for the treatment of oral infections [19]. On the other

	Microorganisms				
Tested substances/MIC	***S. mutans***	***S. mitis***	***S. sanguis***	***S. sobrinus***	***L. casei***
L. ferrea (Jucá) 1:8 (62.5 mg/mL)	15	14	14	15	15
A. chica (Crajiru) (500 mg/mL)	0	0	0	13	13
O. micranthum (alfavaca) (1000 mg/mL)	0	0	16	12	0
C. multijuga (copaíba) (1000 mg/mL)	20	19	19	20	22
Chlorhexidine 0.12% 1:8 (0.15 mg/mL)	12	13	13	12	13
A. nitidum (carapanaúba)	0	0	0	0	0
C. guianensis (andiroba)	0	0	0	0	0
E. ayapana (Japana-branca)	0	0	0	0	0
S. acmella (jambu)	0	0	0	0	0

Table 2.
Diameter of the inhibition halos (mm) of the substances against the microorganisms.

hand, *L. ferrea* stem bark extract exhibited better antimicrobial activity than fruit extract when tested against the same oral microorganisms in planktonic cells [45].

Dental biofilm is a dense, whitish, noncalcified aggregate of bacteria, with desquamated epithelial cells and food debris creating conditions for an imbalance of resident oral microflora, favoring the destruction of hard and soft tissues by the development of oral pathologies such as caries and gingivitis. Recently, an *L. ferrea* extract was standardized according to the current Brazilian legislation with regard to pH, sedimentation, density, and stability, along with microbiological tests of the extract. The microbial test was used to verify the presence of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, fungi, yeasts, coliforms, and minimum inhibitory concentrations of *S. mutans* and *S. oralis* strains. Thus, this *L. ferrea* extract was shown to have antibacterial activity against the oral microorganisms tested and satisfactory stability and quality, enabling the formulation of a mouthwash using this extract to control dental biofilm [20].

4.3 Standardization of the orabase and mouthwash formulations

After the preliminary studies of the *L. ferrea* extract, orabase and mouthwashes based on this extract were analyzed.

The use of oral antimicrobial formulations as an adjunct treatment to mechanical means of dental biofilm and gingival inflammation control has been well established [46]. However, acceptance of the use of plant-based oral products still faces obstacles due to a lack of quality control, since the profile of the end-product constituents has implications in phytotherapeutic efficiency and safety [47]. Thus, the Brazilian ANVISA [48] has established that all phytotherapeutic medication must be submitted to formulation stability tests. Production operations must follow operational procedures with clearly defined and approved standards, in conformity with the notification or registration of traditional phytotherapeutic products with the competent sanitary agency. The final objective is to obtain products that are within the quality standards demanded.

Therefore, Marreiro et al. [20], in a preliminary study, evaluated the antimicrobial activity of aqueous extracts of the fruits, stem bark, and an orabase formulation of *L. ferrea* against biofilm microorganisms by the agar diffusion and broth microdilution methods and evaluated cytotoxicity by hemolysis assay on fibroblast cell

culture. This study endeavored to find an alternative material as a way to guarantee the supply of raw vegetable matter independent of the seasonality of the fruits. The microorganisms used for determining the MICs were *S. salivarius*, *S. mutans*, *S. oralis*, *L. casei*, and *C. albicans*. The extract of the stem bark and the orabase formulation of *L. ferrea* showed antimicrobial activity against microorganisms of the biofilm and were not toxic when tested on erythrocytes and in cell cultures (**Figure 8**).

In addition to these results, Venâncio et al. [21] evaluated the in vitro pharmacological stability of a phytotherapeutic mouthwash based on *L. ferrea* extract with regard to the microbiological parameters of control, organoleptic characteristics, sedimentation, pH, and density. Using methods in accordance with the legislation, the study determined the total number of microorganisms and *Salmonella* sp., *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*; stability characteristics (color, odor, brightness, and consistency); sedimentation test (centrifuge); the pH measurement (pH meter); and density evaluation (pycnometer). The results demonstrated the *L. ferrea* mouthwash was shown to be free of contamination for the tested microorganisms and was within the standards of safety demanded for its use.

In addition to the reported studies, with the aim of finding another purpose for its use, Matos [49] evaluated a formulation of *L. ferrea* orabase for use in the healing of oral ulcers. The overall aim of this study was the physical-chemical quality control of an orabase ointment formulation of *L. ferrea*. For physicochemical evaluation, centrifugal tests, pH, mass, relative density, microbiological assessment, and organoleptic character of contaminant tests were performed. The physical conditions tested were storage at room temperature (±25.9°C), room temperature, protected from light (±28.8°C), and air conditioning (±23.7°C); the experimental time intervals were 0, 30, and 60 days. The results showed that in the centrifugation test, phase separation was observed at all times and in all storage environments. In the pH test, only the formulation stored at room temperature obtained a lower pH value, mean (1.95) after 60 days of manipulation. The density test showed the mean value of 0.809 g/cm^3 when tested at time 0 and after 30 days of formulation values under air conditioning (0.746 g/cm^3), room temperature (0.702 g/cm^3), and room temperature, protected from light, dark (1.022 g/cm^3). The evaluation of contaminants showed that there was no bacterial growth in any environment and experimental time, but macroscopically, the increase in cotton wool colonies compatible with fungal colonies was observed in the formulation stored under air conditioning in the time interval of 30 days and under all storage locations in the time interval of 60 days. In the organoleptic assessment, the ointment showed changes at 60 days after formulation. Based on the results, the formulation tested maintained the best stability of the tested characteristics when stored at room temperature in the dark. However, after 60 days of storage, the formulation presented chemical and physical instability and growth of contaminants.

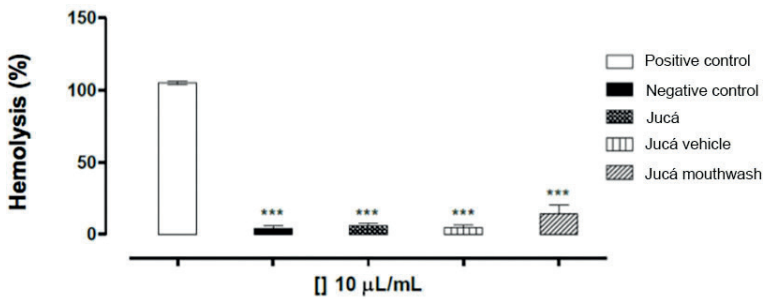


Figure 8.
*Hemolysis assay: Triton™ X-100 (positive control). *** Statistically significant in comparison with the positive control (P < 0.05).*

5. Conclusions

Given the growth of the products derived from medicinal plants in Brazil, it was necessary to implement a statute that covered the requirements for all medicines and biomaterials to ensure the quality, efficacy, and safety of these products. Based on the researches, *C. multijuga,* in the form of copaiba oil, was an effective agent against the etiological agents of caries disease. Copaiba oil emulsions as dentin biomodifiers with their properties of antibacterial activity and inhibition of MMPs may contribute to the stability of the hybrid layer in caries-affected dentin treated with this emulsion.

Propolis demonstrated an effective and controlled inflammatory response in the periodontal tissue such as the absence of bone resorption, blood vessels without congestion, and presence of dense gingival fibers.

The *L. ferrea* extract showed antibacterial activity against the oral microorganisms tested, and satisfactory stability and quality, enabling the formulation of a mouthwash using this extract to control dental biofilm. The results demonstrated that the *L. ferrea* mouthwash was shown to be free of contamination for the tested microorganisms and was within the standards of safety demanded for/to allow its use.

As propolis showed a reduction in bone resorption, and the other medicinal plants studied were in compliance with the safety standards, future investigations into the effectiveness of adding these herbal medicines to bone substitutes will be conducted. Via an effective biodegradation delivery system, the release of phytotherapic agents is expected to promote the growth of bone tissue. Pharmacological tests will examine the phytotherapic ability to generate an osteotropic effect, stimulating bone cell proliferation and differentiation while decreasing osteoclast activity. Thus, future studies will be in search of herbal devices that stimulate the innate regenerative capacity of bone and can be used in the regeneration of bone tissue.

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Conflict of interest

The authors declare no competing interests.

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