ORIGINAL ARTICLE

Effect on color stability and surface roughness of nanofiller dental composite after soaking in Bidara leaf (*Ziziphus mauritiana Lam)* ethanol extract*:* an experimental study

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Received: 15 September 2025
Revised: 25 October 2025
Accepted: 22 November 2025
Published: 30 November 2025
DOI: 10.24198/pjd.vol37no3.61351

p-ISSN 1979-0201
e-ISSN 2549-6212

Citation:
Fitriyani, S, Nazilla, CS, Sundari, I, Diansari, V, Subhaini, S, Ningsih, DS, Rahmayani, L, Qamaruzzaman, JB. Effect on color stability and surface roughness of nanofiller dental composite after soaking in Bidara leaf (Ziziphus mauritiana Lam) ethanol extract: an experimental study. Padjadjaran J. Dent, November. 2025; 37(3): 310-320.

ABSTRACT

Introduction: Dental composite resins offer aesthetic appeal and high wear resistance; however, their physical properties may diminish after exposure to colored foods or mouthwashes. We explore Bidara leaf (*Ziziphus mauritiana Lam*) extract as an alternative mouthwash due to its rich content of bioactive compounds. The study aims to analyze the color change and surface roughness of nanofiller composite resins after immersion in Bidara leaf ethanol extract. **Methods**: This research was an experimental study. The resin composite samples were divided into four groups; each group consisted of 3 specimens (n=3): Group 1 (negative control) immersed in distilled water; Group 2 (positive control) immersed in 0.2% chlorhexidine; and Groups 3 and 4 soaked in 4.5% and 20% ethanol extracts of Bidara leaf, respectively. The specimens were cylindrical with a diameter of 10 mm and a thickness of 2 mm. All specimens were the same size, shape, and color, with flat surfaces and no cracks or stains. Samples were immersed for two minutes daily over one month. Color changes were analyzed using a stereomicroscope and the CIELab color system with Adobe Photoshop, while surface roughness was assessed using atomic force microscopy (AFM). **Results**: Color changes show $\Delta E>3.3$ indicating a visible difference. The most significant color change occurs in Group 4, followed by Groups 3, 2, and 1. One-way ANOVA shows a statistically significant color change ($p<0.05$). Surface roughness also increases, with Group 4 (0.138±0.066 µm) displaying the greatest roughness compared to Group 3 (0.122±0.061 µm), Group 2 (0.122±0.076 µm), and Group 1 (0.054±0.012 µm). Based on the one-way ANOVA test, the results show no significant differences ($p>0.05$) between the pre- and post-immersion. **Conclusion**: Although changes in surface roughness were not statistically significant, the ethanol extract of Bidara leaf influences the color and surface roughness of the nanofiller dental composite.

KEYWORDS:
Color stability, surface roughness, nanofiller dental composite, Bidara leaf, discoloration

INTRODUCTION

Composite resin restorative materials are essential in modern dentistry. They are used for the rehabilitation of teeth by restoring their biological, functional, and aesthetic properties. These materials owe their superior aesthetic qualities and visual appeal to the refinement of filler technology, specifically the reduction of filler particle size, which not only enhances translucency but also improves their resistance to abrasive wear.[1,2] In addition, the clinical performance of composite resins depends on several mechanical properties, including compressive strength, diametral tensile strength, flexural strength, fracture toughness, and abrasion resistance. [3,4]

Color is a key aesthetic property of teeth, and dental composites are designed to provide excellent color matching and optical performance. Thus, color stability serves as a direct indicator of the overall quality of a composite material. Any reduction in color stability reflects a decline in the material's aesthetic performance. Discoloration of composite resins can be attributed to both intrinsic and extrinsic factors. The intrinsic factors include changes in the filler components, degradation of the resin matrix, and the release of filler particles onto the composite's external surface. This degradation typically increases surface roughness, causing the material to be more prone to staining and encouraging plaque and biofilm accumulation, further exacerbating discoloration and reducing longevity.[4–9]

Conversely, the extrinsic factors involve the absorption or adsorption of dyes from external sources such as tea, coffee, nicotine, and mouthwashes like chlorhexidine.[3–5,10] These substances interact with the surface of composite materials, resulting in visible discoloration.[3] The susceptibility of dental materials to discoloration remains a persistent issue because they are constantly exposed to chromogenic substances found in foods and beverages. Additionally, environmental factors such as smoking and antibacterial treatments further contribute to staining by introducing additional staining agents onto the restoration surface.[3,6,8,11]

Chlorhexidine, a widely prescribed antimicrobial agent, is often regarded as the gold standard for antibacterial treatments due to its broad-spectrum activity.[12–14] However, a major drawback of chlorhexidine is its chromogenic potential, leading to brown staining of hard and soft tissues and resin-based restorations. Therefore, plant-derived mouthwashes have gained attention as alternatives, offering antimicrobial benefits without the chromogenic side effects associated with chlorhexidine.[13,14] They are also often more affordable and environmentally friendly compared to chemical alternatives.[14,15]

Ziziphus mauritiana Lam., commonly known as Bidara commonly utilized for tea or vegetable (cooking) [16], is one such plant that is well recognized in traditional medicine for its therapeutic properties. Several bioactive compounds had been identified in Bidara leaves, including flavonoids, terpenoids, tannins, saponins, alkaloids, and steroids, all of which contribute to its antimicrobial and therapeutic properties. Its leaf extract has demonstrated antimicrobial activity against a range of pathogens, including *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Aspergillus niger*, and *Candida albicans*.[15,17,18] Previous reports indicate that 4.5% Bidara leaf extract exhibited notable antibacterial activity, as did the 20% ethanol extract.[15] Bidara leaf extract has the potential to be used as an herbal mouthwash with antimicrobial properties in the oral cavity. However, despite this potential, Bidara leaf extract, which contains chromophore compounds, may also cause staining or discoloration of the restorations.

This study evaluated the potential *of* 4.5% and 20% *Ziziphus mauritiana Lam*. (Bidara) leaf extract as a natural alternative to synthetic mouthwashes, focusing on its effects on composite resin restorations. The primary objective was to assess the impact of Bidara leaf extract on staining and surface roughness of nanofiller composite resins. This research seeks to understand whether Bidara leaf extract can mitigate or exacerbate staining of composite resins compared to conventional agents like chlorhexidine. Based on these considerations, the study aims to analyze the color changes and surface roughness of nanofiller composite resins following immersion in Bidara leaf ethanol extract.

METHODS

This research was an experimental laboratory study employing a pre-test-post-test group design. Twelve composite resin specimens were divided into four groups based on the immersion solutions. Group 1 (negative control) was immersed in aquadest Group 2: (positive control) was also immersed in 0.2% chlorhexidine, and

Groups 3 and 4 received the experimental solutions: 4.5% and 20% *Ziziphus mauritiana Lam* leaf extracts, respectively. Color changes were assessed first, followed by the evaluation of surface roughness for each specimen.

A total of 12 composite resin disc samples were fabricated using a standardized mold template (2 mm height and 10 mm diameter). Using a bulk-fill technique, uncured resin composite (Filtek Z350 XT, 3M ESPE, USA) was packed into the mold. Mylar strips were placed over the top and bottom surfaces of the uncured resin composite to prevent the formation of an oxygen inhibition layer, and the excess material was extruded by condensing the mould in between two glass plates. The specimens were light polymerised for 20 seconds at a 1mm distance perpendicular to the sample using the D-mate LED Light Cure (Dentmate Technology, Taiwan) with a light intensity of 1200mW/cm^2 nm according to the manufacturer's instructions. The specimens were removed from the mold 1 hour after the light-curing cycle and rinsed under tap water at 37^{o}C.

The Bidara leaves (*Ziziphus mauritiana Lam*) were sourced from Aceh Besar. Bidara leaves were collected during peak photosynthesis hours, between 8:00 and 11:00 a.m., selecting exclusively fully developed leaves while excluding those still in bud form. Approximately 5 kg of leaves were gathered and thoroughly rinsed with clean, running water to remove any surface impurities. The leaves were then air-dried indoors under well-ventilated conditions for 7 days. For extraction, 1 kg portion of the dried leaves was immersed in 5 L of 96% ethanol in an Erlenmeyer flask. This mixture was stored for 5 days with occasional stirring before filtering. The residue was then re-macerated for an additional 3 days. Finally, the filtrate was concentrated using a rotary evaporator to obtain a viscous extract, which was diluted with distilled water to create 4.5% and 20% solutions.[15]

Composite resin specimens were divided into four groups (n = 3 per group): Group 1 (negative control): aquadest, Group 2 (positive control): 0.2% chlorhexidine, Group 3: 4.5% ethanol extract of *Ziziphus mauritiana Lam* leaves, and Group 4: 20% ethanol extract of *Ziziphus mauritiana Lam* leaves. Prior to immersion, the pH of each solution was measured using a pH meter, with values recorded as 7.4, 5.2, 6.9, and 5.8, respectively. Each composite specimen was placed in an individual plastic vial corresponding to its group and immersed in the designated solution for 2 minutes daily over a 1-month period. Solutions were refreshed daily, and specimens were incubated at 37°C throughout the study.

Color change was assessed using a stereomicroscope (Olympus SZ61, Japan) at 15x magnification. The device was calibrated with its internal calibration scale prior to measurements to ensure accuracy. Images were subsequently processed using Adobe Photoshop software. Each specimen was divided into 16 sections (Figure 1), and the color values L, a, and b were recorded for each section.

Using a VITA Easyshade 3D Master's lightness (VITA Zahnfabrik), the color of each sample was captured. For uniformity, measurements were made against a white backdrop. Twenty color measurements were taken both at the beginning and at the end of the immersion period. The equipment was calibrated before use in accordance with the manufacturer's instructions. The color data were measured using the CIE Lab system, in which the b* coordinate indicates saturation on the yellow (+) and blue (-) axes, the a* coordinate indicates saturation on the red (+) and green (-) axes, and the L* value depicts brightness (black to white axis; 0 to 100). These values were then used to calculate the color change (ΔE) according to the CIELab color system using the formula (Equation 1).[6]

A color change value ΔE ≤1 color change is considered visually invisible, while ΔE between 1 and 3 indicates a color change is visible to the examiner. A ΔE >3.3 represents a clinically noticeable color change.

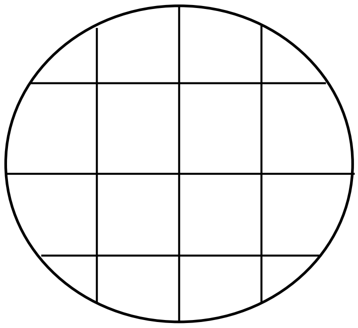

Figure 1. Division of 16 sections on each specimen

Average surface roughness (Ra) was measured before and after specimen immersion using Atomic Force Microscopy (AFM) (Nano surft easy scan 2, nanosurf, Switzerland) in contact mode, with cantilevers calibrated to a spring constant of 10 nN. The cantilever tip traversed the specimen surface during scanning to capture detailed surface topography. For each specimen, three images of 3.2x2.7 mm were acquired at a resolution of 352x288 pixels. The roughness measurement for each specimen was calculated as the average Ra value obtained from the three images.[19]

After data collection, the results for the experimental and control groups were compared using the Statistical Package for the Social Sciences (SPSS) version 26.0. To assess the influence of each treatment group, data were analysed using one-way analysis of variance (ANOVA) and the Kruskal-Wallis test, with a significance level set at 5%.

RESULTS

Table 1 presents the color change values observed after immersion in aquadest, 0.2% chlorhexidine, and ethanol extracts of Bidara leaf at concentrations of 4.5% and 20%, respectively. The results show a visible color change, with ΔE values greater than 3.3, indicating that the color changes are perceptible to the naked eye.[4]

Table 1. Average value of color change of nanofiller dental composite with One-Way ANOVA test

Groups	specimen	ΔE	$\bar{X}$ ± SD	p
Group 1: aquadest	1	8.712	7.878 ± 2.045	0.047*
	2	9.375		
	3	5.548		
Group 2: 0.2% chlorhexidine	4	10.189	10.983 ± 1.948	
	5	13.203		
	6	9.557		
Group 3: 4.5% Bidara leaves extract	7	13.091	12.171 ± 0.855	
	8	12.024		
	9	11.400		
Group 4: 20% Bidara leaves extract	10	13.063	12.718 ± 2.174	
	11	10.393		
	12	14.777		

The color change data were initially assessed for normality with the Shapiro-Wilk test, confirming a normal distribution ($p > 0.05$). A One-Way ANOVA subsequently revealed a significant effect of the immersion solution on color change (ΔE) across the four groups ($p = 0.047$). Post-hoc analysis with Tukey's HSD indicated that Group 4 (20% Bidara leaves extract) exhibited significantly greater color change than Group 1 (aquadest) (Table 2)

Tabel 2. The data of the post hoc test between group research

(I) Groups		(J) Groups	Mean Difference (I-J)	Std. Error	Sig.
Aquadest		CHX 0,2%	-3.104667	1.496474	.240
	-	EEDB 4.5%	-4.293333	1.496474	.080
		EEDB 20%	-4.840333*	1.496474	.048*
CHX 0,2%		Aquadest	3.104667	1.496474	.240
	-	EEDB 4.5%	-1.188667	1.496474	.855
		EEDB 20%	-1.735667	1.496474	.666
EEDB 4.5%		Aquadest	4.293333	1.496474	.080
	-	CHX 0,2%	1.188667	1.496474	.855
		EEDB 20%	-.547000	1.496474	.982
EEDB 20%		Aquadest	4.840333*	1.496474	.048*
	-	CHX 0,2%	1.735667	1.496474	.666
		EEDB 4.5%	.547000	1.496474	.982

*. The mean difference is significant at the 0.05 level.

Table 3 shows that the average surface roughness of the nanofilled dental composite increased after immersion in aquadest, chlorhexidine 0.2%, and 4.5% and 20% ethanol extracts of *Ziziphus mauritiana Lam* leaves for 2 minutes daily over a period of 1 month. Notably, immersion in the 20% *Ziziphus mauritiana Lam* extract exhibited the highest surface roughness value compared to the other groups. The Kruskal-Wallis test results revealed no statistically significant differences in surface roughness after immersion in any solutions (p > 0.05).

Table 3. Surface roughness values of the nanofiller dental composite before and after immersion with the One-Way ANOVA test

Surface roughness (μm)							
Groups	Specimen	Before	After	X̄ ± SD		p	
						Before	After
Group 1: aquadest	1	0.053	0.053	0.046	0.054	0.081	0.473
	2	0.028	0.043	±	±		
	3	0.059	0.068	0.016	0.012		
Group 2: 0.2% chlorhexidine	4	0.043	0.21	0.042	0.122		
	5	0.030	0.076	±	±		
	6	0.055	0.080	0.012	0.076		
Group 3: 4.5% Bidara leaves extract	7	0.062	0.068	0.1	0.122		
	8	0.09	0.11	±	±		
	9	0.15	0.19	0.044	0.061		
Group 4: 20% Bidara leaves extract	10	0.08	0.11	0.084	0.138		
	11	0.064	0.065	±	±		
	12	0.11	0.24	0.034	0.066		

* Significant value of one-way ANOVA statistical test (p<0.05)

Table 4. Differences in surface roughness of nanofiller dental composite before and after immersion with Kruskal Wallis test

Surface roughness (μm)		
Group	Mean surface roughness difference before and after immersion	p
Group 1: aquadest	0.008	0.392
Group 2:0.2% chlorhexidine	0.080	
Group 3:4.5% Bidara leaves extract	0.022	
Group 4: 20% Bidara leaves extract	0.054	

* Significant values from the Kruskal-Wallis statistical test (p<0.05)

The mean surface roughness difference of nanofiller dental composite resins before and after immersion in the solutions was analysed using ANOVA after a Shapiro-Wilk test, which confirmed a normal data distribution (p > 0.05). The ANOVA results showed no significant differences (p > 0.05), indicating that immersion in these solutions (aquadest, 0.2% chlorhexidine, and 4.5% and 20% ethanol extracts of *Ziziphus mauritiana Lam* leaves) did not significantly affect the surface roughness of the composite resins (Table 4). This is illustrated in Figure 2 where the bars show the mean surface roughness values remain relatively consistent across all groups, with no significant variation observed.

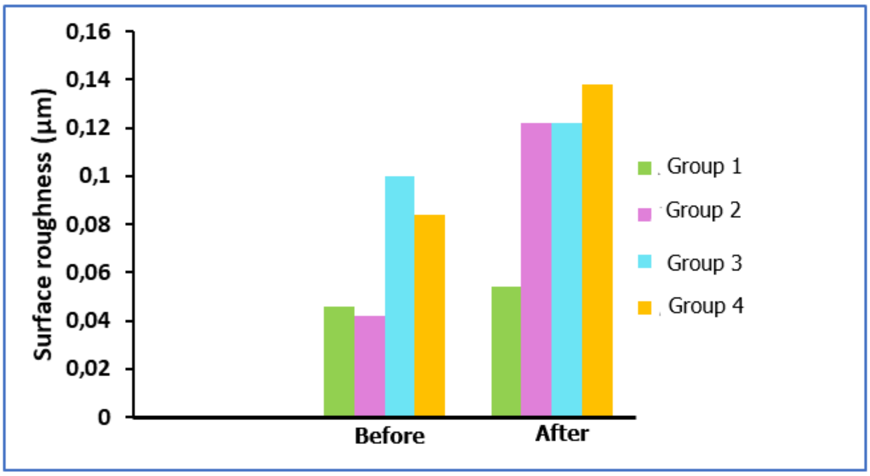

Figure 2. Value of surface roughness nanofiller dental composite before and after immersion in group 1, group 2, group 3 and group 4.

Figure. 3 illustrates the surface topography of nanofiller dental composite resin viewed in both three-dimensional (3D) and top-down perspectives. The images show the AFM images before and after immersion in Aquadest, 0.2% Chlorhexidine, 4.5% and 20% of ethanol extract of Bidara leaves. Notably, the surface roughness observed before immersion exhibits fewer dark areas, while the post-immersion surface reveals a greater number of dark regions. The abundance of the dark areas corresponds to deeper and rougher surface features, as highlighted in Figure 3.

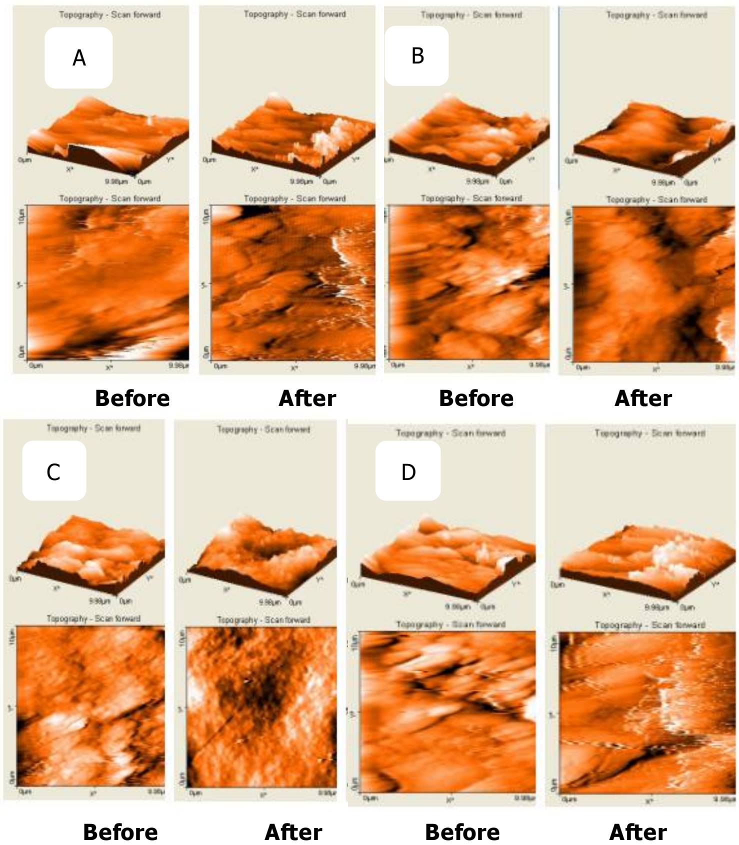

Figure 3. Changes in surface roughness of nanofiller dental composite before and after immersion in A. Aquadest, B. 0.2% Chlorhexidine, C. Bidara leaf ethanol extract 4.5%, D. Bidara leaf ethanol extract 20%

DISCUSSION

Based on Table 1, the results show that aquadest, 0.2% chlorhexidine, and *Ziziphus mauritiana Lam* ethanol extracts at concentrations of 4.5% and 20% influence the color of nanofiller dental composites. This is demonstrated by a color change value (ΔE > 3.3), which indicates that these changes are perceptible to the eye and impact the composite's aesthetic quality. Additionally, the results show a statistically significant color change ($p < 0.05$).

In a previous study, Shetty et al. reported that color change exhibited a significant difference ($p<0.05$) in various solution immersions (staining media).[8] The dental literature describes acceptable and noticeable color change criteria in dental composites. The term "perceptible thresholds" refers to the ΔE value at which an observer perceives a color shift; most observers can identify a change at ΔE of 1. On the other hand, the majority of observers accept a shift at ΔE of 3.7, referred to as the acceptable threshold.[20]

The color change in nanofiller dental composites can be attributed to their filler type and resin primarily due to water absorption and the hydrophilicity of the composite resin matrix.[5,19,20] The organic matrix of the nanofiller composite resin consists of the monomers BisEMA, UDMA, and TEGDMA. Because of their ether linkages (O-O) in BisEMA and TEGDMA and hydrogen bonding (N-H) in UDMA, these monomers enhance the resin's tendency to absorb water.[5,12,20,21,22]

This water sorption not only promotes staining from external sources but also reduces the long-term color stability and aesthetic properties of the composite. Bidara leaves contain various secondary metabolites, including tannins, similar to those found in substances like coffee and tea. Tannins possess staining potential because they can adhere to and be absorbed by the surface of dental materials, such as composite resins, due to the hydrophilic properties and porosity of the resin.[15,16,17,23-27]

The staining ability of Bidara (*Ziziphus mauritiana Lam*) leaf extract in this study is in accordance with the findings of Aulia et al., who reported that tannin compounds in red betel leaves (*Piper crocatum*) contribute to color changes in dental composite resins.[24] In this study, the tannins in the ethanol extract of Bidara leaves were likely absorbed by the composite resin through its ether linkages and hydrogen bonds, resulting in noticeable staining.

Furthermore, our results show that the extent of color change is concentration-dependent, with the 20% ethanol Bidara leaf extract producing a significantly greater color change compared to the 4.5% and 0.2% of extract, as shown in Table 2. Previous studies have demonstrated that the potential of dental composites to discolor increases with the percentage of staining or chromogenic agents in solution. According to Ersoz et al., after seven days of immersion, microhybrid dental composite exhibits more discoloration in hot coffee than cold coffee. Hot coffee contains more chromogen agents than cold coffee. It is more adsorbed and absorbed into dental composite nanofiller when there are several chromogen agents present.[28]

Although the composite resin samples in this study were cured under a Mylar strip, a method known to yield smoother surfaces that are less susceptible to staining due to the absence of an oxygen-inhibited layer. However, research shows that this technique does not eliminate microscopic irregularities entirely.[29] These subtle irregularities, as illustrated in Figure 3A, may serve as sites for pigment retention and stain accumulation over time, particularly after immersion in solutions with staining potential such as chlorhexidine 0.2% and ethanol extract of Bidara leaves.

In this study, 0.2% chlorhexidine (CHX) was used as a positive control, due to its well-established ability to cause tooth discoloration, both on natural dentition and composite restorations, after extended exposure. Our results align with Janisch et al., Tanthanuch et al., and Aiane et al., as the samples immersed in CHX exhibited a color change greater than 3.3 ΔE, which is visible to the naked eye. The primary mechanism behind extrinsic staining caused by cationic antiseptics like CHX is the precipitation of dietary chromogens onto dental surfaces. Studies have demonstrated that chromogens from foods and beverages such as tea, coffee, and red wine bind to CHX-treated surfaces, leading to noticeable discoloration.[6,7,30]

Moreover, the Bidara leaves used in this study contain tannins, which are also found in tea and coffee. Tannins can increase the staining potential by interacting with CHX, forming complex, pigmented molecules that further adhere to composite surfaces, thus intensifying discoloration. This interaction between CHX, tannins, and dietary chromogens likely contributed to the pronounced discoloration observed in our composite samples. Other possible mechanisms for the composite staining are (1) the Maillard reaction; and (2) the formation of colored metal sulphides. The Maillard reaction has been demonstrated to occur on dental composites in vitro, particularly when these materials are exposed to antimicrobial agents like chlorhexidine (CHX), which catalyze the reaction. Research has shown that CHX increases the formation of melanoidins through this process, further contributing to discoloration.[13,30]

According to Figure 2 and Table 3, the results show that immersion in aquadest, 0.2% chlorhexidine solution, and Bidara leaf ethanol extract at concentrations of 4.5% and 20% increases the surface roughness of nanofiller dental composites. Before immersion, the mean surface roughness (Ra) values were 0.046 ± 0.016 µm for group 1, 0.042 ± 0.012 µm for group 2, 0.1 ± 0.044 µm for group 3, and 0.084 ± 0.034 µm for group 4. Following immersion, the Ra values rose to 0.054 ± 0.012 µm for group 1, 0.122 ± 0.076 µm for group 2, 0.122 ± 0.061 µm for group 3, and 0.138 ± 0.066 µm for group 4. Based on Table 4, statistical analysis using one-way ANOVA revealed no significant differences in surface roughness values before and after treatment. The surface roughness measured was similar to a previous study, which described no significant effects of surface roughness after immersion of the dental resin composite.[31]

Composite resin restorations are generally durable in the oral cavity, where material properties and environmental factors such as oral moisture, temperature fluctuations, and exposure to oral fluids all play significant roles in their longevity. It has been shown that the degradation of composite resins, due to the breakdown of filler components when exposed to acidic solutions, leads to surface roughness changes and reduced strength of the nanofiller composite resins[24,30].

In this study, the highest roughness values were observed in samples soaked in chlorhexidine 0.2% (group 2) and 20% ethanol extract of Bidara leaves (group 4) (Table 3). This may be attributed to the acidic nature of these solutions. Previous researchers found that the surface roughness of resin composites increased to various degrees if exposed to alcoholic or acidic solutions. The staining process may be enhanced and the composite resin surface softened by the sorption of acid or alcohol molecules into the resin matrix. Acids may increase extrinsic discoloration by influencing surface smoothness.[23] This suggests that the acidic nature of these solutions contributes significantly to surface roughness, potentially reducing the composite's resilience over time.

In this study, the increase in roughness correlates with the pH of the solutions: aquadest (pH 7.4), 0.2% chlorhexidine (pH 5.3), and Bidara leaf ethanol extracts at 4.5% and 20% concentrations (pH 6.9 and 5.8, respectively). Janson et al 2025 stated that surface roughness is not only affected by staining agents, but acidic media can also induce the matrix to soften, degrade, and release filler in dental composite resins.[31,32] The acidity of the ethanol extract of Bidara leaves is likely due to the active compounds contained in leaves, such as flavonoids, alkaloids, polyphenols, tannins, and saponins.[3] As Shetty et al. Observed, lower pH solutions promote the degradation of the nanofiller composite surface, resulting in increased surface roughness and potentially reducing the longevity of the dental restoration.[8,31-3]

Moreover, commercial mouthwashes may contain substances such as organic acids, emulsifiers, and detergents that weaken and deteriorate the surface and discolor it.[14,15,27,30] Mouthwashes that contain organic acids, such citric and phosphoric acids, lower pH levels in the mouth, break down the polymer matrix, and reduce microhardness composite resins.[5,12,30] Alcohol-containing or low pH mouthwashes can deteriorate composite resins by influencing their physical and mechanical properties. The release of monomeric residue and the collapse of polymer matrix contribute to discoloration.[13,16]

The hydrophilicity of composite resins makes them susceptible to reductions in both physical and mechanical properties due to water absorption.[20-3] This absorption initiates hydrolytic degradation, which is associated with increased surface roughness and compromised material integrity. As Morais et al., describe, water diffuses into the composite resin, particularly at the interface between the resin matrix and filler particles, where it accumulates and promotes breakdown of filler particles and the matrix-filler bond. Over time, this process degrades the composite's structural stability, leading to weakened mechanical properties and increased surface roughness, ultimately affecting the composite's esthetic and functional longevity in the oral environment. [3,5,12,30]

The limitation of this research is that it did not include temperature as a variable affecting the color stability of dental composite resin. Theoretically, temperature can induce the water sorption on the surface of the material. This factor will be explored in future studies.

CONCLUSION

The Bidara leaf extract influences the color stability of the dental composite nanofiller. The higher the concentration of Bidara leaf extract, the greater the color change observed. However, there was no significant change in the surface roughness of the nanofiller dental composite after immersion in Bidara leaf extract. The implication of using Bidara leaf extract in dental applications should be carefully evaluated, particularly because of its potential to cause discoloration of the dental composite, which can reduce its esthetic appearance.

Acknowledgement: No declare
Author Contributions: Conceptualisation, S.F. and C.S.; methodology, C.S, I.S and V.D.; software, D.S.N and S.; validation, S.F., C.S. and L.R.; formal analysis,S.F; investigation, S.F.; resources, J.; data curation, S.; writing original draft preparation, S.F and C.S; writing review and editing, J.; visualisation, S.F.; supervision, S.F.; project administration, C.S.,; funding acquisition, no funding. All authors have read and agreed to the published version of manuscript.
Funding: This research did not receive external funding
Institutional Review Board Statement: Not applicable
Informed Consent Statement: Not applicable
Data Availability Statement: The data that support the findings of this researchare available from the corresponding author, upon reasonable request.
Conflicts of Interest: The authors declare no conflict of interest.

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