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Antibacterial effect of an experimental endodontic cement with the incorporation of natural compounds (mint, *muña*, propolis tincture, and Jalk propolis): an *in vitro* experimental study

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ABSTRACT

Objective: To determine *in vitro* the antibacterial effect of an experimental endodontic cement based on different natural compounds (mint, *muña*, propolis tincture, and Jalk propolis): against *Enterococcus faecalis*. **Materials and methods:** The antibacterial effect was determined against the E. faecalis strain (ATCC 2982), using the disk diffusion method. In Petri dishes with brain heart agar (BHA), the strain was disseminated, and the four natural compounds were placed on Whatman paper disks to incubate for 24 hours under anaerobic conditions. The diameters of the inhibition halos were measured with a digital vernier caliper. The data obtained were statistically examined using analysis of variance (ANOVA) and Tukey's *post-hoc* test. **Results:** The *muña* compound showed the greatest inhibition halo (5.975 ± 0.050 mm), followed by mint, Jalk propolis, and propolis tincture, in that order. The control endodontic cement showed the greatest inhibition halo (18.050 ± 0.451 mm), followed by the mint-containing cement (16.498 ± 0.460 mm). **Conclusions:** The *muña* compound presented the highest antibacterial activity against *E. faecalis*, while propolis tincture had the lowest antibacterial activity. On the other hand, experimental endodontic cement with natural compounds decreased the antibacterial activity concerning pure cement.

Keywords: endodontics; mint; propolis; *Enterococcus faecalis*.

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INTRODUCTION

The root canal preparation procedure begins with access opening, biomechanical preparation, disinfection, drying, and obturation, during which materials such as cements or sealers are used (1, 2). Root canal obturation materials must be "biocompatible, non-resorbable, impermeable to dissolution by tissue fluids, provide a hermetic seal, and allow for adequate handling" (3). Proper root canal obturation with suitable materials increases treatment effectiveness by reducing microleakage and eliminating residual bacteria within the root canal, thereby improving the prognosis of the treated tooth (4). The success of an obturation material is closely related to its ability to achieve a hermetic seal of the root canal spaces, which depends on its physicochemical properties (5).

Currently, the search continues for an optimal material that combines multiple favorable properties for obturation and stands out among those already available. According to the literature, certain compounds such as gel-based chlorhexidine exhibit a high level of disinfection against *E. faecalis*, followed by propolis (6). Likewise, propolis, 2% morinda, povidone-iodine, and calcium hydroxide, evaluated at depths of 200 µm and 400 µm, have demonstrated antibacterial activity against *E. faecalis*, showing inhibition halos after 21 days of evaluation (7). Another study compared the *in vitro* apical sealing ability of a cement formulated with *Minthostachys mollis* (muña) essential oil and zinc oxide against Grossman-type cement (8), showing acceptable levels of apical sealing. Similarly, the addition of *Melaleuca* (tea) to the physicochemical properties of zinc oxide-eugenol cement and a bioceramic cement was assessed (9), demonstrating no alteration in any of the evaluated properties. Finally, an *in vitro* study demonstrated that mint exhibits antibacterial activity against *Streptococcus mutans* and *Streptococcus pyogenes* (10).

In this context, the literature reports a number of natural compounds with potential antibacterial activity. Therefore, the present study aims to determine *in vitro* the antibacterial effect of an experimental endodontic cement formulated with different natural compounds (mint, muña, propolis tincture, and Jalk propolis) against *E. faecalis* strains.

MATERIALS AND METHODS

An experimental *in vitro* study was conducted. The natural compounds evaluated were muña EOP (Aceites Esenciales del Perú SAC, Lima, Peru), Ekala mint (Es Aromaterapia EIRL, Lima, Peru), Jalk propolis (Distribuidora Jalk EIRL, Lima, Peru), and propolis tincture (Magistral Pharma, Bauru, Brazil). The sample was obtained using a modified Kirby-Bauer method (including at least two tests of each element to be evaluated, along with a positive and a negative control), incorporating two additional repetitions to increase the reliability of the results. Group selection was based on an exhaustive review of the scientific literature highlighting their potential as antibacterial agents.

For the preparation of the experimental cements, a methodology based on the commercial endodontic cement Polifil was used (11, 12). A mixture was prepared by combining 8 mg of L-iditol, 22 mg of zinc oxide, 60 mg of calcium carbonate, and 18 µL of castor oil (Sigma-Aldrich, Missouri, USA) for 20 seconds until a base paste was formed. To this, 30 µL of each natural compound were added; finally, 0.04 mL of diphenylmethane and 0.01 mL of isophorone diisocyanate (Sigma-Aldrich, Missouri, USA) were incorporated, mixing for an additional 20 seconds until the optimal working viscosity was achieved. An experimental cement without any natural compound was also prepared as a control group.

Preparation of bacterial culture medium and disk diffusion method

To assess the antibacterial effect, the disk diffusion method (modified Kirby-Bauer technique) was used. Brain-heart infusion (BHI) broth was prepared by diluting 4 g in 100 mL of distilled water and stirring circularly for 20 seconds. This dilution was divided into 16 test tubes containing 6 mL each, which were sterilized in an autoclave at 125 °C. The *E. faecalis* strain (ATCC 2982) was activated by thawing for 1 hour at 4 °C. Then, 1 mL of it was added to each BHI tube to allow culture growth for 24 hours until reaching a 0.5 McFarland standard. Separately, 22.5 g of brain-heart agar (BHA) were diluted in 400 mL of distilled water and distributed into 40 sterile Petri dishes (10 mL each). The plates were left at room temperature to allow the agar to solidify.

Evaluation of the inhibition halo

Each solidified Petri plate was inoculated with the cultured *E. faecalis* strain using the swab technique to cover the entire surface. Subsequently, 0.8 µL of each natural compound were applied to Whatman paper disks, which were then placed on the surface of the inoculated plates in four replicates. Each process included a positive control (2% chlorhexidine) and a negative control (9‰ sodium chloride). The plates were incubated under anaerobic conditions in an anaerobic chamber (an area controlled at 37 °C for 24 hours at 1 atmosphere). The antibacterial effect was observed 24 hours after the inoculation of the plates, in order to clearly distinguish the inhibition halo produced by each natural compound. The obtained diameters were measured using a Vernier caliper (Bel-Art Products, Warminster, USA).

Statistical analysis

The data obtained from the antibacterial inhibition assays of both the natural compounds and the experimental cements were processed using SPSS v.26 software (IBM Company, New York, USA) through descriptive (mean and standard deviation) and inferential statistical analyses. The Shapiro-Wilk normality test was applied to verify the distribution of the data. Subsequently, one-way ANOVA revealed a statistically significant difference ($p < 0.001$). Finally, Tukey's post hoc test indicated significant differences among the groups, with a significance level of $p < 0.05$.

RESULTS

The descriptive statistical analysis provided the mean values and standard deviations of the inhibition halos produced by the natural compounds against *E. faecalis*. One-way ANOVA showed significant differences among the study groups ($p < 0.001$). Tukey's post hoc test revealed differences between the groups, whit the muña compound exhibited the largest inhibition halo (5.975 ± 0.050 mm), followed by mint (4.450 ± 0.129 mm) and Jalk propolis (4.250 ± 0.129 mm), while the propolis tincture (2.200 ± 0.071 mm) showed the lowest antibacterial activity (Table 1). Statistically significant differences ($p < 0.05$) are indicated by indices in Figure 1.

Once the natural compounds were added to the experimental endodontic cement, the inhibition halo results showed some variations compared to their pure counterparts. One-way ANOVA revealed significant differences among the experimental cements, with a value of $p < 0.001$. Tukey's post hoc test showed differences among all groups, except between the experimental cements containing muña (14.475 ± 0.419 mm) and tincture (14.350 ± 0.351 mm). The control endodontic cement exhibited the largest inhibition halo (18.050 ± 0.451 mm), followed by the cement containing mint (16.498 ± 0.460 mm). The experimental cement with propolis showed the smallest inhibition halo (6.075 ± 0.650 mm) (Table 2). Statistically significant differences ($p < 0.05$) are indicated by indices in Figure 2.

Table 1. Inhibition halos (mean and standard deviation) of natural compounds against *E. faecalis*.

Natural compound	Inhibition halo (mm)		
	Mean	SD	Sig.*
Mint	4.450	0.129	p < 0.001
Muña	5.975	0.050	
Jalk propolis	4.250	0.129	
Propolis tincture	2.200	0.071	

* Significance according to ANOVA test. SD: standard deviation.

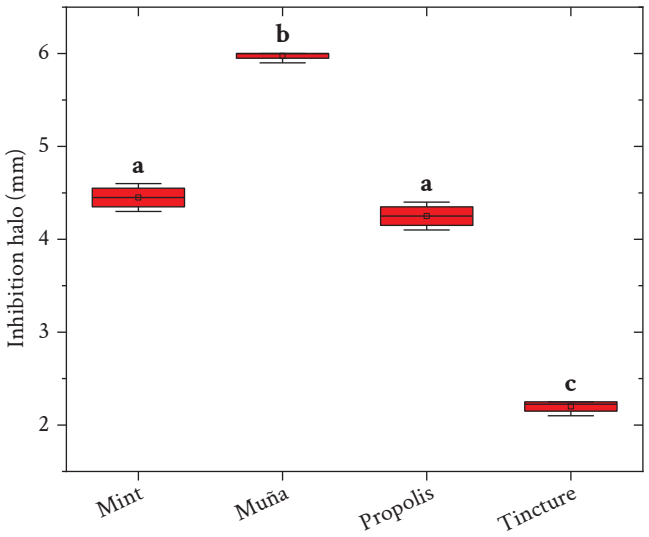

Figure 1. Box-and-whisker plot of the inhibition halos of natural compounds against *E. faecalis*. Different letters (a, b, and c) indicate statistically significant differences according to the Tukey test at the $p < 0.05$ level.

Table 2. Inhibition halos (mean and standard deviation) of the experimental endodontic cements against *E. faecalis*.

Experimental cement	Inhibition halo (mm)		
	Mean	SD	Sig.*
Cement + mint	16.498	0.460	p < 0.001
Cement + muña	14.475	0.419	
Cement + Jalk propolis	6.075	0.650	
Cement + tincture	14.350	0.351	
Cement	18.050	0.451	

* Significance according to the ANOVA test. SD: standard deviation.

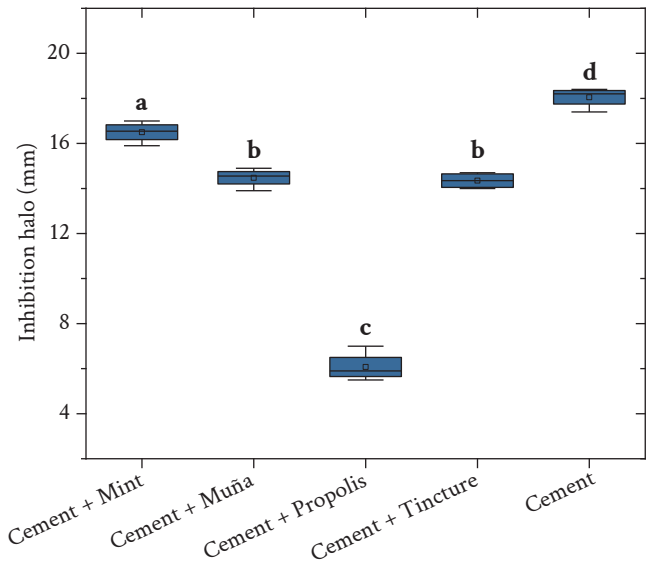

Figure 2. Box-and-whisker plot of the inhibition halos of the experimental cements against *E. faecalis*. Different letters (a, b, and c) indicate statistically significant differences according to the Tukey test at the $p < 0.05$ level.

DISCUSSION

E. faecalis strains are present in approximately 44% of infectious processes and apical lesions, and were found in a higher proportion (60%) in teeth with failed endodontic treatments (13, 14). Based on this, antibacterial sensitivity tests were performed on the natural compounds and subsequently incorporated into an experimental endodontic cement, as studied by Kitagawa et al. (2021) (15), and subjected to the same test using *E. faecalis* (ATCC 2982).

Several authors have determined, through similar studies, the antibacterial effect of the natural products evaluated in this research: muña (6.0 mm), a value similar to that found by Cecchini et al. (16) in 2021; mint (4.5 mm), higher than that found by Barros et al. (11) in 2015, who obtained 1.0 mm, and similar to that reported by Shayegh et al. (17) in 2008; Jalk propolis (4.3 mm) and propolis tincture (2.2 mm), both comparable to the results obtained by Vasudeva et al. (6) in 2017, who used propolis gel. Regarding other comparable values, it should be noted that natural products may vary in composition depending on factors such as climate, altitude, irrigation conditions, and even plant-to-plant differences; therefore, standardization is essential.

Previous studies on the antibacterial sensitivity test of an endodontic cement in which an element was incorporated reported values ranging from 9 mm to 18.42 mm (18-20). The control endodontic cement showed the largest inhibition halo, followed by those containing mint, muña, propolis tincture, and Jalk propolis, in that order. These results confirmed the antibacterial properties of all the substances incorporated into the endodontic cement (21, 22).

Regarding the experimental cement with propolis, it showed the smallest inhibition halo; however, studies indicate that the flavonoids present in propolis can alter the bacterial cell wall membrane and inhibit bacterial motility, thus contributing to synergism with certain antibiotics (23). Likewise, it is also capable of inhibiting the synthesis of mediators of the inflammatory process, such as prostaglandins and leukotrienes, as well as promoting phagocytic activity (24).

In terms of the percentage of flavonoids in each compound, research indicates that propolis contains the highest average (4.3%), while only about 1.0% originates from mint and muña (25-27). The notable antibacterial activity of the cement containing mint contrasts with studies suggesting that this effect derives from the presence of phenolic compounds, phenolic acids, flavonoids, and the combination of ketones and monoterpenes, mainly menthone, pulegone, and menthol (22).

An important limitation of this study was the lack of additional research with a similar design, partly due to ethical restrictions regarding the use of animals in research established by the Institutional Ethics Committee of Universidad Peruana Cayetano Heredia. The limited experience and knowledge in applying these methodologies at the university reduce investment in acquiring additional bacterial strains, thereby restricting the depth of research on these products.

CONCLUSIONS

This *in vitro* study determined that the natural compounds evaluated exhibit antibacterial activity against *E. faecalis*. The muña compound showed the largest inhibition halo, while the propolis tincture displayed the lowest antibacterial activity. The experimental endodontic cements containing natural compounds showed reduced antibacterial activity compared to the pure cement, followed by the cement with added mint; the cement containing propolis exhibited the lowest activity among them.

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EDPGP: data curation, formal analysis, research, methodology, resources, funding acquisition, software, visualization, writing - original draft.
KHM: validation, writing - original draft, writing - review & editing.
JAD: conceptualization, research, methodology, project administration, supervision, validation, writing - review & editing.

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