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In vitro Performance of a Pomegranate Dentifrice on Complete Dentures: A Step toward Natural Oral Care

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This study aimed to evaluate the effects of brushing with an experimental pomegranate dentifrice (PD) on heat-polymerized acrylic resin specimens, comparing it with a commercial dentifrice (CD) not specifically designed for dentures and distilled water (DW).

Place and Duration of Study: Research Laboratory of the Graduate Program in Dentistry at the Federal University of Ceará, located in Fortaleza, Ceará, Brazil.

Methodology: Thirty acrylic resin specimens (n = 10) underwent five cycles of 17,800 brush strokes, simulating 60 months of accumulated brushing. The specimens were assessed for roughness, surface microhardness, mass loss, and color stability initially (T0) and after each cycle (T1: 12 months; T2: 36 months; T3: 60 months). The data were analyzed using repeated measures ANOVA, followed by the Bonferroni post-test.

Results: Intergroup comparisons revealed no significant differences for the assessed parameters. However, intragroup analysis indicated a significant increase in microhardness for the PD group ($P = 0.01$) and heightened surface roughness in the CD group ($P < 0.01$). Overall, the results suggest that this experimental pomegranate-based dentifrice is a viable option for brushing complete dentures without compromising their structural integrity, representing a promising candidate for future denture cleaning protocols.

Conclusion: The pomegranate dentifrice neither adversely affected surface roughness nor caused significant changes in mass or color of the acrylic resin. As such, it shows potential as a natural alternative to conventional denture cleansers, although further studies are needed to assess its biofilm removal efficacy and confirm its fungicidal effects.

Keywords: Denture bases; tooth brushing; Punica granatum; microhardnesss; color.

1. INTRODUCTION

Denture stomatitis (DS) is an inflammation of the oral mucosa predominantly associated with the species *Candida albicans* (Gendreau & Loewy, 2011; Patil et al., 2015). Its estimated prevalence is approximately 70% among removable denture wearers (Manikandan et al., 2022). Despite its multifactorial etiology, DS is strongly associated with poor oral hygiene and the continuous use of complete or partial dentures, particularly during sleep (Bianchi et al., 2016; McReynolds et al., 2023; Ribeiro et al., 2024). Generally, DS is asymptomatic; however, some patients may report symptoms such as burning sensation or taste alterations (Altarawneh et al., 2012; Bianchi et al., 2016; Gendreau & Loewy, 2011; McReynolds et al., 2023).

The management of denture stomatitis (DS) may include topical or systemic antifungal treatments, combined with patient instructions on denture biofilm cleaning and disinfection. Nystatin and miconazole are the main topical agents used to relieve signs and symptoms. For recurrent infections or in immunocompromised patients, systemic antifungals such as fluconazole, itraconazole, and amphotericin B may be prescribed (Hilgert et al., 2016). It is crucial to emphasize the growing resistance of several microorganisms to antimicrobial agents. In addition, antifungals are associated with significant hepatotoxic effects, particularly with repeated administrations (Badaró et al., 2020).

As an alternative to conventional drugs, some studies have suggested the use of certain medicinal plants, with proven antimicrobial effects, for the management and prevention of oral candidiasis, through physical and chemical denture hygiene strategies (Badaró et al., 2020; Barreto et al., 2021; Patel et al., 2008; Pérez-Nicolás et al., 2023; Veilleux & Grenier, 2019; Villalta et al., 2006).

An example of this is *Punica granatum* Linné, commonly known as pomegranate, which has shown a potent fungicidal effect against Candida species for the prevention and treatment of denture stomatitis (Almeida et al., 2017; Singh et al., 2002; Vasconcelos et al., 2006; Vasconcelos et al., 2003), even at relatively low concentrations (6.25%) of the fruit peel extract (Vasconcelos et al., 2006). The pharmacological properties of *Punica granatum* L. have been extensively documented, highlighting its potent oral antifungal and antibacterial activities (Jurenka, 2008; Gomes et al., 2016; Bassiri-Jahromi et al., 2018). These effects are primarily attributed to its rich bioactive composition, in which polyphenols represent the predominant phytochemical class. Among them, tannins, particularly punicalagin, stand out for their significant biological relevance. The antifungal action of tannins is believed to involve interactions with and disruption of fungal cell membranes, thereby impairing cellular integrity and function (Brighenti et al., 2021; Ferreira et al., 2025).

Several denture cleaning methods are available on the market; however, brushing remains the simplest and most cost-effective method. It is known that brushing can lead to mass loss and increased surface roughness of acrylic resin over time (Campos et al., 2023; Fouda et al., 2025), which may promote plaque retention, loss of gloss and smoothness, as well as facilitate denture staining. Thus, the availability of a dentifrice with low abrasiveness and natural-origin fungicidal properties appears to be an interesting product for the hygiene of complete dentures.

Hence, the present study sought to investigate the impact of brushing heat-polymerized acrylic resin specimens with an experimental dentifrice containing 6.25% *Punica granatum* Linné peel extract on microhardness, surface roughness, mass loss, and color stability. The null hypothesis adopted was that brushing with the experimental pomegranate dentifrice would not differ from brushing with conventional dentifrices for any of the aforementioned parameters.

2. MATERIALS AND METHODS

2.1 Experimental Design

This randomized in vitro study was conducted to evaluate the effects of brushing with a pomegranate-based experimental dentifrice on heat-polymerized acrylic resin specimens. A total of 30 specimens were prepared and randomly allocated into three groups (n = 10 each): a negative control group (specimens brushed with distilled water); a positive control group (specimens brushed with a low-cost commercial dentifrice not specifically designed for dentures); and the experimental group (specimens brushed with the pomegranate-based experimental dentifrice).

Subsequently, the specimens underwent a brushing regimen in a mechanical simulator for a period corresponding to 60 months. Before and after the simulated brushing, the specimens were weighed using an analytical balance and assessed for surface roughness, Knoop microhardness, and color change. The experimental workflow is illustrated in Fig. 1.

2.2 Sample Calculation

The sample size calculation was performed using the public domain website OpenEpi, based on surface loss data from a previous study (Carvalho-Neto et al., 2021). Considering that roughness and color stability differed significantly compared to the group treated with distilled water (0.897 ± 0.026 vs. 0.059 ± 0.053 / 11.560 ± 0.216 vs. 2.899 ± 0.992), it was determined that 10 samples per group would be required to achieve a 95% confidence interval with 80% power.

2.3 Specimens Preparation

Thirty heat-polymerized acrylic resin specimens were fabricated in a rectangular shape with average dimensions of 30 × 20 × 5 mm. The specimens were obtained from plaster molds prepared within flasks. To create the molds, rectangular silicone matrices were produced using Zetaplus (Zhermack SpA, Badia Polesine, RO, Italy). These matrices were then embedded in type IV dental stone (Densite, Dentsply Ind. Com. Ltda., Petrópolis, RJ, Brazil). After the stone had set, its surface was treated with a separating medium (Cel-Lac, SS White Artigos Odontológicos Ltda., Rio de Janeiro, RJ, Brazil). The counter-mold was filled with an additional portion of stone poured over the matrices, and the flask was compressed for approximately 40 minutes. Upon opening the flask, the matrices were removed, leaving the plaster molds.

The acrylic resin (Artigos Odontológicos – Clássico, Campo Limpo Paulista, SP, Brazil) was mixed at a ratio of 10 g of powder to 10 ml of liquid. The mixture was placed into the flask during its plastic phase, and the flask was subsequently compressed again. The assembly was then immersed in hot water to initiate the heat-polymerization process. After the resin had set, the rectangular specimens were removed for finishing and polishing. Polishing was performed using a polishing machine (Aropol 2V, Arotec Indústria e Comércio, Cotia, Brazil) with water sandpapers of varying grits (Norton Indústria Brasileira, São Paulo, Brazil) – 600, 1200, and 2000 – each applied for one minute. Felt discs with pumice paste were also used during the polishing procedure. All materials used in the fabrication of the specimens were handled according to the manufacturers' instructions.

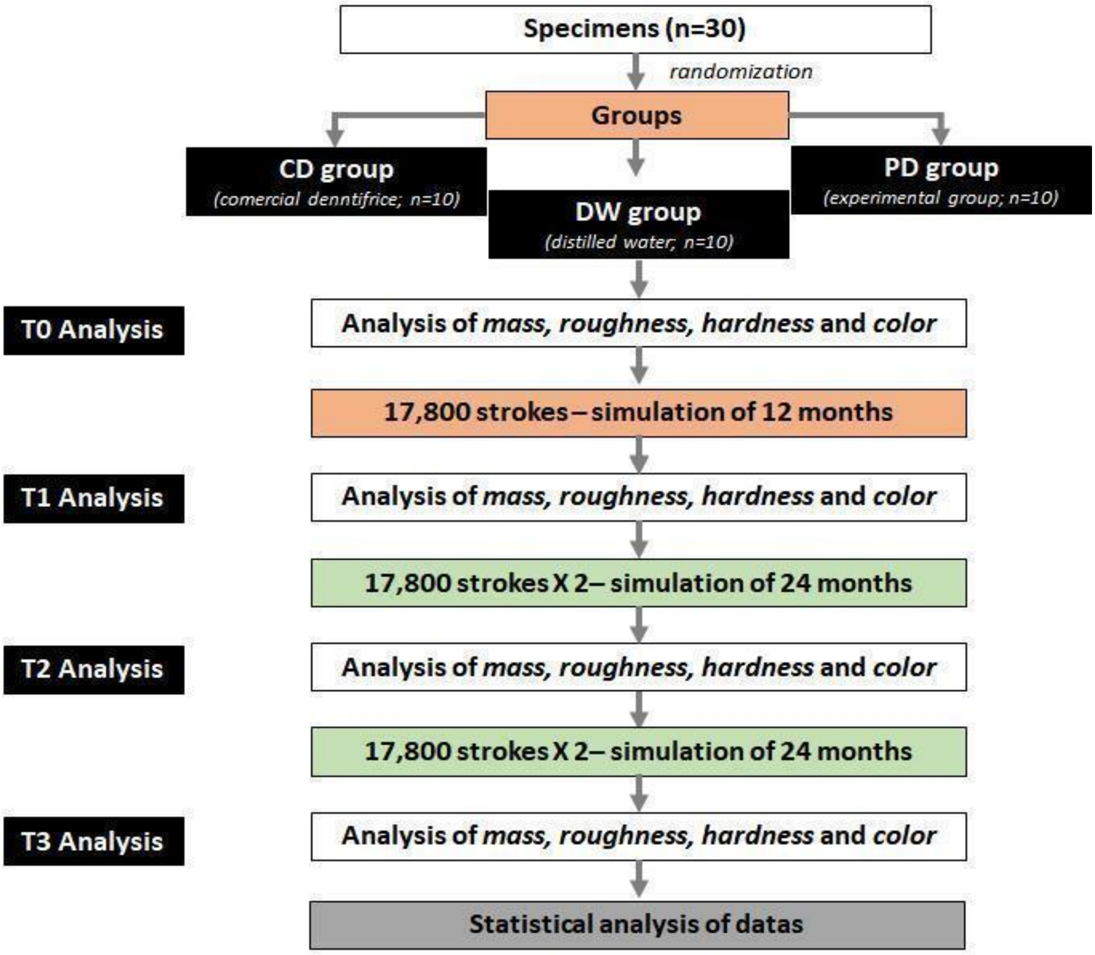

Fig. 1. Flowchart of the in vitro study. Distribution of specimens for each analysis (n = 10 per group)

Distilled Water (DW) = Negative Control Group = Specimens brushed with distilled water; Commercial Dentifrice (CD) = Positive Control Group = Specimens brushed with a commercially available, non-specific, low-cost denture toothpaste; Pomegranate Dentifrice (PD) = Experimental Group = Specimens brushed with an experimental pomegranate dentifrice with low abrasiveness (6.25% concentration)

2.4 Tested Substances

Experimental Pomegranate Dentifrice (PD Group): A dentifrice was developed using a hydroalcoholic extract derived from the peel of *Punica granatum* Linné fruit at a concentration of 6.25% (according to Invention Patent BR1020170212203, National Institute of Industrial Property, Federal University of Ceará, Brazil). The extract was provided by All Chemistry Brasil Ltda (R. Cocáis, 300 - Jardim Oriental, São Paulo - SP, ZIP 04347-190). The commercial extract exhibited a chromatographic profile similar to that of the extract collected from the georeferenced and characterized botanical garden source, being standardized by the tannin punicalagin. The dentifrice formulation includes micronized calcium carbonate (noted for its low abrasiveness), glycerin, carboxymethylcellulose, nipagin, saccharin, sodium lauryl sulfate, menthol, peppermint flavor, and the hydroalcoholic pomegranate extract.

Commercial Dentifrice (CD Group): The selected commercial dentifrice was Even (produced by Indústrias Reunidas Raymundo da Fonte S/A, Vila Torres Galvão Paulista, PE, Brazil). Its list of ingredients includes propylparaben, calcium carbonate, sodium lauryl sulfate, flavoring agents, water, and sodium monofluoro-phosphate.

Distilled Water (DW Group): For the negative control, specimens were treated with distilled water.

2.5 Brushing Test

Soft nylon-bristled toothbrushes (Medfio Indústria e Comércio de Artigos Odontológicos Ltda., Pinhais, Paraná, Brazil) were used for the test. Each brush contained 34 individual tufts. The handles were removed to facilitate adaptation to the brushing machine supports. Before and after the simulated brushing, the specimens were placed in an ultrasonic bath (Ultracleaner 1400, Unique, Indaiatuba, São Paulo, Brazil) for 5 minutes. A simulation of five years of hygiene for the heat-polymerized resin specimens was performed using a brushing machine (Elquip – MSEI, São Carlos, São Paulo, Brazil). In this simulation, 12 months of brushing corresponded to 17,800 cycles. Brushing strokes were applied under a 200 g load, with a movement amplitude set to 20 mm and a speed of 4.5 strokes per second. Every 30 seconds, the tested substance was dispensed onto the specimens for 4 seconds. Throughout the test, the machine temperature was maintained at 37°C.

To simulate the diluting effect of saliva in the oral cavity and reduce friction, a suspension was prepared by mixing 100 ml of distilled water with 100 ml of each dentifrice, creating a 1:1 ratio maintained at room temperature. After dilution, the solution was loaded into 20 ml syringes and connected to the brushing machine.

2.6 Color Stability Test

Color evaluation was performed using a portable spectrophotometer (Vita Easyshade, Vita Zahnfabrik H. Rauter GmbH & Co, Germany). This device quantified the color differences, represented as ΔE. The formula for ΔE is given by: $\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$. In this equation, L* indicates lightness; a* represents red-green chromaticity; and b* denotes yellow-blue chromaticity.**

The color difference of each specimen was determined based on the observation standard recommended by the CIE (Commission Internationale de l'Éclairage, 1978), comparing post-brushing changes with their initial characteristics. To ensure consistent readings and minimize ambient light interference, a condensation silicone mold (Reflexdenso, Yller Biomateriais S/A, Pelotas, RS, Brazil) and a white background screen were used, focusing the measurement on the center of each specimen.

ΔE values were interpreted as follows: values below 1 were considered imperceptible to the human eye; values between 1 and 3.3 were perceptible to trained observers; and values above 3.3 were perceptible to the average observer, being classified as clinically unacceptable (Johnston, 2009; Ruyter et al., 1987; Seghi et al., 1989).

2.7 Evaluation of Mass

An electronic analytical balance (MARK 210A, Bel Engineering, Piracicaba, São Paulo, Brazil) with a sensitivity of 0.1 g was used. Before each measurement, the balance was calibrated and allowed a 30-minute warm-up period. Mass variation (Δm) was calculated in grams using the formula: $\Delta m = m_1 - m_2$, where m_1 represents the initial weight and m_2 corresponds to the post-brushing weight. Measurements were performed before and after brushing with each substance, ensuring that the specimens were previously sonicated with distilled water and dried with absorbent paper.

2.8 Surface Roughness

Surface roughness was measured using a benchtop profilometer (T1000, Hommel Tester, Hommelwerke, Gbh, Schwenningen, Schwarzwald-Baar, Germany), which moved a diamond tip with a 5 µm radius along a 4.8 mm path for 10 seconds. Each specimen underwent three roughness (Ra) measurements on the flattest surface. The arithmetic mean of these readings was considered as the statistical unit (Sartori et al., 2008).

2.9 Microhardness Knoop

Microhardness levels were evaluated using a microhardness tester (FM-ARS 9000 and FM-100, Future-Tech Corp., Kawasaki, Kanagawa, Japan) connected to a computer with image analysis software. Each specimen was subjected to a calibrated vertical load of 10 g for 5 seconds. Five random indentations were made on each specimen. The mean values of the control group were calculated for subsequent comparison with the means of the experimental group (Carvalho-Neto et al., 2021).

2.10 Statistical Analysis

Data obtained from surface roughness, Knoop microhardness, color change, and mass variation tests were tabulated in Microsoft Excel and transferred to GraphPad Prism 5.0 for statistical analysis. A 95% confidence level and a significance threshold of $P < 0.05$ were adopted. After verifying data normality using the Kolmogorov-Smirnov test, results were expressed as mean values with standard errors. The data were analyzed by repeated-measures ANOVA, followed by Bonferroni post hoc tests.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Evaluation of mass

Table 1 presents the mass results recorded before and after the simulated brushing over a period corresponding to 60 months. No statistically significant differences in mass loss were observed among the experimental groups ($P = 0.560$).

3.1.2 Surface Roughness (Ra)

At the end of the simulated brushing period, a significant increase in surface roughness (Ra) was observed in the commercial dentifrice (CD) group ($P < 0.001$) compared to the other groups ($P = 0.009$). Table 2 details the Ra values for all groups.

3.1.3. Microhardness analysis

At the end of the 60-month simulated brushing period, the mean microhardness did not differ significantly among any of the groups. Table 3 presents the microhardness means throughout the study.

Table 1. Means and standard deviation of mass, in grams, according to the treatment and time.

	Time				
	T0	**T1**	**T2**	**T3**	***P*-Value**
Mass					
DW	3.15±0.21^{Aa}	3.14±0.22^{Aa}	3.14±0.22^{Aa}	3.14±0.22^{Aa}	1.000
PD	3.13±0.22^{Aa}	3.05±0.33^{Aa}	3.04±0.33^{Aa}	3.05±0.33^{Aa}	0.792
CD	3.14±0.21^{Aa}	3.16±0.18^{Aa}	3.17±0.18^{Aa}	3.17±0.19^{Aa}	0.893
P-Value	1.000	0.567	0.529	0.560	

Different lowercase letters = significant difference between evaluation periods of the same group. Different capital letters = significant difference between groups in the same evaluation period.

Table 2. Means and standard deviation of roughness (μm), according to the treatment and time.

	Time				
	T0	**T1**	**T2**	**T3**	***P*-Value**
Roughness					
DW	0.22±0.15^{Aa}	0.22±0.15^{Aa}	0.21±0.15^{Aa}	0.22±0.13^{Aa}	0.810
PD	0.19±0.08^{Aa}	0.20±0.06^{Aa}	0.21±0.07^{Aa}	0.22±0.09^{Aa}	0.206
CD	0.21±0.13^{Aa}	0.27±0.13^{Aa}	0.38±0.19^{Bb}	0.39±0.16^{Bb}	< 0.001
P-Value	0.123	0.451	0.020	0.009	

Different lowercase letters = significant difference between evaluation periods of the same group. Different capital letters = significant difference between groups in the same evaluation period.

Table 3. Means and standard deviation of microhardness according to the treatment and time.

	Time				
	T0	**T1**	**T2**	**T3**	***P*-Value**
Microhardness					
DW	20.40±3.90^{Aa}	19.14±2.59^{Aa}	18.06±2.14^{Aa}	20.14±3.67^{Aa}	0.274
PD	16.34±1.72^{Aa}	18.00±2.24^{Aa}	18.43±2.92^{Ab}	16.43±2.97^{Aa}	***0.010***
CD	16.88±3.16^{Aa}	18.18±3.16^{Aa}	17.34±3.11^{Aa}	18.34±2.96^{Aa}	0.634
P-Value	0.810	0.600	0.671	0.053	

Different lowercase letters = significant difference between evaluation periods of the same group. Different capital letters = significant difference between groups in the same evaluation period.

Table 4. Means and standard deviation for ΔE, in different periodos in relation to the baseline, according to the treatment and time

	Time				
	T0	**T1**	**T2**	**T3**	***P*-Value**
Color					
DW	-	2.22±1.53^{Aa}	1.40±0.91^{Aa}	1.85±0.90^{Aa}	0.207
PD	-	1.67±0.85^{Aa}	1.76±1.31^{Aa}	2.17±1.01^{Aa}	0.220
CD	-	1.55±1.26^{Aa}	2.14±1.02^{Aa}	1.87±0.98^{Aa}	0.522
P-Value		0.456	0.339	0.712	

Different lowercase letters = significant difference between evaluation periods of the same group. Different capital letters = significant difference between groups in the same evaluation period

3.1.4 Color stability

The mean color variations are presented in Table 4. No statistically significant differences in color changes were observed among the experimental groups, and none of the changes were considered clinically unacceptable (ΔE > 3.3).

3.2 Discussion

Based on the obtained data, the null hypothesis was accepted for the parameters of color, mass, and microhardness, as no significant differences were observed between the pomegranate dentifrice (PD) and commercial dentifrice (CD) groups. However, for surface roughness, the null hypothesis was rejected, as the CD group exhibited significantly higher Ra values compared to the PD and distilled water (WD) groups.

An increase in the surface roughness of complete dentures may promote the retention of microorganisms such as *Candida albicans* on their surfaces. Furthermore, adequate microhardness of the denture material is essential to withstand wear caused by daily use and the abrasive action of cleaning agents (Bianchi et al., 2016; Carvalho-Neto et al., 2021; Gad; Fouda, 2020). The physical structure of dental materials and the prognosis of applied treatments can be negatively affected by the mechanical movements and temperature changes in the oral cavity. For this reason, the in vitro evaluation of dental materials has been preferred to simulate oral cavity conditions (thermocycling, water aging, and chewing forces) in a laboratory environment, mimicking the natural aging process, since in vivo evaluation has been difficult in clinical trials (Akcay & Aktoren, 2024). Studies indicate that adverse changes in these surface properties of denture base materials are directly associated with a higher incidence of denture stomatitis (Bianchi et al., 2016; Freire et al., 2018).

Increased surface roughness is often attributed to the high abrasiveness of dentifrices routinely used for denture cleaning, due to their cost-effectiveness, simplicity, and wide availability (Bianchi et al., 2016; Freitas-Pontes et al., 2016; Gad & Fouda, 2020; McReynolds et al., 2023). The abrasive nature of a dentifrice is critical for removing organic deposits and preventing staining on dentures (Freire et al., 2018). Therefore, the abrasive capacity of dentifrices for dentures must balance cleaning efficiency without excessively affecting the surface roughness of the material.

In this study, the pomegranate-based experimental dentifrice did not significantly alter the surface roughness of the acrylic resin after a simulated five-year brushing period. However, specimens cleaned with the commercial dentifrice exhibited a significant increase in surface roughness (*P* = 0.009).

The low abrasiveness of the pomegranate-based experimental dentifrice can be attributed to its composition, which includes only one abrasive agent: micronized calcium carbonate. In contrast, the Even® dentifrice incorporates two abrasive agents: calcium carbonate and sodium silicate. A study highlighted that the calcium carbonate particles in conventional dentifrices, used as abrasives, are spherical and exhibit irregular sizes and weights, contributing to more uneven and aggressive wear (Pisani et al., 2010). Conversely, micronized calcium carbonate particles have more uniform sizes and are less abrasive. Ramadhan, Damiyanti and Triaminingsih (2018) observed that the complexity of abrasive agents in conventional dentifrices was positively correlated with increased surface roughness in acrylic resin specimens. Based on these findings, Sorgini et al. (2015) emphasized the importance of using denture-specific cleaning agents, as they observed significantly lower surface roughness following denture hygienewith these products compared to conventional options.

Although brushing is a widely adopted method, it should be applied with caution. The main precaution is to use denture-specific dentifrices containing mild abrasives, along with soft-bristled brushes, which should be replaced frequently for denture hygiene. Furthermore, it is crucial that dentures be cleaned outside the mouth at least once daily and stored in clean water when not in use (Felton et al., 2011; Freitas-Pontes et al., 2009; Freitas-Pontes et al., 2016; McReynolds et al., 2023).

Regarding color stability, an essential factor from an aesthetic perspective, no clinically significant changes were observed in any group, indicating the adequate aesthetic durability of the material, particularly under the conditions simulated in this study. The preservation of the acrylic resin's surface polish in denture bases is also crucial for aesthetics, as materials with increased surface roughness can absorb and retain food dyes, becoming discolored or stained (Bajunaid et al., 2022, Costa et al., 2025, Takhtdar et al., 2023).

In this study, no notable color changes were detected after five years of brushing with the pomegranate-based dentifrice. Ideally, a denture cleaner should not alter the color of the acrylic resin, ensuring the longevity of the prosthesis (Badaró et al., 2019). However, another study using a similar pomegranate-based dentifrice reported different results in artificial teeth. Carvalho-Neto et al. (2021) observed significant color variations in artificial teeth used in complete dentures cleaned with a pomegranate-based dentifrice, although the mean ΔE values remained within clinically acceptable limits (below 3.3). Based on these acceptable variations, the pomegranate-based dentifrice remains a promising candidate for future denture-cleaning protocols.

Mass variations highlighted remarkable stability in this material after the 60-month simulated brushing test. This suggests that the structural integrity of the heat-polymerized acrylic resin is minimally affected by abrasion from brushing and the abrasiveness of the tested substances. This characteristic is important for prosthetic materials, ensuring longevity and reducing the need for frequent replacements (Campos et al., 2023).

Regarding microhardness, the study showed some fluctuations in values, particularly notable in the PD group. Although microhardness increased during the first 36 months, it returned to values close to the initial levels by the end of the study, showing no statistical difference from the negative control after 60 months. Material microhardness is intrinsically linked to its strength and wear resistance over time. Panariello et al. (2015) emphasized in their study the correlation between abrasion resistance and surface microhardness. These fluctuations may be attributed to the properties of the dentifrice or the effects of the brushing machine.

The findings of this in vitro study indicate that the pomegranate dentifrice may represent a promising alternative for the hygiene of removable dentures. Its low abrasiveness and preservation of acrylic resin properties after prolonged use simulation (Carvalho-Neto et al., 2021), combined with the previously reported antifungal effect against Candida albicans (Vasconcelos et al., 2003; Vasconcelos et al., 2006), suggest potential clinical application in the prevention of denture stomatitis. However, validation of these benefits requires clinical trials to confirm antimicrobial efficacy and user acceptability.

Although the results reported thus far suggest the feasibility of using this experimental pomegranate dentifrice for brushing complete dentures without damaging their structure, the present study was limited to evaluating laboratory parameters related to the polymer's structural integrity. Therefore, it is also relevant to assess, through further studies, the dentifrice's biofilm removal capacity, as well as to confirm the initially reported fungicidal effects.

4. CONCLUSION

After a simulated five-year brushing regimen with a *Punica granatum* Linné-based dentifrice on heat-polymerized acrylic resin specimens, no significant changes were observed in color, mass variation, surface roughness, or microhardness compared to the negative control. These findings suggest that this experimental dentifrice preserves the properties of complete denture acrylic resin, even after prolonged use, and shows promise as a natural alternative to conventional denture cleansers.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare 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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