



Effect of Addition of Green Propolis on Microbiological and Shear Properties of Ionomer Cement

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Authors' contributions

This work was carried out in collaboration among all authors. Authors TF-S and CCdOS designed the study. Authors AAdS, RdSTJ and ACOT conducted the experiments. Authors TF-S and CCdOS analyzed the data. Authors AAdS, ACOT and TF-S drafted the article. Authors LDdAeS and CCdOS revised and translated the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To modify the GIC with green propolis extract to evaluate its antimicrobial and shear strength effects.

Study Design: This is an experimental, *In vitro* study.

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Place and Duration of Study: The stages of this project were carried out at the Department of Dentistry of the Federal University of the Jequitinhonha and Mucuri Valleys, in the city of Diamantina/MG, Brazil.

Methodology: For the microbiological analysis, cement discs were prepared in triplicate and grouped into four groups (GIC without modification, GIC+1% propolis extract, GIC+5% propolis extract, GIC+10% propolis extract). Azithromycin at 10% was used as a positive control, and GIC without modification was used as a negative control. The discs were embedded in petri dishes containing BHI agar and dental biofilm inoculum. The plates were then placed in an oven at 36°C for 24 hours. The results obtained in the microbiological evaluation were used to define the composition of the groups for the shear tests. Three groups were formed (GIC + 5% propolis extract, GIC + 10% propolis extract and GIC without modification) and the cements were used to bond orthodontic buttons to the enamel surface (n=10 in each group). The specimens were then placed in a container with distilled water and kept at 36°C for 24 hours. The EZ-Test-Shimadzu® universal testing machine was used to perform the mechanical tests. The collected data were tabulated in the SPSS 17.0 software for Windows.

Results: The results showed that the GIC modified with 5% and 10% propolis presented an inhibition zone, proportionally compatible with the positive control. Furthermore, the GIC modified with 10% propolis extract demonstrated greater shear strength when compared to the unmodified GIC.

Conclusion: The GIC modified with 10% propolis extract presents an increase in the shear strength of the cement in addition to enhanced antimicrobial effects.

Keywords: Glass ionomer cement; shear; propolis; dental biofilm.

1. INTRODUCTION

Glass ionomer cement (GIC), formulated in powder-liquid form, is a dental material recognized for its biocompatibility, fluoride release capacity, and adequate adhesion to dental tissues, providing good consistency and high performance in restorations and cementations (Fricker, 2022). Over the years, GIC has been modified to improve its properties and expand its clinical applicability, resulting in conventional, metal-reinforced, high-viscosity, and resin-modified versions, each with specific advantages for different clinical needs (Sidhu & Nicholson, 2016).

Despite this, there are adverse characteristics in its use, such as low mechanical and abrasion resistance, reduced translucency, friability, and sensitivity to the technique, conferring specific limitations to the use of the technique (Makanjuola & Deb, 2023). Studies have investigated the incorporation of different materials into GIC to improve its properties, with emphasis on propolis, known for its antibacterial and antiviral activity due to the flavonoids, aromatic acids, and esters present (Oliveira et al., 2017; Neelima et al., 2020; Lesmana et al., 2022). Green propolis, produced by *Apis spp.* bees from plants of the genus *Baccharis spp.* in the Brazilian savannah (de Barros et al., 2007), has benefits in the treatment of inflammation, antioxidant, antibacterial and immunological

action, in addition to aiding in the recovery of liver dysfunctions and ulcers (Rocha et al., 2012; de Miranda et al., 2019). Studies indicate that Brazilian green propolis is more effective against oral microorganisms in biofilms than European propolis, suggesting that its variable composition influences its properties (Collucia et al., 2022).

The oral environment and clinical demands represent significant challenges for the development of new dental materials, which must be resistant and adaptable to different acidic conditions (Spatafora et al., 2024). In this context, white spot lesions, characterized by localized areas of enamel demineralization without cavitation, represent the first stage of dental caries. They have a prevalence between 10% and 49% and are common in patients undergoing orthodontic treatment due to the accumulation of bacterial plaque (Selwitz et al., 2007; Maxfield et al., 2012). The complexity of orthodontic accessories makes proper hygiene difficult, favoring the retention of bacterial biofilm and making certain regions more susceptible to the dental demineralization process (Rosenbloom & Tinanoff, 1991). In this sense, the mineral loss of the enamel modifies its optical properties, resulting in the appearance of milky-white areas that do not completely recover their original appearance.

The increase in porosity prevents adequate remineralization, hindering the penetration of

calcium ions, phosphate, and salivary proteins essential for this process. Consequently, the persistence of the lesions favors bacterial infiltration, intensifying the demineralization process and potentially compromising the integrity of the enamel (Tufekci et al., 2011). Studies indicate that the addition of propolis to GIC can positively alter its antibacterial properties (Neelima et al., 2020). This characteristic is particularly favorable, considering that GIC is commonly used in the cementation of orthodontic brackets and bands. Propolis, being a natural, non-toxic, low-cost compound with multiple therapeutic functions, has great potential to aid in the treatment, hygiene, and maintenance of oral health (Hatunoğlu et al., 2014; Saputra et al., 2019). Its use in dental materials could, therefore, contribute to the reduction of complications related to oral hygiene during orthodontic treatment and offer additional benefits to the oral health of patients.

Considering the high prevalence of white spots associated with the use of orthodontic appliances, which compromise both the aesthetics and functionality of tooth enamel, it is essential to seek alternatives that minimize these adverse effects. Although studies on GIC with the addition of propolis suggest an increase in antimicrobial potential, others indicate that this action may be negative, with no consensus in the literature for the use of this modification (Panahandeh et al., 2021). Therefore, the objective of this study is to modify the GIC with green propolis extract and evaluate its antimicrobial effects and shear strength.

2. MATERIALS AND METHODS

2.1 Study Design and Sample

This is an in vitro experimental study approved by the Research Ethics Committee of the Federal University of the Jequitinhonha and Mucuri Valleys (UFVJM approval: 2,663,969). It was carried out to evaluate the antimicrobial action and shear resistance of a modified GIC through the addition of freeze-dried green propolis at concentrations of 5% and 10%.

2.2 Microbiological Analysis

For the microbiological analysis, 9 mm diameter GIC discs (Maxxion C; FGM; Joinville/SC) were prepared in triplicate and grouped into five groups: (1) unmodified GIC (negative control); (2)

GIC + 1% propolis extract; (3) GIC + 5% propolis extract; (4) GIC + 10% propolis extract; (5) GIC + 10% azithromycin (positive control).

To perform the test, lyophilized propolis extract was used. To calculate the proportion of propolis in the different concentrations, the simple rule of three was used, where the weight of the discs was considered as 100%. The amount of propolis corresponding to the desired concentration was calculated as the variable x .

The discs were prepared using five molds obtained with condensation silicone and a hot glue stick, with dimensions of 1.1 cm in diameter by 30 cm in length, using five molds. After production, the molds were disinfected with 0.5% sodium hypochlorite, which was sprayed onto them. After 10 minutes, the molds were washed with running water and stored in a sterile container to dry.

After standardizing the portions of the liquid and the glass ionomer powders and standardizing the weight of the test disc, the propolis powder was added to the glass ionomer powder. Propolis powder was added to the different groups in proportions of 1%, 5%, and 10% of the total weight of the disc to the ionomer powder, and then the ionomer was manipulated.

The GIC discs were inserted with sterile tweezers into petri dishes containing Brain Heart Infusion Broth – BHI agar (Kasvi; Pinhais/PR) and inoculated with dental biofilm. Subsequently, the plates were stored in an oven at 36 degrees Celsius for 24 hours.

The inhibitory activity of the extracts was determined when the halo formed had a diameter greater than or equal to the positive control, and the measurement was performed with the aid of a digital caliper (DIGIMESS® model).

2.3 Preparation of Test Specimens and Shear Test

For the analysis of shear strength, 30 extracted human first premolar teeth, healthy, without defects in the formation of the dental enamel or cracks or fractures in the coronal portion, were acquired from the Human Tooth Bank of UFVJM.

To perform the tests, the center of the clinical crown on the vestibular surface of all teeth was determined using a digital caliper (Digimess®-200mm. São Paulo, São Paulo). The height and width of the dental crown were measured, and

the center of the clinical crown of each tooth was determined at the intersection of half of the distances. Then, polyvinyl chloride (PVC) tubes with an internal diameter of 40 mm, a thickness of 2 mm, and an approximate height of 20 mm were filled with acrylic resin, followed by insertion of the tooth root into the acrylic and positioning of the crown with the aid of a set square, so that the vestibular surface was at a 90-degree angle with the base of the PVC tube. After polymerization of the acrylic resin, the test specimens were stored in containers containing distilled water in an oven at 37 °C for 72 hours (Bishara et al., 2000; 2001).

To perform the tests, orthodontic buttons (Morelli®, Sorocaba, São Paulo) were used, cemented with GIC, according to the manufacturer's specifications, in the center of the clinical crown of the vestibular surfaces of the respective test specimens. The test specimens were randomly distributed into three experimental groups, each containing 10 teeth, as follows: (1) GIC without modification (negative control); (2) GIC + 5% propolis extract; (3) GIC + 10% propolis extract.

To cement the button, the GIC was manipulated and applied to the entire base of the apparatus with the aid of an insertion spatula. The orthodontic button was pressed against the test specimen, and excess cement was removed with the aid of a clinical dental probe. After the cement dried, the test specimens were stored in containers with distilled water for 24 hours in an oven at 37°C until the mechanical tests were performed. To perform the mechanical tests, the

universal testing machine, EZ-Test-Shimadzu®, was used with a 200 kgf (kilogram-force) load cell and a speed of 0.5 mm/min in the occlusogingival direction with an active dental chisel tip (Pignatta et al., 2009). The force required to detach each bracket was recorded and expressed in Newtons (N), then divided by the area of the bracket base in square millimeters (mm²) to be converted to Megapascals (Mpa).

2.4 Data Analysis

The collected data were tabulated using SPSS 17.0 software for Windows. Data normality was determined using the Shapiro-Wilk normality test. The comparison between groups for the results of the shear tests was adjusted in a One-Way Analysis of Variance model and subsequently submitted to the Bonferroni Post-Hoc test for multiple comparisons. The significance level adopted in the tests was 95% ($p < 0.05$).

3. RESULTS AND DISCUSSION

The GIC modified with propolis at concentrations of 5% and 10% presented bacterial growth inhibition halos with diameters proportional and comparable to the positive control.

The comparative analysis of the mechanical shear tests showed that the cementation group with GIC modified with 10% propolis extract presented greater shear resistance when compared to the GIC group without modification ($p < 0.05$) (Tables 1 and 2).

Table 1. Descriptive analysis of the shear force of the different groups analyzed

Groups	Maximum Strength	
	Mean	Standard Deviation
GIC	1,672	0,457
GIC + 5% propolis extract	6,747	2,141
GIC + 10% propolis extract	7,185	1,415

Table 2. Comparative analysis of shear strength between different groups

Maximum strength		Mean difference	"p"	Confidence interval (95%)	
				Minimum	Maximum
GIC	GIC + 5% propolis extract	-5,0750	0,073	-10,5102	0,3602
	GIC + 10% propolis extract	-5,5125	0,046	-10,9477	-0,0772
GIC + 5% propolis extract	GIC	5,0750	0,073	-0,3602	10,5102
	GIC + 10% propolis extract	-0,4375	1,000	-5,8727	4,9977
GIC + 10% propolis extract	GIC	5,51250	0,046	0,0772	10,9477
	GIC + 5% propolis extract	0,4375	1,000	-4,9977	5,8727

The present study showed that the GIC modified with 10% propolis extract, in addition to providing an antimicrobial action in contact with the dental biofilm, also increases the shear strength of the cement.

For some specific uses, the insertion of propolis can suppress microbial activity, alleviating bacterial adhesion, reducing the solubility of the GIC, and consequently the production of extracellular polysaccharides, soluble and insoluble in water (Yuan et al., 2022). The antimicrobial activity of propolis has already been evaluated, seeking to successfully combat different bacteria such as *Staphylococcus spp.*, *Streptococci*, facultative anaerobes and Gram-positive cocci in the oral cavity, among others (Mohsin et al., 2015; Tambur et al., 2021). One of the points presented, referring to the microbial action of propolis, is related to the presence of phenolic compounds and the synergistic effect between them (Darwish et al., 2010; Mohdaly et al., 2015) and its use as an “embalming substance”, thus avoiding the deterioration of dead invaders inside the hive (Torete et al., 2013). A good seal is important not only to keep the restoration in place, but also to make the surface impermeable to microleakage and cavities. Furthermore, cementing materials are widely indicated for cementing crowns, inlays, onlays, veneers, multiple-unit fixed prostheses, endodontic pins, and orthodontic appliances (Leung et al., 2022). As propolis has antimicrobial action, it can reduce the bacterial load.

Furthermore, propolis can be considered a suitable biomaterial in the treatment of wound biofilms, since by affecting one or more phases of wound healing, biofilms can delay the healing response (Oryan et al., 2018). Dalenberg et al., (2020) reported that propolis can help improve the health of microbial colonies by shifting the microbiome in favor of commensal bacteria, which outcompete pathogenic bacteria. The incorporation of propolis extract into the GIC aims to improve the basic characteristics of the material, obtaining a superior material after modification. Regarding microbial action, the use of the modified GIC is capable of increasing the GIC antimicrobial potential and consequently reducing the potential for caries recurrence and biofilm proliferation to the detriment of the presence of high concentration flavonoids, caffeic, benzoic and cinnamic acids, capable of acting on bacteria generating structural and functional damage (Beltagy et al., 2018; Andrade

et al., 2019;) These characteristics make these two materials attractive as dental restorative or cementation bases because they are capable of repelling the action of potential caries-causing agents.

The mechanical strength of glass ionomer cement is lower than that of composite resin (Vieira et al., 2006), which becomes a limiting characteristic of its use. However, when evaluating the base composition of propolis and its use within the hive (sealant, thermal insulation, among others), it is possible to establish a direct relationship between its use and increased mechanical strength of the GIC. One of the related points refers to the fact that it is found solid in natura, which may favor the increase in the associated hardness parameters (Marcucci et al., 1996; 2001; Pinto et al., 2011) which draws attention to the presence of inorganic elements in its composition, such as: copper, manganese, iron, calcium, aluminum, vanadium, and silicon. The presence of these elements and/or compounds may establish a valid hypothesis about the increased strength of the GIC with the addition of propolis extract, considering the aggregation of several biological functions and activities.

The addition of propolis to GIC, in relation to mechanical resistance, can favor the resistance of the material, increasing the durability of the cement and consequently reducing the cost in the medium and long term (Subramaniam et al., 2016). Studies indicate that the addition of propolis extract, as a wood treatment method, is capable of increasing its flexural strength and durability when compared to untreated wood (Woźniak et al., 2020). Furthermore, several studies mention that the use of propolis can increase the GIC's chemical adhesion and provide greater adhesion of the cement to the dentin (Hatunoglu et al., 2014; Aguilar-Perez et al., 2023).

Despite the positive aspects evaluated, it is important to emphasize that some parameters should be considered when using the GIC modified with propolis in clinical practice. Propolis, like other bee-related products, can induce allergic reactions in oral tissues. Another associated characteristic refers to aesthetic issues, considering that the incorporation of propolis can change the color of the GIC, making it yellowish or reddish. In the present study, even though green propolis was used, a change in the color of the modified GIC was observed. It is

recommended that additional studies be carried out with sample aging tests and fluoride and propolis release tests over time.

4. CONCLUSION

It is concluded that the GIC modified with 10% propolis extract, in addition to providing an antimicrobial action in contact with the dental biofilm, also increases the shear strength of the cement.

ETHICAL APPROVAL

This study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki, approved by the Human Research Ethics Committee of the Federal University of the Jequitinhonha and Mucuri Valleys (approval number: 2,663,969).

CONSENT

Informed consent was obtained from the patient for scientific dissemination.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declares that no generative ai technologies such as large language models (chatgpt, copilot, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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