Brazilian Dental Science

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UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Instituto de Ciência e Tecnologia
Campus de São José dos Campos

ORIGINAL ARTICLE

DOI: https://doi.org/10.4322/bds.2025.e4697

# Incorporation of red propolis into ionomer restorative cements: an antimicrobial and fluoride release analysis

Incorporação de própolis vermelha em cimentos ionômero de vidro: uma análise antimicrobiana e de liberação de flúor

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**How to cite:** Almeida TMO, Borges MHN, Sette-de-Souza PH, Pinheiro MA, Vieira BR, Sampaio GAM. Incorporation of red propolis into ionomer restorative cements: an antimicrobial and fluoride release analysis. Braz Dent Sci. 2025;28(4):e4697. https://doi.org/10.4322/bds.2025.e4697

## ABSTRACT

**Objective:** This study aimed to evaluate the antimicrobial activity of Glass Ionomer Cements (GICs) with the addition of 11% and 20% Red Propolis Ethanolic Extract (RPEE) against *Streptococcus mutans* cultures and the fluoride release capacity of the cements. **Material and Methods:** Six conventional GICs (Riva, Maxxion R, Vidrion R, Ketac, Vitremer, and Ionolux) were used with the addition of 11% and 20% RPEE (n=10). *S. mutans* bacterial strains were cultured, and the cement samples were placed in contact with the microorganism for 48 hours in a bacteriological incubator. Inhibition zones were measured using a digital caliper. Fluoride release was measured at 2 hours, 24 hours, and 7 days using a selective ion electrode connected to an ion analyzer. The Kruskal-Wallis test with Dunn's post-hoc test was applied for antimicrobial data, and two-way ANOVA with Tukey's test for fluoride release. **Results:** The addition of RPEE significantly increased the antimicrobial effect of Maxxion R, Vitremer and Ionolux cements. The Maxxion R and Vitremer cements showed greater antimicrobial activity compared to Vidrion R in the groups without RPEE addition (controls). With 11% RPEE, the Maxxion R and Vitremer GICs displayed larger inhibition zones than Vidrion R, Riva, and Ionolux. At 20% RPEE, Maxxion R continued to exhibit the best results. A significant increase in fluoride release was observed for Ionolux with 11% RPEE (2h) and for Riva with 11% and 20% RPEE (24h) compared to their controls. **Conclusion:** The addition of 11% RPEE enhanced the antimicrobial effect of the GICs. The Maxxion R and Vitremer GICs with 11% RPEE stood out for their superior antimicrobial activity. The fluoride release capacity of the tested cements was not affected and was even enhanced in some cases.

## KEYWORDS

Antimicrobial; Dental materials; Fluoride; Glass ionomer cement; Propolis.

## RESUMO

**Objetivo:** Este estudo teve como objetivo avaliar a atividade antimicrobiana de Cimentos de Ionômero de Vidro (CIVs) com adição de Extrato Etanólico de Própolis Vermelha (EPR) a 11% e 20% em culturas de *Streptococcus mutans* e a capacidade de liberação de flúor dos cimentos. **Material e Métodos:** Foram utilizados seis CIVs convencionais (Riva, Maxxion R, Vidrion R, Ketac, Vitremer e Ionolux) com adição de RPEE 11% e 20%. Foram cultivadas cepas bacterianas de *S. mutans,* e as amostras de cimento foram colocadas em contato com o microrganismo por 48 horas em uma incubadora bacteriológica (n=10). As zonas de inibição foram medidas usando um paquímetro digital. A liberação de flúor foi medida em 2 horas, 24 horas e 7 dias usando um eletrodo de íons seletivo conectado a um analisador de íons (n=3). O teste Kruskal-Wallis com teste de Dunn's foram aplicados para os dados do antimicrobiano, e two-way ANOVA com teste de Tukey's para liberação de flúor. **Resultados:** A adição de RPEE aumentou significativamente o efeito antimicrobiano dos cimentos Maxxion R, Vitremer e Ionolux. Os cimentos Maxxion R e Vitremer apresentaram maior atividade antimicrobiana em comparação ao Vidrion R nos grupos sem adição de RPEE (controles). Com 11% de RPEE, os GICs Maxxion R e Vitremer apresentaram maiores zonas de inibição do que Vidrion R, Riva e Ionolux. Com 20% de RPEE, o Maxxion R continuou a exibir os melhores resultados.

Um aumento significativo na liberação de flúor foi observado para o Ionolux com 11% RPEE (2h) e para o Riva com 11% e 20% RPEE (24h) em comparação com seus controles. **Conclusão:** A adição de 11% de RPEE aumentou o efeito antimicrobiano dos GICs. Os GICs Maxxion R e Vitremer com 11% de RPEE se destacaram por sua atividade antimicrobiana superior. A capacidade de liberação de flúor dos cimentos testados não foi afetada e foi até mesmo aumentada em alguns casos.

## PALAVRAS-CHAVE

Antimicrobiano; Materiais dentários; Flúor; Cimento de ionômero de vidro; Propolis.

## INTRODUCTION

Glass ionomer cements (GICs) stand out as widely used materials in dentistry due to their various clinical properties, such as fluoride release, adhesion to dental structures, biocompatibility, and linear coefficient of thermal expansion. Despite their excellent characteristics, convencional GICs exhibit certain limitations, including low tensile strength, limited aesthetics, critical initial solubility due to moisture sensitivity—especially during the early stages of setting—and a short working time. Nevertheless, when appropriately indicated and planned, these disadvantages do not compromise their clinical applicability [1-3].

Glass ionomer cements possess cariostatic properties, primarily attributed to the incorporation of fluoride released by the material into the dental structure, making it more resistant to bacterial acid attack. Recent studies, however, have shown that the addition of antimicrobial agents to GICs can significantly enhance their antimicrobial properties [2,4-7].

Propolis, a material produced by bees from resins found on plant stems, has garnered increasing scientific interest due to its biological properties, such as antimicrobial, anti-inflammatory, antioxidant, antiviral, and wound-healing activities [8-10]. In particular, red propolis, predominantly found in the North and Northeast regions of Brazil, especially in coastal areas, has demonstrated promising antimicrobial and antifungal activity, despite being relatively underexplored [11-13].

Red propolis is abundant in Brazil and presents unique flavonoids with strong antimicrobial and antifungal activity. Given its antibacterial effectiveness against oral microorganisms, such as *Streptococcus mutans* [12-14], the incorporation of Red Propolis Ethanolic Extract (RPEE) into GICs emerges as a potential strategy to enhance the anticariogenic effect of the material. This addition may contribute to reducing the bacterial population in areas adjacent to the material [2,5]. However, in higher concentrations, the intense color of red propolis can also affect the aesthetics of restorations, in addition to, it is important to consider that the inclusion of antimicrobial agents in GICs may alter some of their physical and biological properties [15], such as their fluoride release capability.

Therefore, the present study aims to evaluate the antimicrobial activity of GICs containing 11% and 20% RPEE against *S. mutans* cultures, as well as to measure the fluoride release capacity of these materials. The null hypothesis of this study is that the addition of RPEE enhances the antimicrobial properties of GICs without compromising their fluoride release capability.

## MATERIAL AND METHODS

### Experimental design

This is an *in vitro* laboratory study. Two factors were studied—type of GIC (6 brands) and RPEE concentration (0%, 11%, 20%)—using a factorial design. All procedures were performed by a single blinded operator. The analyses were conducted at the Biotechnology Laboratory of the University of Pernambuco (BIOSPE), Arcoverde Campus.

### Preparation of GICs containing RPEE

This study utilized Red Propolis Ethanolic Extract (RPEE), obtained from raw red propolis collected from the coastal region of Alagoas, Brazil (Fernão Velho, Alagoas, Brazil, Batch 03/23). For each 25 g of propolis, this was dissolved in 250 mL of 80% (vol/vol) ethanol solution. The extract was then filtered twice on filter paper to remove excess wax. Then, the EERP was prepared at the concentrations of 11% and 20% and placed in an amber glass bottle at room temperature.

Six self-curing restorative GICs were used: Riva (SDI, Bayswater, Victoria, Australia), Maxxion R (FGM, Joinville, Santa Catarina, Brazil), Vidrion R (SSWhite, Rio de Janeiro, Rio de Janeiro, Brazil), Ketac Molar Easymix (3M ESPE, Seefeld, Bavaria, Germany), Vitremer (3M, St. Paul, Minnesota, USA) and Ionolux (VOCO, Cuxhaven, Lower Saxony, Germany) (Table I).

For the control group, materials were prepared following the manufacturers' instructions. For the test group, RPEE was incorporated into the cement liquid during preparation at a ratio of one drop of RPEE to two drops of liquid, using the same dropper tip [5]. The mixture was then spatulated with the cement powder. The materials were placed into polyethylene molds (10 mm in diameter × 5 mm in thickness) using insertion spatulas, and left to rest with the surface covered by a glass plate for 5 minutes at 25°C.

A total of 234 samples (n=10 for antimicrobial analysis, n=3 for fluoride release analysis) were prepared for each test and distributed into 18 groups: RC (Riva Control), R11 (Riva with 11% RPEE), R20 (Riva with 20% RPEE), MC (Maxxion R Control), M11 (Maxxion R with 11% RPEE), M20 (Maxxion R with 20% RPEE), VC (Vidrion R Control), V11 (Vidrion R with 11% RPEE), V20 (Vidrion R with 20% RPEE), KC (Ketac Control), K11 (Ketac with 11% RPEE), K20 (Ketac with 20% RPEE), VTC (Vitremer Control), VT11 (Vitremer with 11% RPEE), VT20 (Vitremer with 20% RPEE), IC (Ionolux Control), I11 (Ionolux with 11% RPEE), and I20 (Ionolux with 20% RPEE).

## Antimicrobial analysis

*S. mutans* ATCC 25175 bacterial strains were cultured in Brain Heart Infusion (BHI). A $10^{-1}$ dilution containing $1.2 \times 10^{8}$ CFU/mL was prepared, determined through serial dilution in 0.85% saline solution. After incubation at 37°C for 48 hours, the bacterial strain was spread on BHI agar plates and left at room temperature for 30 minutes. Subsequently, the samples were placed in direct contact with the medium and incubated at 37°C for 48 hours in a microaerophilic environment. After this period, the diameters of the inhibition zones were measured at two points, horizontally and vertically, using a digital caliper. Discs soaked with 0.12% chlorhexidine and saline solution were used as positive and negative controls, respectively.

## Fluoride release analysis

After sample preparation, the specimens were stored at 37°C and 100% humidity for 30 minutes. Each specimen was then placed in 2 mL of deionized water obtained from the Milli-Q purification system and kept in an incubator at 37°C. Fluoride release was measured at 2 hours, 24 hours, and 7 days using a selective ion electrode connected to an ion analyzer (Thermo Scientific Orion, USA) calibrated with standards ranging from 0.2 to 5.0 ppm F in 50% TISAB II. Readings were taken in millivolts (mV) and converted to $\mu$g/mL (ppm F) using linear regression of the calibration curve.

**Table I -** Composition of cements

| GIC | Manufacturer | Liquid* | Powder* | Batch |
|---|---|---|---|---|
| **Riva** | SDI (Australia) | Polyacrylic acid, dihydroxybutanedioic acid, water | Chemical glass oxides, polyacrylic acid | 11875203 |
| **Maxxion** | FGM (Brazil) | Polyacrylic acid, water | Fluorine-aluminum silicate glass, tartaric acid, calcium fluoride | 181022 |
| **Vidrion** | SSWHITE (Brazil) | Tartaric acid, water | Sodium-calcium-aluminum fluorosilicate, barium sulfate, polyacrylic acid, pigments | 0061022 |
| **Ketac Molar Easymix** | 3M ESPE (Germany) | Copolymer of acrylic acid and maleic acid, tartaric acid, water | Chemical glass oxides, polyacrylic acid | 10006917 |
| **Vitremer** | 3M (USA) | Polyacrylic acid, hydroethyl methacrylate water | Fluorine-aluminum silicate glass | 2325500381 |
| **Ionolux** | VOCO (Germany) | Polyacrylic acid, tartaric acid, water | Fluorine Aluminosilicate Glass | 2246424 |
| **RPEE** | Fernão Velho (Brazil) | Neutral alcohol, red propolis and purified water | | 03/23 |

*Composition was provided by manufacturers.

### Statistical analysis

The results were organized into a database using Microsoft Excel and then exported to the Statistical Package for the Social Sciences (SPSS, version 20, SPSS Inc., Chicago, IL, USA) for statistical analysis. The non-parametric Kruskal-Wallis test, followed by Dunn's multiple comparison test, was used to compare antimicrobial effect results. Two-way ANOVA with Tukey's multiple comparison test was used for fluoride release results. All tests were conducted with a significance level of 95% ($p<0.05$).

## RESULTS

### Antimicrobial analysis

For Maxxion R and Vitremer GICs, data analysis showed an increase in inhibition zone size in the groups with 11% RPEE, with significant differences between M11 and MC ($p=0.004$) and between VT11 and VT20 ($p=0.001$). For Ionolux, an increase in inhibition zone size was observed in the group with 20% RPEE, with a significant difference between I20 and IC ($p=0.005$). For Vidrion R and Ketac, inhibition zone size increased in the groups with red propolis, but the difference was not statistically significant. For Riva, a slight reduction in inhibition zone diameter was noted, but without statistical significance (Table II).

When comparing the tested RPEE concentrations, it was observed that an increase in RPEE concentration did not necessarily correspond to larger inhibition zones. Only for Ionolux did the 20% RPEE group exhibit significantly larger zones than the 11% group ($p=0.005$). For Vidrion R, this increase was not statistically significant ($p=0.835$), and for the other cements, a decrease in inhibition zone size was observed in the 20% RPEE group compared to the 11% group, with significant differences between VT11 and VT20 ($p=0.001$) (Table II).

When comparing the results among different GIC brands, Maxxion R and Vitremer exhibited greater antimicrobial activity, with significant differences compared to Vidrion R ($p=0.018$) in the control groups. For the 11% RPEE groups, Maxxion R and Vitremer had significantly larger inhibition zones than Vidrion R, Riva, and Ionolux ($p=0.001$). For the 20% RPEE groups, Maxxion R continued to show the best results, with significant differences compared to Riva and Vitremer ($p=0.001$), followed by Ionolux and Ketac, which had significantly larger inhibition zones than Riva ($p=0.001$) (Table III).

**Table II -** Mean and standard deviation of inhibition zone measurements (mm) – intragroup comparisons

| GIC | Control | RPEE 11% | RPEE 20% | p |
|---|---|---|---|---|
| **Riva** | 1.6 (1.4) | 1.0 (1.2) | 0.5 (0.6) | 0.185 |
| **Maxxion R** | 2.4 (1.0)$^{B}$ | 5.6 (2.1)$^{A}$ | 3.1 (1.2)$^{AB}$ | **0.004** |
| **Vidrion R** | 1.3 (0.7) | 1.3 (0.6) | 1.6 (1.0) | 0.835 |
| **Ketac** | 2.0 (0.5) | 2.2 (0.2) | 2.1 (0.7) | 0.295 |
| **Vitremer** | 2.3 (0.5)$^{A}$ | 3.9 (1.6)$^{A}$ | 1.0 (0.9)$^{B}$ | **0.001** |
| **Ionolux** | 1.7 (0.3)$^{AB}$ | 1.2 (0.4)$^{B}$ | 2.5 (0.5)$^{A}$ | **0.005** |

Means followed by identical letters indicate no statistically significant difference ($p > 0.05$). Means followed by different letters indicate statistically significant differences ($p < 0.05$). $^{A,B}$Uppercase letters represent differences between control and test groups within the same cement (row). Non-parametric Kruskal-Wallis test followed by Dunn's multiple comparisons test.

**Table III -** Mean and standard deviation of inhibition zone measurements (mm) between-group comparisons

| GIC | Control | RPEE 11% | RPEE 20% |
|---|---|---|---|
| **Riva** | 1.6 (1.4)$^{ab}$ | 1.0 (1.2)$^{b}$ | 0.5 (0.6)$^{b}$ |
| **Maxxion R** | 2.4 (1.0)$^{a}$ | 5.6 (2.1)$^{a}$ | 3.1 (1.2)$^{a}$ |
| **Vidrion R** | 1.3 (0.7)$^{b}$ | 1.3 (0.6)$^{b}$ | 1.6 (1.0)$^{abc}$ |
| **Ketac** | 2.0 (0.5)$^{ab}$ | 2.2 (0.2)$^{ab}$ | 2.1 (0.7)$^{ac}$ |
| **Vitremer** | 2.3 (0.5)$^{a}$ | 3.9 (1.6)$^{a}$ | 1.0 (0.9)$^{bc}$ |
| **Ionolux** | 1.7 (0.3)$^{ab}$ | 1.2 (0.4)$^{b}$ | 2.5 (0.5)$^{ac}$ |
| **p** | **0.018** | **0.001** | **0.001** |

Means followed by identical letters indicate no statistically significant difference ($p > 0.05$). Means followed by different letters indicate statistically significant differences ($p < 0.05$). $^{a,b}$while lowercase letters represent comparisons between cements (column). Non-parametric Kruskal-Wallis test followed by Dunn's multiple comparisons test.

### Fluoride release analysis

The addition of EEPV did not affect the fluoride release capacity of the tested cements, as there was no significant reduction in fluoride release in the groups with propolis compared to the control groups. Additionally, a significant increase in fluoride release was observed in the Ionolux P11 group compared to the Ionolux control group at the 2-hour mark (p=0.021) and in the Riva P11 and Riva P20 groups compared to the Riva control group at the 24-hour (p=0.006) and 7-day (p=0.005) marks (Tables IV, V and VI).

When comparing the different GIC brands tested, it was observed that at the 2-hour mark, the Vidrion R cement groups exhibited significantly higher fluoride ion release than the other cements for both the control groups (p=0.001) and the groups with 20% EEPV addition (p=0.001). The Maxxion R and Riva control cements showed lower fluoride release than Vidrion R but significantly higher release than the Ionolux and Ketac cements (p=0.001). The same pattern was observed for the groups with 20% EEPV addition. When comparing the cements with 11% EEPV addition, Vidrion R and Maxxion R cements showed significantly better results than the other cements (p=0.001) (Table VII).

At the 24-hour mark, among the control groups, Vidrion R continued to show the highest fluoride release, with significant differences compared to the Riva and Ketac cements (p=0.001). However, among the cements with EEPV addition, Riva cement exhibited the highest fluoride release, with significant differences compared to Ketac at the 11% concentration (p=0.002) and compared to both Ketac and Ionolux at the 20% concentration (p=0.010) (Table VIII).

**Table IV -** Fluoride release results for cements at the 2-hour mark (µg/mL) – intragroup comparisons

| GIC | Control | RPEE 11% | RPEE 20% | p |
|---|---|---|---|---|
| **Riva** | 4.20 (0.09) | 4.24 (0.08) | 4.56 (0.21) | 0.076 |
| **Maxxion R** | 4.78 (0.31) | 5.00 (0.12) | 4.55 (0.03) | 0.596 |
| **Vidrion R** | 5.26 (0.03) | 5.25 (0.01) | 5.26 (0.01) | 1.000 |
| **Ketac** | 3.60 (0.14) | 3.85 (0.01) | 3.74 (0.09) | 0.518 |
| **Ionolux** | 3.75 (0.15)$^{A}$ | 4.17 (0.08)$^{B}$ | 3.90 (0.05)$^{AB}$ | **0.021** |

Means followed by the same letters indicate no statistically significant difference (p > 0.05). Means followed by different letters indicate a statistically significant difference (p < 0.05). $^{A,B}$ Uppercase letters indicate differences between the control and test groups within the same cement (row). Two-way ANOVA test with Tukey's multiple comparison test.

**Table V -** Fluoride release results for cements at the 24-hour mark (µg/mL) - intragroup comparisons

| GIC | Control | RPEE 11% | RPEE 20% | p |
|---|---|---|---|---|
| **Riva** | 4.26 (0.10)$^{B}$ | 4.77 (0.17)$^{A}$ | 4.80 (0.15)$^{A}$ | **0.006** |
| **Maxxion R** | 4.55 (0.91) | 4.60 (0.16) | 4.62 (0.15) | 1.000 |
| **Vidrion R** | 4.84 (0.06) | 4.56 (0.12) | 4.60 (0.14) | 0.806 |
| **Ketac** | 3.94 (0.20) | 4.18 (0.01) | 4.29 (0.05) | 0.801 |
| **Ionolux** | 4.55 (0.07) | 4.48 (0.22) | 4.36 (0.22) | 1.000 |

Means followed by the same letters indicate no statistically significant difference (p > 0.05). Means followed by different letters indicate a statistically significant difference (p < 0.05). $^{A,B}$Uppercase letters indicate differences between the control and test groups within the same cement (row). Two-way ANOVA test with Tukey's multiple comparison test.

**Table VI -** Fluoride release results for cements at the 7-day mark (µg/mL) – intragroup comparisons

| GIC | Control | RPEE 11% | RPEE 20% | p |
|---|---|---|---|---|
| **Riva** | 4.59 (0.32)$^{B}$ | 5.04 (0.09)$^{A}$ | 5.15 (0.13)$^{A}$ | **0.005** |
| **Maxxion R** | 4.32 (0.06) | 4.27 (0.04) | 4.26 (0.05) | 1.000 |
| **Vidrion R** | 4.27 (0.05) | 4.33 (0.16) | 4.30 (0.08) | 1.000 |
| **Ketac** | 4.46 (0.12) | 4.69 (0.15) | 4.28 (0.18) | 0.821 |
| **Ionolux** | 4.70 (0.02) | 4.96 (0.10) | 4.65 (0.24) | 1.000 |

Means followed by the same letters indicate no statistically significant difference (p > 0.05). Means followed by different letters indicate a statistically significant difference (p < 0.05). $^{A,B}$Uppercase letters indicate differences between the control and test groups within the same cement (row). Two-way ANOVA test with Tukey's multiple comparison test.

**Table VII -** Fluoride release results for cements at the 2-hour mark (µg/mL) - intergroup comparisons

| GIC | Control | RPEE 11% | RPEE 20% |
|---|---|---|---|
| **Riva** | 4.20 (0.09)$^{c}$ | 4.24 (0.08)$^{b}$ | 4.56 (0.21)$^{b}$ |
| **Maxxion R** | 4.78 (0.31)$^{b}$ | 5.00 (0.12)$^{a}$ | 4.55 (0.03)$^{b}$ |
| **Vidrion R** | 5.26 (0.03)$^{a}$ | 5.25 (0.01)$^{a}$ | 5.26 (0.01)$^{a}$ |
| **Ketac** | 3.60 (0.14)$^{d}$ | 3.85 (0.01)$^{b}$ | 3.74 (0.09)$^{c}$ |
| **Ionolux** | 3.75 (0.15)$^{d}$ | 4.17 (0.08)$^{b}$ | 3.90 (0.05)$^{c}$ |
| p | **0.001** | **0.001** | **0.001** |

Means followed by the same letters indicate no statistically significant difference (p > 0.05). Means followed by different letters indicate a statistically significant difference (p < 0.05). $^{a,b,c,d}$while lowercase letters indicate the comparison between the cements (column). Two-way ANOVA test with Tukey's multiple comparison test.

**Table VIII -** Fluoride release results for cements at the 24-hour mark (µg/mL) - intergroup comparisons

| GIC | Control | RPEE 11% | RPEE 20% |
|---|---|---|---|
| **Riva** | 4.26 (0.10)$^{bc}$ | 4.77 (0.17)$^{a}$ | 4.80 (0.15)$^{a}$ |
| **Maxxion R** | 4.55 (0.91)$^{ab}$ | 4.60 (0.16)$^{ab}$ | 4.62 (0.15)$^{ab}$ |
| **Vidrion R** | 4.84 (0.06)$^{a}$ | 4.56 (0.12)$^{ab}$ | 4.60 (0.14)$^{ab}$ |
| **Ketac** | 3.94 (0.20)$^{c}$ | 4.18 (0.01)$^{b}$ | 4.29 (0.05)$^{b}$ |
| **Ionolux** | 4.55 (0.07)$^{ab}$ | 4.48 (0.22)$^{ab}$ | 4.36 (0.22)$^{b}$ |
| p | **0.001** | **0.002** | **0.010** |

Means followed by the same letters indicate no statistically significant difference (p > 0.05). Means followed by different letters indicate a statistically significant difference (p < 0.05). $^{a,b,c}$while lowercase letters indicate the comparison between the cements (column). Two-way ANOVA test with Tukey's multiple comparison test.

**Table IX -** Fluoride release results for cements at the 7-day mark (µg/mL) - intergroup comparisons

| GIC | Control | RPEE 11% | RPEE 20% |
|---|---|---|---|
| **Riva** | 4.59 (0.32) | 5.04 (0.09)$^{a}$ | 5.15 (0.13)$^{a}$ |
| **Maxxion R** | 4.32 (0.06) | 4.27 (0.04)$^{b}$ | 4.26 (0.05)$^{b}$ |
| **Vidrion R** | 4.27 (0.05) | 4.33 (0.16)$^{b}$ | 4.30 (0.08)$^{b}$ |
| **Ketac** | 4.46 (0.12) | 4.69 (0.15)$^{ab}$ | 4.28 (0.18)$^{b}$ |
| **Ionolux** | 4.70 (0.02) | 4.96 (0.10)$^{a}$ | 4.65 (0.24)$^{b}$ |
| p | 1.000 | **0.001** | **0.001** |

Means followed by the same letters indicate no statistically significant difference (p > 0.05). Means followed by different letters indicate a statistically significant difference (p < 0.05). $^{a,b}$while lowercase letters indicate the comparison between the cements (column). Two-way ANOVA test with Tukey's multiple comparison test.

At the 7-day mark, no significant differences were observed among the control groups. However, for the cements with 11% EEPV, Riva and Ionolux cements showed significantly higher fluoride release than Maxxion R and Vidrion R cements. Among the cements with 20% EEPV, Riva cement exhibited significantly higher fluoride release than the other cements (Table IX).

## DISCUSSION

Previous research observed that 25% concentrations of RPEE can increase the antimicrobial capacity of GICs without affecting the mechanical properties of the cements [5]. However, due to the strong coloration of red propolis, high concentrations of RPEE may alter the color of the cements, thus affecting the aesthetics of the restoration. In the present study, concentrations of 11% and 20% RPEE were used to determine whether lower concentrations of RPEE could also significantly increase the antimicrobial effect of the cements. Pilot tests performed in our laboratory also indicated that concentrations higher than 20% impaired the setting reaction and consistency of some GICs. Therefore, we chose these intermediate concentrations to ensure a balance between antibacterial efficacy and adequate cement manipulation.

The findings suggest that the GIC modified with propolis shows great promise as a restorative material due to its antibacterial properties. Previous studies have investigated the antibacterial properties of GICs containing propolis against *S. mutans*, as well as its effect on biofilm formation *in vitro*. The results revealed notable antibacterial efficacy and a clear reduction in biofilm formation when the GIC contained propolis compared to the control group [16]. In the present study, GICs with RPEE concentrations of 11% and 20% were used, and the results showed a significant increase in the antimicrobial capacity of the GIC with 11% RPEE. Other authors also observed positive results, with a significant increase in antimicrobial effectiveness against S. mutans and C. albicans, after incorporating propolis into GICs at concentrations of 11%, 25%, and 50% [5].

Aguilar-Perez et al. [17] evaluated the effect of incorporating different concentrations of propolis on the antibacterial, mechanical, and adhesive properties of a commercial cement. The results highlighted the remarkable antiseptic potential of the modified material against *S. mutans*, as well as offering anti-inflammatory properties. Especially in atraumatic restorative procedures, such as those in deep cavities, this material shows promise as a viable alternative.

Incorporation of red propolis into orthodontic adhesives has been shown to increase their antimicrobial activity against Streptococcus mutans, without compromising physicomechanical properties such as degree of conversion and shear bond strength [18]. Comparable results were observed in a study where conventional glass ionomer cements containing 25% RPEE exhibited significantly enhanced antimicrobial activity against S. mutans and Candida albicans, while maintaining their mechanical properties and fluoride release capacity [5].

Fluoride release is one of the main characteristics of this material and a decisive factor for its clinical use. The present study aimed to quantify fluoride release over periods of 2h, 24h, and 7 days, since during the first 24 hours, fluoride release from the GIC is most intense, gradually stabilizing over the following days until reaching a steady state [19-21]. The fluoride release from cements with RPEE addition did not show statistical differences compared to control groups, suggesting favorable clinical use. Other authors, such as one by other authors, also demonstrated that yellow propolis extract improved the antimicrobial properties of GICs while maintaining the fluoride ion release characteristics [22].

The Ionolux cements (at 2 hours) and Riva cements (at 24 hours and 7 days) showed a significant increase in fluoride amounts in the groups with RPEE addition. Other authors have also observed increased fluoride release when propolis is used with glass ionomer cement [23]. It is believed that when propolis is added to glass ionomer, fluoride from both the ionomer and propolis is released into the surrounding environment, resulting in increased fluoride release compared to conventional glass ionomer use.

The nature of GICs can affect their fluoride release capacity, depending on whether they are light-cured or chemically activated, restorative, base, or cementing, and whether they are conventional, anhydrous, or resin-modified [24]. The anhydrous GIC is similar to the conventional one with a few modifications, while the conventional one is made from powder and a polyacid liquid. The anhydrous variant differs in that acid is incorporated after lyophilization and vacuum drying of the powder, with the liquid being only distilled water [25]. In the present study, Vidrion R cement showed significantly higher fluoride release than the other cements at 2 and 24 hours, likely because it is an anhydrous type. The variation in results between the different GIC brands may have occurred due to the difference in composition between the cements and a possible interaction between the flavonoids in RPEE and the polyacid matrix of the cements.

This study has limitations as it is an in vitro study, where the oral environment is not faithfully replicated. Additionally, only one antimicrobial test was performed. Therefore, further antimicrobial tests using biofilm models, in situ tests, and clinical trials are recommended. Finally, the results obtained are promising and indicate that the use of GICs associated with red propolis is an effective alternative in dental practice. It presents itself as a cost-effective alternative, as red propolis is low-cost and available regionally. Continued research in this area could be crucial for the development of innovative dental materials, offering better therapeutic and preventive outcomes for patients.

## CONCLUSIONS

The incorporation of 11% RPEE enhanced the antimicrobial effect of the GICs, particularly Maxxion and Vitremer. Fluoride release varied among materials: while most were unaffected, Riva and Ionolux showed increased fluoride release at specific time points.

## Author's Contributions

GAMS: Conceptualization. TMOA, MHNB: Data Curation. PHSS, MAP, BRV: Formal Analysis. GAMS: Funding Acquisition. TMOA, MHNB: Investigation. TMOA, MHNB: Methodology. GAMS: Project Administration. PHSS, MAP, BRV, GAMS: Supervision. PHSS, MAP, BRV: Validation. TMOA, MHNB: Writing – Original Draft Preparation. PHSS, MAP, BRV, GAMS: Writing – Review & Editing.

## Conflict of Interest

No conflicts of interest declared concerning the publication of this article.

## Funding

This work was supported by the Pernambuco State Foundation for Science and Technology (FACEPE).

## Regulatory Statement

This study did not utilize hazardous substances, animal or human subjects. As a result, adherence to specific regulatory laws or guidelines concerning occupational health, safety, or environmental protection was not required. All appropriate steps were taken to uphold ethical research standards and ensure laboratory safety.

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**Editor: Anuradha Prakki**

Date submitted: 2025 Feb 20
Accept submission: 2025 Sept 11