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Enhancing antimicrobial properties of a resin-based material via incorporation of a powdered phytotherapeutic extract: an *in vitro* experimental study

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ABSTRACT

Objectives: This study aimed to evaluate the degree of conversion (DC), immediate enamel bond strength (IEBS), antimicrobial activity, and release of the active principle of a resin-based material (RBM) enriched with the powdered *Schinopsis brasiliensis* (Braúna) stem antibacterial extract.

Methods: The RBM was enriched with 0, 1.25, 2.5, 5, 10, and 20 wt% powdered Braúna extract. The DC (n = 7) was assessed using micro-Raman spectroscopy. The IEBS (n = 7) was determined through the microshear test until failure, and failure modes were examined under a stereomicroscope. The antimicrobial activity (n = 15) was assessed by quantifying colony-forming units, and the release of the active principle was determined using ultra-high-performance liquid chromatography. One-way analysis of variance/Tukey and Kruskal-Wallis/Dunn tests were utilized to analyze the data (p < 0.05).

Results: Materials with 10 wt% and 20 wt% extract showed the lowest DC statistically. However, for IEBS, there were no statistically significant differences among the different groups. All materials released the active principle, but only those with 20 wt% and 10 wt% extract could inhibit biofilm formation similarly to 0.12% chlorhexidine.

Conclusions: Adding powdered Braúna extract between 10 wt% and 20 wt% is a promising alternative to provide an antimicrobial function to RBMs.

Keywords: Composite resins; Dental caries; Phytotherapy

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INTRODUCTION

Resin-based dental restorative materials (RBDMs) are a category of composites designed for direct or indirect tooth restorations, featuring a polymerizable resin matrix reinforced with inorganic fillers or fibers [1]. These materials are typically formulated using methacrylate monomers, polymerization initiators, and reinforcing fillers and undergo curing through radical polymerization, producing durable and rigid final structures [2]. RBDMs are commonly used in dentistry for a range of applications, owing to their advantageous properties. These include light-controlled curing time, a more conservative preparation technique, the ability to mimic natural tooth appearance closely, and adequate mechanical strength to withstand the forces of mastication [3]. The use of such composites has increasingly supplanted metal-based restorative materials in recent years [2].

However, RBDMs do not offer any advantages in terms of modulating caries risk factors, which tend to promote greater biofilm accumulation compared to other restorative materials like amalgam and glass ionomer [4]. Caries lesions around restorations remain one of the most frequent causes of failure and replacement of RBDMs, representing a major challenge to the longevity of restorations [5]. A systematic review reported that, among studies evaluating posterior restorations, 78.6% identified caries around restorations and/or fractures as the primary reasons for failure [6]. Notably, while the global prevalence of primary caries has decreased over the past decades, the incidence of caries lesions around restorations has remained relatively stable, raising concerns about potential overdiagnosis or misinterpretation of marginal defects and staining as active lesions [5]. This clinical ambiguity may result in unnecessary restoration replacements. As bacterial colonization of RBDMs plays a key role in lesion development, improving the antimicrobial properties of these materials has become a focus of research [5]. The development of RBDMs capable of inhibiting microbial activity could enhance the prevention of caries lesions around restorations and extend restoration longevity.

Efforts to develop antimicrobial resin composites have shown promising results, with a review identifying 34 active ingredients. Dimethylaminododecyl methacrylate (DMAHDM) is a well-studied example, consistently demonstrating positive antibacterial effects and mechanical properties, making it a promising candidate for clinical use. In contrast, some substances, such as graphene oxide decorated with ZnO nanoblasts and titanium dioxide nanoparticles, have been shown to negatively impact the composites' degree of conversion (DC) [7]. This highlights the need to investigate alternative agents, particularly natural ones. An innovative approach involves embedding curcumin, a natural photosensitizer, into methacrylate-based resin systems to enable antimicrobial photodynamic therapy, which has shown promising antibacterial activity against *Streptococcus mutans,* at low concentrations with minimal effect on mechanical properties [8]. The use of herbal medicines with confirmed antimicrobial activity is also a promising research avenue [8].

In this context, *Schinopsis brasiliensis* (Braúna), a native tree from the semi-arid region of northeastern Brazil, has been traditionally used for medicinal purposes and has recently gained scientific attention due to its diverse biological properties. Phytochemical analyses revealed the presence of bioactive compounds such as tannins, flavonoids, phenols, and polyphenols, which are associated with antimicrobial, antioxidant, and anti-inflammatory effects [9]. Recent evidence has indicated that the stem extract of Braúna possesses various biological activities, including *in vitro* activity against oral microorganisms associated with the caries process, such as *S. mutans*, *Streptococcus mitis*, and *Streptococcus salivarius*, with effects comparable to chlorhexidine [10]. Moreover, it was found that the extracts of *S. brasiliensis* have low cytotoxicity and a good selectivity index, suggesting their potential incorporation into dental materials [9].

However, it remains unknown whether the addition of *S. brasiliensis* extract to resin-based materials can confer antimicrobial properties and alter their physical and mechanical behavior. While the DC is often evaluated, a significant gap in this field is the lack of information on immediate bond strength to dental enamel, a property crucial for optimal performance in the oral environment [7]. Therefore, this study aimed to evaluate the DC, immediate enamel bond strength (IEBS), antimicrobial ac-

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tivity, and release of the active principle of a resin-based material enriched with powdered *S. brasiliensis* (Braúna) stem extract. The null hypothesis posits that incorporating *S. brasiliensis* stem extract in the resin-based material does not alter the tested properties.

METHODS

Ethical considerations

This study followed the CRIS (Checklist for Reporting *In-vitro* Studies) guidelines of the 2014 concept note for *in vitro* studies [11]. This study was approved by the Research Ethics Committee of Universidade Federal do Rio Grande do Norte (certificate of presentation for ethical appreciation No. 59712722.2.0000.5537).

Experimental design, sample size, and randomization

This *in vitro* experimental study investigated the dependent variables: DC, IEBS, failure mode, antimicrobial activity, and release of the active principle. The independent variables consisted of different concentrations of the powdered *S. brasiliensis* stem extract (0 wt%, 1.25 wt%, 2.5 wt%, 5 wt%, 10 wt%, and 20 wt%). The sample size was defined for each test according to previous investigations [12–16], and the specimens underwent testing in a randomized manner through a selection process utilizing numerical identifiers corresponding to the specimens within each group. The materials and instruments used in this investigation are shown in Table 1.

Extract preparation

The extract of *S. brasiliensis* preparation followed a previously described method [10]. Stem barks of *S. brasiliensis* were collected from the herbarium of the Universidade Federal da Paraíba (EAN-14049) in the semi-arid region of Sertão da Paraíba, Brazil. The barks were dried at 40°C ± 1°C (Figure 1) and then subjected to extraction using ethanol (Merck, Darmstadt, Germany) as the solvent. The percolation process was conducted in three cycles at 30°C for 5 days. The ethanolic extract underwent concentration in a rotary vacuum evaporator operating at 40°C and 55 revolutions/min until complete solvent removal.

Modified resin-based material preparation

The modified resin-based materials were prepared by homogenizing the powdered *S. brasiliensis* extract with Fluroshield (Dentsply Sirona, Petrópolis, RJ, Brazil), a commercial light-cured fluoride-releasing resin sealant, in the following proportions: 1.25 wt%, 2.5 wt%, 5 wt%, 10 wt%, and 20 wt%. According to the manufacturer, the Fluroshield composition includes urethane-modified bisphenol A-glycidyl methacrylate (Bis-GMA) dimethacrylate (<40%), Bis-GMA (<20%), other polymerizable dimethacrylate resins (<20%), barium boron alumino silicate glass (<30%), sodium fluoride (<5%), and dipentaerythritol pentaacrylate phosphate (<5%). Initially, a 220-g ATY224 Precision Analytical Balance (Shimadzu, Kyoto, Japan) weighed 1 g of the material. The extract was macerated with a pestle in a grater (Chiarotti, Mauá, SP, Brazil) to ensure complete disintegration of the grains, and the resulting powder was weighed according to the proportion established for each group (Figure 1). Homogenization was performed manually using a

Table 1. Specifications of materials and instruments used in the research

Material	Trade Name	Shade	Lot	Composition/ pecifications
Fissure sealant	FluroShield (Dentsply Sirona, Petrópolis, RJ, Brazil)	Matte	376737N	Urethane modified Bis-GMA dimethacrylate (<40%); barium boron alumino silicate glass (<30%); polymerizable dimethacrylate resins (<20%); Bis-GMA (<20%); sodium fluoride (<5%); dipentaerythritol pentaacrylate phosphate (<5%)
Adhesive system	Adper Single Bond 2 (3M ESPE, Maplewood, MN, USA)	-	2100700761	Ethyl alcohol (25%–35%); Bis-GMA (10%–20%); silane treated silica (10%–20%); HEMA (5%–15%); copolymer of acrylic and itaconic acids (5%–10%); glycerol 1,3 dimethacrylate (5%–10%); UDMA (<5%); water (<5%); diphenyliodonium hexafluorophosphate (<0.5%)
Phosphoric acid	UltraEtch, (Ultradent, Salt Lake City, UT, USA)	-	-	Phosphoric acid (<40%); cobalt aluminate blue spinel (<1%); siloxane (<1%)
Light-curing unit	Valo Grand Cordless, (Ultradent)	-	-	Standard power 1,000 mW/cm^2

Bis-GMA: bisphenol A-glycidyl methacrylate; HEMA: 2-hydroxyethyl methacrylate; UDMA, urethane dimethacrylate.

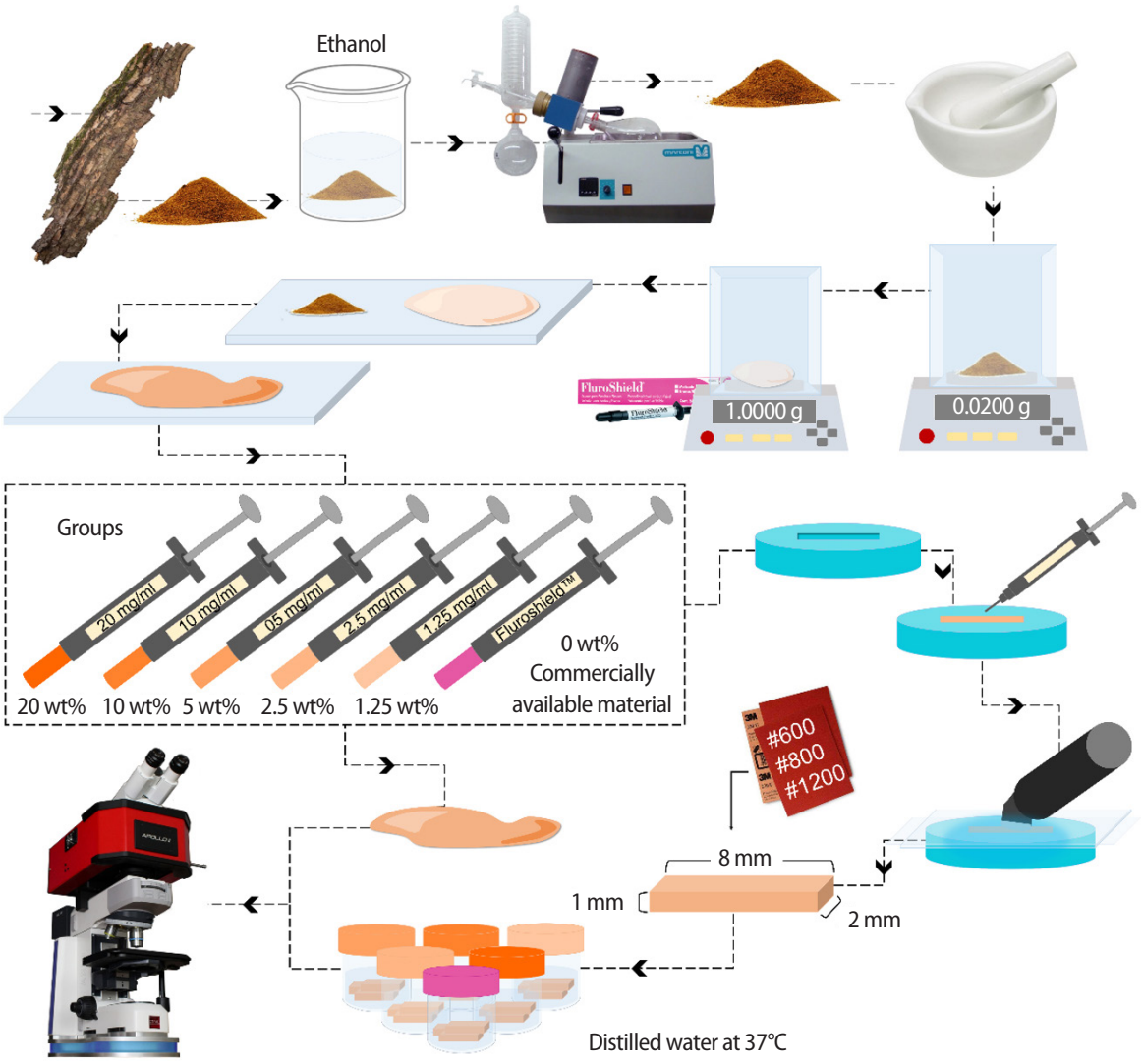

Figure 1. Schematic representation of the extract preparation and fissure sealant handling. Stem bark dried and crushed with ethanol, followed by rotary evaporation to obtain pure powder. The powder was macerated and filtered to remove grains, weighed with the sealant, and mixed. The mixture was then placed in syringes for group allocation. For the degree of conversion, specimens were prepared using a silicone matrix, covered with a polyester strip and glass slide, photoactivated for 40 seconds, sanded, and stored for micro-Raman analysis.

spatula on a glass plate and occurred in an environment protected from light exposure to prevent unwanted photoactivation of the product. Subsequently, the mixtures were labeled and stored in disposable syringes, protected from light, until further testing.

Degree of conversion

DC was measured using micro-Raman spectroscopy. Specimens were prepared by forming bars (n = 7 per group) using a silicone matrix (Express XT, Soft Dense Paste; 3M ESPE, Maplewood, MN, USA) with dimensions of 8 mm (length)× 2 mm (width)× 1 mm (height). The material application was carried out with syringe applicator tips. A polyester strip and a glass slide were placed over the material to standardize the photoactivation distance, which was photoactivated for 40 seconds using a third-generation light-emitting diode (LED) device (Valo Grand Cordless, 1,000 mW/cm^2; Ultradent, Salt Lake City, UT, USA). Subsequently, all specimens were manually finished and polished using #600, #800,

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and #1200 water sandpaper (3M ESPE). Figure 1 presents a schematic illustration of the extract preparation and specimen fabrication procedures.

The bars were immersed in distilled water at 37°C for 24 hours and analyzed using micro-Raman spectroscopy (FORAM, CRAIC Technologies, San Dimas, CA, USA) with 785 nm laser excitation, 50× lens, and 50% power (90 mW) for 60 seconds. For unpolymerized materials, three readings were taken, and the average was used to calculate the DC. For polymerized materials, readings were taken on each bar. Measurements were performed at least 1 mm away from the edges of the specimens. The DC was calculated by comparing the relative change in the peak height of the vinyl C=C band (1,640 cm^{-1}) before (unpolymerized) and after polymerization (polymerized) using the following equation:

$$DC\ (\%) = 100 \times [1 - R_{polymerized}/R_{unpolymerized}]$$

where R is the ratio of aliphatic (1,640 cm^{-1}) and aromatic (1,610 cm^{-1}) peak intensities.

Immediate enamel bond strength

The IEBS was assessed through the shear bond strength test. Proximal surfaces of human third molars were sectioned with standardized dimensions of 3 mm × 3 mm × 2 mm thickness, yielding 42 enamel specimens (n = 7 per group). These specimens were obtained using diamond discs under refrigeration (KG Sorensen, Cotia, SP, Brazil), following a method previously described [17]. The specimens were affixed in circular holders for adaptation in the universal testing machine, using epoxy-based resin (Redelease, Vila Arcadia, SP, Brazil), ensuring only the flat surface of the dental enamel was exposed (Figure 2).

The enamel surface of each specimen underwent conditioning with phosphoric acid (Ultradent) for 15 seconds, followed by a 30-second rinse and air drying at 15 cm for 10 seconds. Two layers of Adper Single Bond 2 adhesive (3M ESPE) were applied with friction

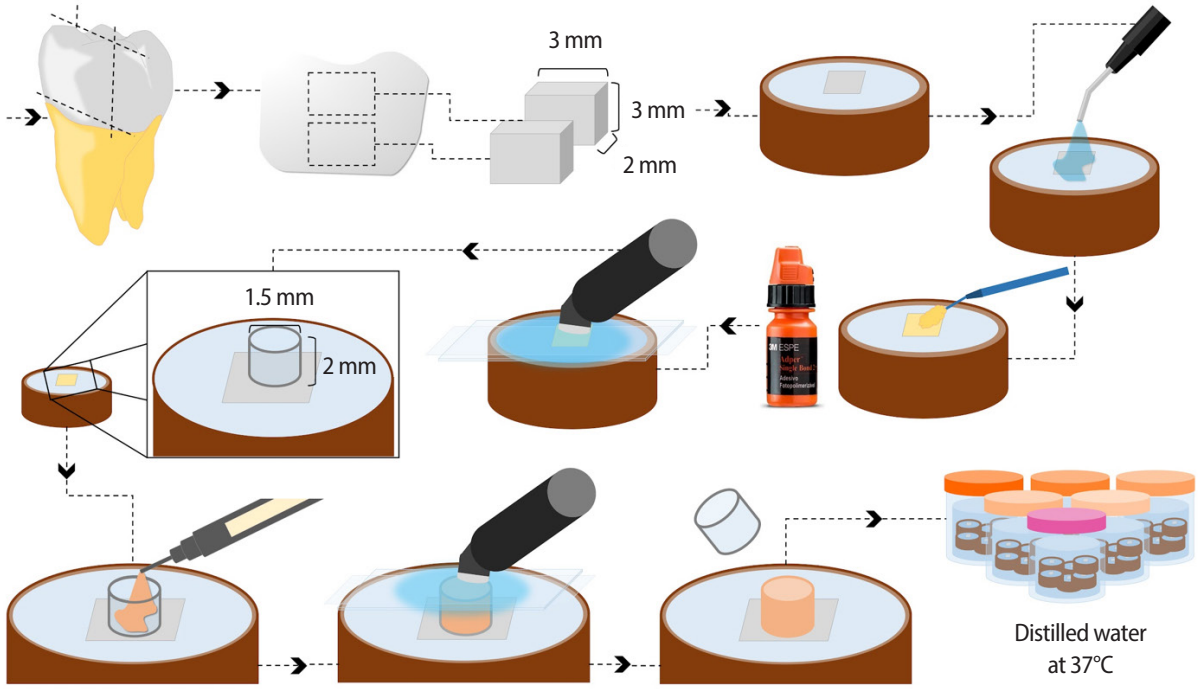

Figure 2. Schematic representation of immediate enamel bond strength specimen preparation. Sectioning of the third molars was followed by proximal cuts that generated sub-fragments with standardized sizes. The sub-fragments were soaked in polystyrene resin, exposing the enamel. A 35% phosphoric acid solution was applied for 15 seconds, followed by washing for 30 seconds and air drying. Two layers of adhesive were then applied. A polyester strip and glass slide were positioned, and photoactivation was performed for 10 seconds. A plastic microtubule was then placed on the enamel and filled with the experimental sealants. The strip and slide were repositioned, followed by photoactivation for 40 seconds. Finally, the plastic tubule was removed, leaving a cylindrical sample of sealant on the enamel, which was stored in distilled water.

on the etched surface, followed by photoactivation for 10 seconds using a third-generation LED device (Valo Grand Cordless, 1,000 mW/cm^2) [18,19]. Subsequently, a cylindrical plastic tubule with a diameter of 1.5 mm (Labor Import, Osasco, SP, Brazil) was cut into standardized pieces of 2 mm height and positioned over the tooth. The modified resin-based material was deposited carefully inside the tubule to avoid bubble formation. A polyester strip and a glass slide were used to ensure a standardized light-curing distance, and photoactivation was performed for 40 seconds. Following photoactivation, the plastic tubule was removed, resulting in cylindrical specimens composed only of the materials of the studied groups on the dental enamel (1.5 mm in diameter, 2 mm in height), which were stored in distilled water for 24 hours in an incubator at 37°C. Figure 2 presents a schematic illustration of the IEBS specimen preparation.

To assess the IEBS, the specimens were positioned in the Universal Testing Machine OM100 (Odeme, Luzerna, SC, Brazil), and a hard steel orthodontic wire (Morelli, Sorocaba, SP, Brazil) was aligned parallel to the dental surface, encircling the cylindrical specimen. This arrangement subjected the specimen to a shear force using a load cell with a capacity of up to 50 Kgf at a speed of 0.5 mm/min. The recorded data included the load required to rupture the specimen. The maximum value of micro-shear strength was determined by dividing the force needed to rupture the specimen (N) by the adhesive surface area (mm^2).

Failure mode

The failure mode was analyzed using a stereomicroscope (Infinity 1, SMZ800; Nikon, Melville, NY, USA) with a 50× magnification lens. Failures were classified into adhesive, cohesive (in enamel or the material), and mixed categories. Adhesive failure occurs at the interface between the material and the tooth. Cohesive enamel failure happens within dental enamel without affecting the bond with the material. Cohesive material failure involves a fracture within the material itself. Mixed failure combines two or more patterns [17].

Antimicrobial assay

1. Saliva collection

Stimulated saliva was collected in a sterile 2-mL Eppendorf tube from a randomly selected donor registered in the research laboratory after signing the informed consent form regarding the donation of the material. Before collection, the donor refrained from oral hygiene for 12 hours. The collected saliva was centrifuged at 2,000 ×*g* for 10 minutes. Subsequently, 50 μL of the supernatant was diluted in 35 mL of brain heart infusion (BHI) broth, following a predefined proportion [20].

2. Antimicrobial activity

Antimicrobial activity was assessed through the colony-forming unit (CFU) count test. Circular specimens (n = 15 per group) measuring 5 mm × 1 mm were created using a silicone matrix (Express XT, Soft Dense Paste). The material for each experimental group was applied to the circular matrix with a syringe applicator tip. A polyester strip and a glass slide were then placed over the material to standardize the photoactivation distance, and photoactivation was performed for 40 seconds (Valo Grand Cordless, 1,000 mW/cm^2). All specimens underwent manual finishing and polishing with #600, #800, and #1200 water sandpaper (3M ESPE).

The specimens were positioned at the bottom of wells in 48-well plates and covered with culture medium containing the inoculum (BHI broth and saliva). The positive control included 0.12% chlorhexidine digluconate (Sigma-Aldrich, Jurubatuba, SP, Brazil). In addition to the groups with specimens made from materials with different concentrations of the extract, the same mass concentrations used to create the materials' groups were diluted in distilled water, resulting in five other control groups: E1, E2, E3, E4, and E5. The plates were then incubated at 37°C ± 1°C for 24 hours. After this period, 10-μL aliquots from each well were taken, seeded onto a Petri dish containing BHI agar (in triplicate), and incubated for 24 hours at 37°C to assess microbial growth. The CFUs were counted to verify microbial viability. Figure 3 presents a schematic illustration of the antimicrobial assay.

Release of the active principle

The release of the active principle was evaluated through ultra-high-performance liquid chromatography (UHPLC), following previously established standards [21,22]. Gallic acid was a marker substance [23]. The proximal

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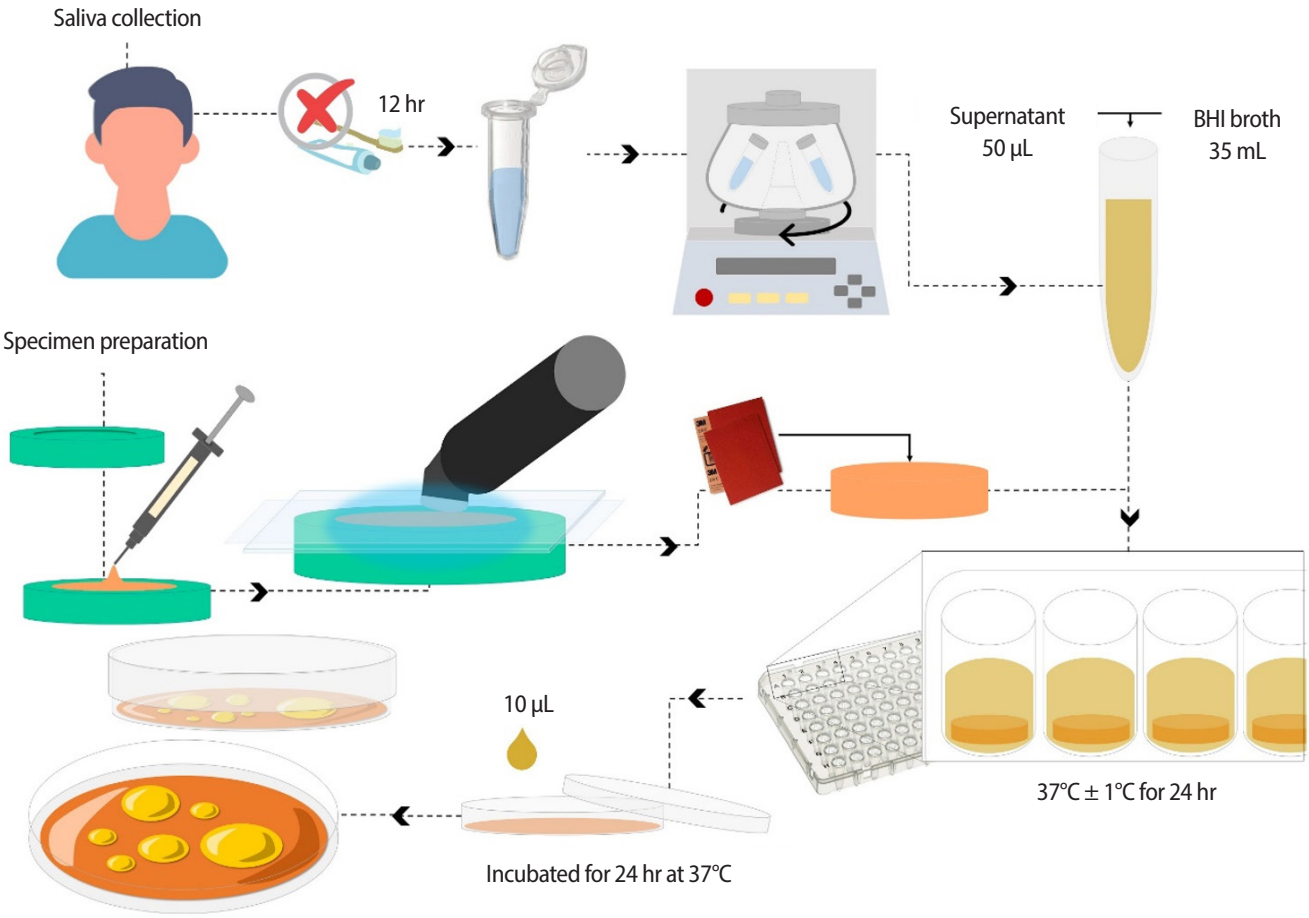

Figure 3. Schematic representation of the antimicrobial assay. Saliva was collected from the donor after 12 hours without oral hygiene and placed in an Eppendorf tube containing 2 mL of saliva. The sample was centrifuged at 2,000 ×g for 10 minutes, and 50 µL of the supernatant was mixed with 35 mL of brain heart infusion (BHI) broth to prepare the inoculum. Sealant specimens were made using a circular matrix, covered with a polyester strip and glass plate, and photoactivated for 40 seconds. The specimens were finished and inserted into wells containing the inoculum, where they remained in contact for 24 hours. Then, 10-µL aliquots were transferred in triplicate to Petri dishes, and colony-forming unit counting was performed after 24 hours.

surfaces of nine human third molars donated after signing a consent statement were utilized to produce rectangular specimens with dimensions of 5 mm × 2 mm with a thickness of 3 mm ($n = 3$ per group), simulating the size of the main groove of a molar that would be sealed with the resin-based material. Figure 4 presents a schematic representation of the release of the active principle assay.

These specimens were placed in a silicone mold positioned one mm above the specimen surface, standardized with a 150-mm digital caliper rod (MTX, Guarulhos, SP, Brazil), resulting in a volume of 10 mm^3. The material was applied to this space using syringe applicator tips, covered with a polyester strip and a glass slide, and photoactivated for 40 seconds (Valo Grand Cordless, 1,000 mW/cm^2). Following photoactivation, the specimens were manually sanded with #600, #800, and #1200 water sandpaper (3M ESPE) and stored in a solution of methanol (75%)/H_2O (25%) for 48 hours.

The gallic acid release was quantified using a high-performance liquid chromatography (HPLC) with diode array detector (DAD) coupled to a UHPLC (Shimadzu). The equipment included a binary analytical pump (LC-20A3 XR), an automatic injector (SIl-20AD XR), a degasser (DGU-20A3), a column oven (CTO-20AC), and a DAD (SPD-M20A). The analysis utilized a Zorbax

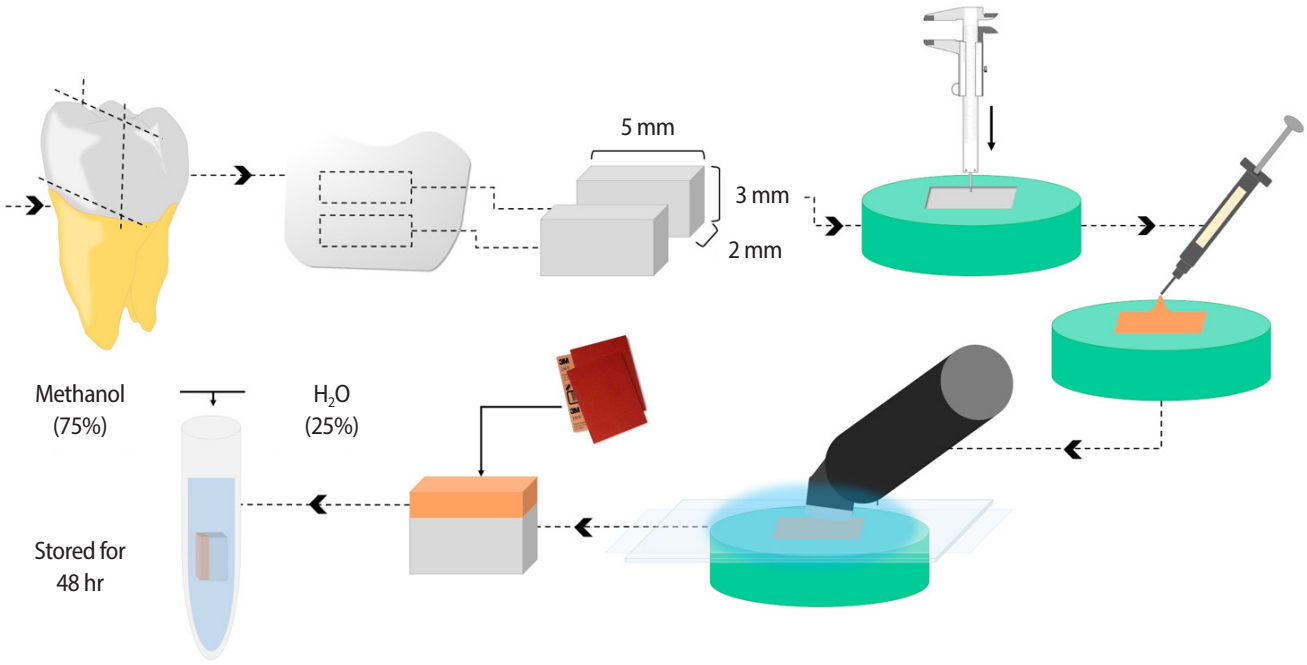

Figure 4. Schematic representation of the release of the active principle assay. Healthy molars were sectioned, and the specimens were cut to standard sizes. Each specimen was immersed in silicone, leaving a 1-mm (10 mm^3) gap that was measured using a caliper. The fissure sealant was then inserted, followed by the positioning of a polyester strip and glass slide. Photoactivation was performed for 40 seconds. The specimens were manually finished with sandpaper, and the final appearance of the tooth and sealant was documented. Finally, the specimens were immersed in a water-methanol solution for 48 hours.

Eclipse Plus C18 column (Phenomenex, Torrance, CA, USA) controlled by the LC Solution software (Shimadzu). The wavelength employed was 272 nm, with a flow rate of 1.0 mL/min and a temperature of 30°C. The mobile phase consisted of formic acid 1%: methanol 90:10 (v/v), and the specimen injection volume was 0.4 µL.

Statistical analysis

The final values of the samples for each test underwent a normality assessment using the Shapiro-Wilk test ($p > 0.05$) and the Levene test. Parametric data underwent analysis of variance, followed by the Tukey test ($p < 0.05$). Nonparametric data underwent the Kruskal-Wallis test ($p < 0.05$), followed by the Dunn test. All analyses were conducted using Prism 8 software (Insight Venture Partners, GraphPad Holdings, LLC, San Diego, CA, USA) and Microsoft Excel 2018 (Microsoft, Redmond, WA, USA). Parametric data are presented as means ± standard deviations. Nonparametric data are presented as median (range). The quantity of the active principle released and failure modes were analyzed descriptively.

RESULTS

Degree of conversion, immediate enamel bond strength, and failure modes

Statistically significant differences were observed among the concentrations of powdered *S. brasiliensis* stem extract, regarding the DC ($p < 0.05$). Table 2 presents the group comparisons. The control group exhibited the highest mean value (68.34 ± 2.5), followed by concentrations of 1.25 wt% (64.09 ± 3.16), 2.5 wt% (61.71 ± 5.2), groups, with no statistically significant differences among them. The 5 wt% group (50.0 ± 7.0) showed a statistically lower value than the control, while the 10 wt% (37.11 ± 5.6) and 20 wt% (33.84 ± 7.7) groups exhibited the lowest mean values, differing significantly from the lower-concentration groups.

Regarding immediate bond strength to dental enamel, no statistically significant differences were observed among the tested concentrations of powdered *S. brasiliensis* stem extract ($p > 0.05$). The mean and standard deviation values for each group, as detailed in Table 2,

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Table 2. The degree of conversion, IEBS, and failure mode according to the different concentrations of powdered *Schinopsis brasiliensis* stem extract ($p < 0.05$)

Group	Degree of conversion (%)	IEBS (MPa)	Failure mode (%), adhesive/mixed
Control (0 wt%)	68.34 ± 2.5^{A}	4.04 ± 0.92^{A}	85/15
1.25 wt%	64.09 ± 3.16^{AB}	4.30 ± 1.91^{A}	70/30
2.5 wt%	61.71 ± 5.2^{AB}	3.71 ± 1.02^{A}	85/15
5 wt%	50.0 ± 7.0^{B}	3.24 ± 0.64^{A}	70/30
10 wt%	37.11 ± 5.6^{C}	4.31 ± 1.90^{A}	70/30
20 wt%	33.84 ± 7.7^{C}	4.11 ± 2.03^{A}	85/15

Values are presented as mean ± standard deviation.
Different capital letters indicate statistically significant differences between groups in each column ($p < 0.05$).
IEBS, immediate enamel bond strength.

were comparable, indicating that the incorporation of the extract did not have a measurable effect on the bond strength to enamel. A notable finding, observed across all groups, was the predominance of adhesive failure, as also presented in Table 2. This suggests that the bond interface was the weakest point, a characteristic that was consistent regardless of the extract concentration used.

Antimicrobial activity

Statistically significant differences were observed among the tested groups ($p < 0.05$). The materials groups with 20 wt% (0; range, 0–0) and 10 wt% extract (45.5; range, 16–79) demonstrated an ability to inhibit microbial growth statistically similar ($p > 0.05$) to the positive control, 0.12% chlorhexidine digluconate (0; range, 0–0). Other groups exhibited uncountable CFU values (999; range, 999–999), which were statistically similar ($p > 0.05$) to the negative control, distilled water (999; range, 999–999).

Solutions containing water and 20 wt% (0; range, 0–0), 10 wt% (0; range, 0–0), and 5 wt% extract (246.5; range, 115–362) showed a statistically similar ability to inhibit microbial growth ($p > 0.05$) compared with the positive control, 0.12% chlorhexidine digluconate (0; range, 0–0). Other solutions exhibited uncountable CFU values (999; range, 999–999), statistically similar to the negative control, distilled water (999; range, 999–999).

Release of the active principle

Qualitative analysis of the experimental groups revealed that all groups, regardless of the concentration of the powdered *S. brasiliensis* stem extract, were capable of releasing the active principle. The successful release was consistently demonstrated by measuring the presence of the chemical marker, gallic acid, in the aqueous medium (methanol 75%/H_2O 25%). This consistent finding across all tested concentrations highlights that the incorporation of the extract into the material did not hinder the release mechanism. Thus, the extract's ability to be released from the material was maintained, providing a foundation for potential biological activity in subsequent analyses.

DISCUSSION

The null hypothesis, which stated that incorporating powdered *S. brasiliensis* stem extract would not alter the tested properties, was rejected based on statistically significant reductions in the DC at higher concentrations (10 wt% and 20 wt%), and enhanced antimicrobial activity observed at these same concentrations, compared to the control ($p < 0.05$).

In this study, higher concentrations of powdered *S. brasiliensis* stem extract were associated with a lower DC, showing an approximate 30% reduction compared to the control group. Higher concentrations of the extract can act as a physical barrier to the passage of light in the material, potentially reducing the percentage of monomer-to-polymer conversion. However, even the 5 wt% group (50.0 ± 7.0) was inferior to the 10 wt% and 20 wt%, it still demonstrated an acceptable conversion value (50%) for resin-based materials, falling within 50% to 75% [24]. The selection of a pit and fissure sealant as the resin-based composite material to be modified in this study is supported by previous research that highlights

the use of sealants as a foundation for incorporating antimicrobial agents [25].

In addition to monomer conversion, evaluating the impact of extract addition on adhesion potential becomes crucial. While Fluroshield is a resin-based sealant that does not mandate the use of an adhesive system, a systematic review with meta-analysis has shown that applying a conventional two-step adhesive system, such as Adper Single Bond 2, enhances the retention of the material [19]. Consequently, given that the application of adhesive systems is a standard procedure for resin-based restorations, Adper Single Bond 2 was used prior to the sealant to replicate a common clinical protocol designed to improve adhesion. This also ensured standardized and reproducible adhesion conditions across all samples, facilitating reliable comparative analysis. In this study, IEBS results indicate no statistically significant differences between the experimental materials and the highly positive control group. These findings suggest that incorporating the *S. brasiliensis* extract did not impair the adhesive performance of the resin-based sealant. This outcome is particularly relevant given that bond strength is a critical factor in ensuring the success and longevity of sealants [26]. Similar results have been reported in studies evaluating non-self-adhesive sealants, where conventional bonding protocols are employed to enhance retention and minimize microleakage. Pitchika *et al.* [26] demonstrated that a separate bonding step prior to sealant application significantly improved shear bond strength compared to self-etch systems applied alone, highlighting the importance of the adhesive interface in clinical performance. Although immediate bond strength is an important indicator, it does not account for long-term degradation phenomena such as hydrolysis or mechanical fatigue. Therefore, future studies should focus on evaluating bond durability over time, especially in aged specimens, to determine whether the addition of plant-derived components affects adhesive stability under intraoral conditions.

The occurrence of adhesive and mixed failures aligns with the typical observation of debonded fissure sealants [27]. The predominance of adhesive and mixed failures observed in this study is consistent with previous findings for conventional fissure sealants [26], where similar failure modes were identified following shear bond strength testing. Notably, the control group, without the *S. brasiliensis* extract, exhibited failure patterns comparable to the experimental groups, suggesting that the incorporation of the extract did not negatively affect the adhesive interface or compromise the material's cohesive strength. The retention of sealant remnants within the deeper fissures, even after partial failure, may maintain the release of bioactive compounds, potentially prolonging antimicrobial action at the tooth–material interface. Nevertheless, this hypothesis warrants further investigation, particularly through studies evaluating sustained release and long-term antibacterial efficacy.

It is noteworthy that the characteristics of plant-derived secondary metabolites can be influenced by several factors, such as the climatic conditions of the collection period (rainfall index during that period), seasonality, and the presence of natural predators [28,29]. Thus, adding the powdered pure extract diluted in a water-based environment aimed to validate its isolated antimicrobial effect in the same concentrations included in the resin-based material. Considering the wide array of chemical combinations that can occur when adding a plant extract to a resin-based material, it is essential to characterize this extract as isolated chemically. In a previous study using HPLC, gallic acid was obtained as the main active principle of *S. brasiliensis* stem extract [23], so it was utilized in this study as well.

Incorporating powdered functional substances into resin-based materials, such as a fissure sealant, which undergoes a polymerization process resulting in a highly dense polymeric network, raises uncertainty about whether these molecules can detach from these chains and be released into aqueous environments. The release into a water-based environment was evaluated using UHPLC, simulating what would occur in saliva in the oral environment. Upon qualitative analysis of the results, it was observed that all experimental groups could release gallic acid into the aqueous medium where the specimens were immersed for 48 hours.

In the present study, all concentrations of powdered *S. brasiliensis* stem extract added to resin-based material were higher than the previously determined minimum inhibitory concentration of 0.5 mg/mL for *S. mutans*, *Streptococcus oralis*, *S. mitis*, and *S. salivarius* [9,21]. However, only extract concentrations above 10 wt%

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demonstrated antimicrobial activity in the resin-based materials tested. It can be inferred that concentrations below 10 wt% of extract in the material, although releasing the chemical marker, do not sufficiently impair bacterial growth.

On the other hand, the two highest concentrations of extract into the material (10 wt% and 20 wt%) provided statistically similar values to the positive control (0.12% chlorhexidine digluconate), confirming the release of antimicrobial agents from the polymer chain into the water-based environment in therapeutic quantities. Thus, although all materials released the active principle, they may have been released in different quantities, which should be further evaluated.

This study presents limitations that should be acknowledged. Although a multispecies biofilm model derived from stimulated saliva was employed, the specific microbial taxa affected by the antimicrobial activity of the extract were not identified, despite previous studies demonstrating proven activity against *S. mutans*, *S. oralis*, *S. mitis*, and *S. salivarius* [9,30,31]. Therefore, additional investigations are necessary to deepen the understanding of the interactions between caries-associated microorganisms and the bioactive compounds released from the resin-based material containing powdered *S. brasiliensis* stem extract. Moreover, long-term outcomes, such as the stability of the antimicrobial effect and the sustained release of active components, were not addressed in this study. Future research should include microbial profiling and prolonged evaluation periods to validate and expand the current findings.

The methods and results presented in this study empower researchers to investigate a range of alternative extract concentrations and synthesize a drug delivery system. Also, further investigations might develop a drug delivery system by conjugating gallic acid to a nanocarrier and incorporating it into resin-based dental materials. This proposed approach would be an alternative to formulating antimicrobial materials, including resin-based fissure sealants, resin composites, adhesive systems, luting agents, and resin-modified glass ionomer, which should be further evaluated.

CONCLUSIONS

Incorporating powdered *S. brasiliensis* stem extract at concentrations of 10 wt% and 20 wt% showcased the capability to augment the antimicrobial effect of the tested resin-based material. While higher concentrations led to a reduction in conversion, the immediate bonding potential remained constant, and the primary type of failure observed was adhesive. All experimental groups demonstrated the ability to release the active agent when immersed in a water-based environment.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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AUTHOR CONTRIBUTIONS

Conceptualization, Project administration, Supervision, Validation: Borges BCD; Data curation: Borges BCD, Sousa-Lima RX. Formal analysis: Marinho MELN, Sousa-Lima RX; Investigation: Silva JCC, Marinho MELN, Sousa-Lima RX, Costa MJF, Sette-de-Souza PH. Methodology: Borges BCD, Sousa-Lima RX, Sette-de-Souza PH. Writing - original draft: Marinho MELN, Sousa-Lima RX. Writing - review & editing: Lima GS, Borges BCD. All authors read and approved the final manuscript.

DATA SHARING STATEMENT

The datasets are not publicly available but are available from the corresponding author upon reasonable request.

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