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Effectiveness of *Cordia verbenacea* and Propolis as Irrigation Solution on the Bond Strength of Filling Material to the Root Dentin: *In vitro* Study

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Authors' contributions

This work was carried out in collaboration among all authors. Authors TFC and CCdOS designed the study. Authors GKB and AFS conducted the experiments. Authors TFS and CCdOS analyzed the data. Authors TFS, CCdOS and GKB drafted the article. Author KHS revised and translated the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: Endodontic treatment aims to eliminate pathological microbiota from root canals to prevent or treat infections in periapical tissues. Irrigation is a crucial step in this process, serving important mechanical, chemical, and biological functions.
Objective: This study aimed to evaluate the effect of irrigation with *Cordia verbenacea* and propolis solutions compared to chlorhexidine and sodium hypochlorite on the bond strength of the filling material to the root dentin.

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Materials and Methods: This *In vitro* experimental study involved 40 healthy human premolars subjected to the push-out test. The teeth underwent decoronation, followed by biomechanical endodontic preparation using mechanized files. The teeth were then divided into four groups according to the irrigating solution: NaOCl 5.25%, chlorhexidine 2%, aqueous extract of *Cordia verbenacea* and aqueous extract of propolis. Data were statistically analyzed using analysis of variance and the Post-Hoc Bonferroni test.

Results: The group irrigated with propolis showed greater resistance to the push-out test in the middle and apical thirds when compared to the other groups ($p<0.05$). There were no statistically significant differences among the other groups.

Conclusion: Irrigation with propolis extract resulted in higher bond strength of the obturating material to the root canal, while irrigation with *Cordia verbenacea* showed similar results to chlorhexidine and sodium hypochlorite.

Keywords: Endodontics; natural products; phytotherapy; root canal irrigants.

1. INTRODUCTION

Endodontic treatment aims to eliminate the pathological microbiota within the root canals to prevent or treat infections in the periapical tissues. Irrigation plays a crucial role in achieving this objective, serving several mechanical, chemical and biological functions. It is considered the only way to impact the areas of the root canal wall not reached by mechanical actions [1].

One of the most commonly used irrigants in endodontic treatments is sodium hypochlorite (NaOCl) due to its broad-spectrum antimicrobial activity [2,3]. However, it can be toxic to the periapical tissues if extruded [4], and it may reduce the mechanical resistance of dentin [5]. Chlorhexidine (CHX) is also used in endodontics as an irrigant because of its antimicrobial action [6], lower toxicity, substantivity [7] and suitability for patients with hypersensitivity to NaOCl. Nevertheless, it has limited efficacy in dissolving organic compounds, which is a desirable property for an irrigant [8].

Currently, there is a growing interest in plant-based products to overcome the limitations of conventional chemical irrigants. These plant-based options are gaining attention due to their biocompatibility [9] and antimicrobial activity against important endodontic pathogens [10,11]. This has spurred research into the production of high-value chemicals and medicines through green and sustainable processes, offering an alternative and complementary approach to existing products [12].

Cordia verbenacea (CV) is native to Central and South America. In Brazil, it is primarily found in the Atlantic and Amazon forests [13]. This plant is widely used in folk medicine, particularly for its antimicrobial [14], anti-inflammatory, and analgesic properties [15]. CV has shown promising results against endodontic pathogens at minimum inhibitory concentrations of up to 4000µg/ml [15]. The scarcity of scientific articles addressing the use of CV in dentistry, especially in the field of endodontics, underscores the importance of further studies in this area. This research has the potential to provide valuable insights for future clinical applications.

Propolis is a substance produced by bees from the resin of flowers, tree leaves, and plants. It becomes a sticky mixture after being mixed with bee saliva [16,17]. Propolis is utilized in both medical and dental fields due to its chemical composition and therapeutic properties [18], including antibacterial, antiviral, antifungal and antiprotozoal activities, as well as its ability to stimulate regenerative processes in the dental pulp [19,20]. In dentistry, especially in endodontics, its effects on pulp regeneration have been studied, including its use as irrigant and intracanal medication, among other applications [19]. Flavonoids, chemical compounds found in propolis, play a fundamental role in its antioxidant activity, primarily by eliminating free radicals [21]. As an irrigant, propolis demonstrates excellent bond strength [21]. This strength is attributed to the antioxidant capacity of flavonoids, which help eliminate the adverse effects of NaOCl, known to inhibit the polymerization of resin monomers [22]. While the results are promising, additional studies are necessary to provide reliable outcomes for clinical practice.

It is acknowledged that microorganisms may persist within the root canal system even after chemical and mechanical preparations [23].

Effective filling aims to seal the root canals system to prevent residual microorganisms, ensuring the success of endodontic treatment and preventing root canal reinfection [24]. Therefore, it is essential for the root canal filling materials to adhere to the dentin, providing an effective seal [25]. The push-out adhesion strength test is widely used in endodontic research [26] to evaluate the bond between the cement and the intracanal filling material, measuring the force required to displace the filling material at the interface with the root dentin [27].

In light of the above, the aim of the present study was to evaluate the effect of irrigation with *Cordia verbenacea* and propolis solutions in comparison to solutions of chlorhexidine and sodium hypochlorite on the bond strength of the filling material to the dentin walls.

2. MATERIALS AND METHODS

A total of 40 healthy human premolars with single, straight roots were selected from the Human Teeth Bank at UFVJM. The teeth included in this study exhibited no enamel formation defects, cracks, or fractures in either the crown or root portions. Teeth with calcified root canals were excluded. The cleaned teeth, free of any remnants of periodontal ligament or calculus, were stored in an aqueous solution at 5°C until the experimental phase.

Sample preparation was conducted by a single researcher. The teeth were sectioned at the amelo-cemental junction using a carborundum disc while continuously cooled with water. They were subsequently stored in distilled water until the moment of biomechanical endodontic preparation. Upon accessing the root canal, the root length was determined using a K-file #15, inserted into the canal until its tip was visible at the apical foramen. The working length was established by subtracting 1 mm from the total root length. The canal was instrumented with rotatory files from the ProT System (MK Life) attached to an endodontic motor (X-Smart Plus). This step was done using the crown-down technique with a torque of 2 N, 300 rotations per minute, and a 16:1 reduction ratio.

For biomechanical preparation, the teeth were randomly divided into four groups, each receiving specific irrigating solutions. Each group received 5 ml (DESCARPACK Slip Syringe with Needle, 05ml; 25x07mm) of the corresponding irrigant, which was gradually added with each file change. Group 1 (n=10) received 5.25% NaOCl, Group 2 (n=10) received 2% CHX, Group 3 (n=10) received an aqueous extract of CV obtained through infusion (active principles were extracted from the leaves through maceration with a 7:3 v/v ethanol-water mixture, using 100g of plant material per 1000 ml) and Group 4 (n=10) received an 18% aqueous propolis extract (MN Própolis - Indústria, Comércio e Exportação Ltda - Mogi das Cruzes – SP). The smear layer was removed using 5 ml of 17% Ethylenediaminetetraacetic Acid (EDTA) for 5 minutes, followed by canal washing with 5 ml of distilled water. Drying of the canal was achieved with absorbent paper cones. For the obturation procedure, the canal was filled with endodontic sealer (Sealer 26, Dentsply, Rio de Janeiro, Brazil) and Gutta-Percha (Tanari®, Tanariman Ltda, Manacapuru, Brazil) using single cone technique, following the manufacturers' protocols. After obturation, the apical and coronal regions of the roots were externally sealed with composite resin (Z-100; 3M ESPE, St. Paul, MN, USA). Subsequently, the teeth were stored at 37°C with 100% humidity for 7 days to allow complete cement setting.

To perform the push-out tests, the coronal and apical ends of each sample were removed (2 mm each), and each sample was sliced into 2 mm-thick sections using a cutting machine (Veiyee DTQ-5, China) at a speed of 6, with constant water irrigation. The remaining 2 mm in the coronal region represented the sample of this third, the remaining 2 mm in the apical region represented the sample of this third, and the central 2 mm of the root was used to represent the middle third.

For the mechanical push-out tests, a universal testing machine, EZ-Test-Shimadzu®, with a 500 Kgf (kilogram-force) load cell and a speed of 1 mm/min, in the apical/coronal direction, was used. The force required for adhesive fracture with extrusion of the obturation material was recorded and expressed in Newtons (N).

The collected data were tabulated using the Statistical Package for Social Sciences (SPSS) 17.0 for Windows. A descriptive statistical analysis was performed to describe the data characteristics. Additionally, the Shapiro-Wilk test was conducted to assess data distribution. Group comparisons were made using a One-Way Analysis of Variance (ANOVA) adjusted in the model, followed by the Bonferroni Post-Hoc test for multiple comparisons. The level of significance adopted was 95% (p<0.05).

3. RESULTS

The Fig. 1 shows the graph with numeric results of the mechanical test for maximum force required for adhesive fracture.

The comparative results of the mechanical test for the maximum force in the coronal, middle, and apical thirds, involving the tested irrigant groups, are presented in Table 1. It was observed that the group irrigated with propolis showed higher force in the middle and apical thirds when compared to the other groups (p<0.05), while the remaining groups did not show statistically significant differences among themselves. There was no difference among the groups in the coronal third.

Table 1. Comparative analysis of the force between different irrigants in the coronal, middle, and apical root thirds

Coronal third		Mean difference	"*p*" value	Confidence interval (95%)	
				Minimum	Maximum
NaOCl	CHX	-0,8750000	0,726	-2,420078	0,670078
	CV	0,1100000	1,000	-1,355789	1,575789
	Propolis	-0,0550000	1,000	-1,520789	1,410789
CHX	NaOCl	0,8750000	0,726	-0,670078	2,420078
	CV	0,9850000	0,407	-0,480789	2,450789
	Propolis	0,8200000	0,753	-0,645789	2,285789
CV	NaOCl	-0,1100000	1,000	-1,575789	1,355789
	CHX	-0,9850000	0,407	-2,450789	0,480789
	Propolis	-0,1650000	1,000	-1,546959	1,216959
Propolis	NaOCl	0,0550000	1,000	-1,410789	1,520789
	CHX	-0,8200000	0,753	-2,285789	0,645789
	CV	0,1650000	1,000	-1,216959	1,546959
Middle third					
NaOCl	CHX	0,0550000	1,000	-0,059881	0,169881
	CV	0,0600000	0,921	-0,054881	0,174881
	Propolis	-,4350000*	**0,000**	-0,549881	-0,320119
CHX	NaOCl	-0,0550000	1,000	-0,169881	0,059881
	CV	0,0050000	1,000	-0,109881	0,119881
	Propolis	-,4900000*	**0,000**	-0,604881	-0,375119
CV	NaOCl	-0,0600000	0,921	-0,174881	0,054881
	CHX	-0,0050000	1,000	-0,119881	0,109881
	Propolis	-,4950000*	**0,000**	-0,609881	-0,380119
Própolis	NaOCl	,4350000*	**0,000**	0,320119	0,549881
	CHX	,4900000*	**0,000**	0,375119	0,604881
	CV	,4950000*	**0,000**	0,380119	0,609881
Apical third					
NaOCl	CHX	-0,0375000	1,000	-0,168383	0,093383
	CV	0,0225000	1,000	-0,108383	0,153383
	Propolis	-,4625000*	**0,000**	-0,593383	-0,331617
CHX	NaOCl	0,0375000	1,000	-0,093383	0,168383
	CV	0,0600000	1,000	-0,070883	0,190883
	Propolis	-,4250000*	**0,000**	-0,555883	-0,294117
CV	NaOCl	-0,0225000	1,000	-0,153383	0,108383
	CHX	-0,0600000	1,000	-0,190883	0,070883
	Propolis	-,4850000*	**0,000**	-0,615883	-0,354117
Própolis	NaOCl	,4625000*	**0,000**	0,331617	0,593383
	CHX	,4250000*	**0,000**	0,294117	0,555883
	CV	,4850000*	**0,000**	0,354117	0,615883

Note: One-Way Analysis of Variance (ANOVA) test; Significance at the level of 5% (p<0.05)

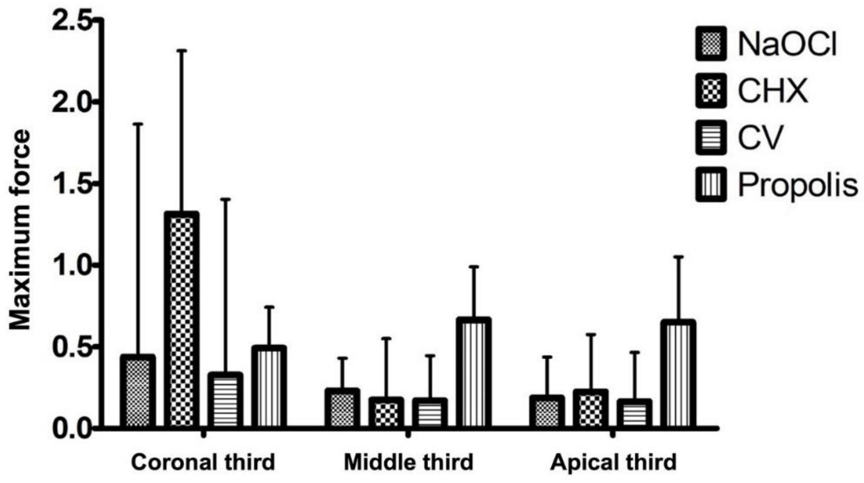

Fig. 1. Average maximum push-out force in the coronal, middle, and apical root thirds of the analyzed groups

4. DISCUSSION

There is a growing interest in the production of high-value chemicals and pharmaceuticals through green and sustainable processes as an alternative and complement to petroleum-based processes [10]. The composition of dentin and its interaction with restorative materials can be affected by the use of chemical solutions during root canal preparation [28]. As a solution, there has been an increase in the number of studies on the use of medicinal plants in endodontics, including the cleaning and disinfection of root canals, intracanal medications between appointments, and endodontic sealers, as well as the evaluation of the therapeutic potential of aromatic substances such as alkaloids, coumarins, saponins, and flavonoids in pulp and dentin repair [9].

In the present study, it was demonstrated that CV and propolis extract do not compromise the mechanical characteristics of canal filling when compared to CLX and NaOCl. Furthermore, root canals irrigated with propolis extract showed greater bond strength of the canal filling, especially in the middle and apical thirds.

The increase in bond strength between the filling material and the tooth, generated by irrigation with propolis extract, may result from the action of the chemical compounds present in the irrigant. The interaction between the components of propolis with dentin tissue may have contributed favorably to the increased bond strength of the filling, especially to the action of the endodontic sealer. The literature supports that flavonoids present in propolis have antioxidant activity and act in the elimination of free radicals [29]. Additionally, the components present in propolis do not inhibit the polymerization of resinous monomers, as occurs in the presence of NaOCl [30]. In this context, it can be suggested that propolis extract acted on the dentin surface, removing free radicals resulting from the instrumentation process and favoring the action of the sealer during canal filling.

The use of propolis in endodontics has generated increasing interest due to its antimicrobial and anti-inflammatory properties [19, 20]. Studies have shown that propolis has a broad activity against pathogenic microorganisms present in the root canal, including those commonly found in failed endodontic treatments, such as Enterococcus faecalis and Candida albicans [31], favoring its use as irrigant solution in endodontic treatments. In addition, its regenerative properties can assist in tissue repair, stimulating dentin formation and favoring the success of the treatment [20].

In a study investigating the effects of propolis on the formation and activation of osteoclast cells, it was shown that propolis plays a significant role in reducing bone loss. It was observed that propolis can reduce the number of giant cells that are positive for TRAP (Tartrate-Resistant Acid Phosphatase) and has an inhibitory effect on the early stage of osteoclastogenesis [19]. This inhibitory activity is dose-dependent.

Furthermore, propolis has been shown to increase the expression of osteoprotegerin and decrease the number of osteoclasts, resulting in the inhibition of osteoclastogenesis [32]. These results highlight the potential of propolis as a substance capable of modulating bone metabolism, suggesting a possible reduction in bone resorption in conditions such as chronic apical periodontitis. However, more studies exploring the mechanisms involved and clinical applications of propolis in this context are needed to confirm and fully understand this effect.

CV showed similar canal filling strength results to CHX and NaOCl. Studies on the antimicrobial activity of CV extract against endodontic microorganisms and its biocompatibility demonstrated positive and promising results [33]. However, there were no studies found that evaluated its effect on filling adhesion. CV extract is predominantly composed of sesquiterpenes, accounting for about 80% of its constituents [34]. These are secondary metabolites of plants composed of three isoprene-forming units and are often associated with plant defense mechanisms due to their antifungal, antibacterial, and antiviral activities. In addition, it has been demonstrated that CV's antioxidant properties inhibit lipid peroxidation and slow down the production of reactive oxygen and nitrogen species, which can enhance dental structure adhesion [35]. Thus, the use of CV as an intracanal irrigant for filling purposes can be considered a viable and effective alternative in endodontic treatment.

The results obtained in this study are promising and suggest that CV and propolis extracts may serve as potential alternative irrigants that do not have a negative impact on the adhesion of endodontic cement to the dentin. Additionally, the antimicrobial, anti-inflammatory, and regenerative properties of propolis, along with the antimicrobial activity and biocompatibility of CV, can enhance the processes of disinfection, repair, and prevention of complications associated with endodontic treatment.

The limitations of this study are associated with its design, which does not replicate a real oral environment, as the root dentin, under clinical conditions, closely interacts with other structures. Additionally, it is important to note that only one type of endodontic sealer was tested in this study. Further studies are necessary to determine the effectiveness of CV and propolis extract as intracanal irrigants in removing the smear layer and their impact on the adhesion of intraradicular posts.

5. CONCLUSION

Root canals irrigated with propolis extract demonstrated greater bond strength between the obturating material and the root dentin in the middle and apical thirds. Additionally, irrigation with CV produced results similar to those of CHX and NaOCl.

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ETHICAL APPROVAL

This *In vitro* experimental study was approved by the Research Ethics Committee of the Federal University of Vales do Jequitinhonha and Mucuri under protocol number 5.676.905.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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