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Effects of Khat Extract and Other Staining Media on Color Change of Composite Resins Subjected to Various Polishing Methods

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Abstract

Objective: To assess the influence of Khat extract and other staining media on color changes in composite resins subjected to various polishing methods.

Methods: 84 disk-shaped specimens (8 mm in diameter and 2 mm thick) were prepared for each composite resin type [nanohybrid (Filtek Z250 XT), nanofilled (Filtek Z350 XT), and microhybrid zirconia/silica (Valux Plus)], for a total of 252 specimens. Each composite group was divided into three subgroups according to polishing methods (n=28): control (no finishing and polishing), polishing Praxis disks, and polishing felt disks. After finishing and polishing, seven specimens from each group were immersed in Khat extract, tea, Pepsi, and distilled water for 7 days. Color parameters were measured before immersion and after the allocated time of immersion. Three-way analyses of variance followed by a one-way ANOVA and Tukey's test were used for the statistical analysis.

Results: A significant interaction between the resin composites, polishing methods, and staining solutions were found ($p<0.05$). The polishing effect on the resin composite color stability was not statistically significant ($p>0.05$). Of the staining solutions, Khat extract induced the highest level of discoloration. Khat extract and tea revealed significantly more color changes than Pepsi and distilled water ($p<0.05$). Of the composite resins, the Valux Plus showed the lowest ΔE^* value, whereas the Filtek Z250 showed the highest ($p<0.05$).

Conclusions: Khat extract showed the highest effects on the color stability of different types of composite resins. Only Khat extract and tea produced clinically perceptible color changes ($\Delta E>3.3$).

Keywords: Discoloration, composite resin, polishing, staining solutions, Khat extract

Background

Among direct restorative materials, composite resin is considered the material of choice when esthetic appearance is a concern [1,2]. However, when this material is present in the oral environment, it becomes vulnerable to the influence of some factors that may result in staining [3]. Composite resin staining might be attributed to the water sorption degree and matrix resin hydrophilicity. If a composite resin can absorb water, it can also absorb other fluids, resulting in color alteration. Furthermore, a rough surface adds to patient discomfort, as a change in surface roughness can be detected by the tip of the tongue. In addition, changes in biofilm accumulation on the surface roughness were reported, which leads to discoloration and/or staining of the restoration or margins [4]. Previous studies have shown that composite resins are susceptible to color instability when exposed to various staining media, especially red wine, coffee, cola, tea, and whisk [5-9].

Khat is a natural stimulant from the Catha edulis plant that is cultivated in the Republic of Yemen and most East African countries. Its young buds and tender leaves are chewed to attain a state of euphoria and stimulation [10]. Khat is an evergreen shrub that is cultivated as a bush or small tree.The leaves have an aromatic odor. The taste is astringent and slightly sweet. The plant is seedless and hardy, growing in a variety of climates and soils. Khat can be grown in droughts where other crops have failed and also at high altitudes. Khat is harvested throughout the year. Planting is staggered to obtain a continuous supply. There is fairly extensive literature on the potential adverse effects of the habitual use of Khat on mental, physical, and social well-being [11]. Many different compounds are found in Khat including alkaloids, terpenoids, flavonoids, sterols, glycosides, tannins, amino acids, vitamins, and minerals. The phenylalkylamines and the cathedulins are the major alkaloids and are structurally related to amphetamine [12].

Some studies have reported high surface roughness of composites, even after finishing, due to irregularly arranged inorganic filler particles, which could result in easier staining over time [6,8,13]. Fundamentally, filler particle size and distribution as well as resin matrix composition have been shown to play an important role in the color stability of composite resins. The filler load improves the physical properties and resistance to functional wear. Due to increasing filler load, the viscosity increases [14,15]. The composites initially were quartz-filled with large filler particles, which make restorations rough and difficult to polish. Because the polishing ability of restorations is a major aesthetic problem, a variety of newer materials have been developed due to the growing needs expressed by dental practitioners. Composite resins derive their physical properties/handling characteristics from the reinforcing filler particles and viscosity of the resin matrix. The majority of direct restorative composite resins fall into one of the following categories: hybrid, flowable, microfilled, packable, and nanofilled composites [16].

Clinically, the longevity of restorations is mostly related to acceptable finishing and polishing properties that provide smooth surfaces. It has been reported that higher surface roughness values (>0.2 μm) are a risk factor for extensive plaque accumulation on dental materials and the main contributor to the multifactorial discoloration of resin restorations [17]. Both esthetics and the longevity of restorations strongly depend on the quality of surface finishing and polishing. Irregularities can influence the appearance, plaque retention, surface discoloration, gingival inflammation, solubility of the organic matrix due to the formation of lactic acids, propionic, and acetic by adhered plaque and secondary caries occurrence [18]. Some mechanical properties such as hardness can be reduced due to the surface roughness of composite restorations and also increase restoration wear. For these reasons, polished and smooth composite resin restorations improve esthetic appearance and increase longevity [18].

Various finishing and polishing techniques have been tested with different types of composite resins to obtain a smooth surface. However, resin-based composites are not yet able to guarantee excellent results when used due to exposure to various staining media in oral environments that vary between different countries. To date, no types of composite resins and finishing and polishing techniques have been developed to eliminate discoloration under various staining media. Therefore, this subject remains controversial. Thus this is the theme of the current study. The purpose of this *in vitro* study was to evaluate the effects of Khat extract and other staining media on the color stability of three different types of composite resins after subjecting them to various polishing methods. The null hypothesis of this study was that the color stability of different composite resins would not be affected by Khat extract and other staining media after subjecting them to various polishing methods.

Materials and methods

Three types of light-cured resin composite resin materials, nanohybrid (Filtek Z250 XT), nanofilled (Filtek Z350 XT), and microhybrid zirconia/silica (Valux Plus), three polishing methods (polyester strip without finishing and polishing, praxis disks, and felt disks in combination with Poligloss aluminum oxide paste), and four storage media (distilled water, Pepsi, tea, and Khat extract) were used (**Figure 1**). **Table 1** shows the characteristics of the composite resin materials and polishing systems used in this study.

For each type of composite resin, 84 disk-shaped specimens were prepared, for a total of 252 disks. The composite discs were fabricated using Teflon molds [19]. A Teflon plate with a hole (8 mm in diameter and 2 mm thick) was prepared [20-22]. After a composite resin material was packed into the hole in the Teflon plate, a polyester strip was placed on the composite surface to smooth it. A glass slide was placed over the polyester strip to ensure excess composite resin material was extruded after pressure was applied [8,23]. All of the composite disk specimens were cured for 40 s with light-emitting diodes (Coolight Light Cure Unit, Gyeonggi-do, Korea) with a peak wave length of approximately 470 nm. All of the specimens were then immersed in distilled water for 24 h at 37ºC for rehydration and polymerization completion [24].

Specimens of each composite resin were divided into three groups, each containing 28 specimens. In all of the groups, a control group of 28 specimens received no finishing and polishing. The remaining 56 specimens were finished with fine grit diamond burs applied for 15 s and then extra-fine diamond burs applied for 15 s under running water. The 56 specimens were randomly divided into two groups (n=28/group) according to the polishing method.

The specimens were polished using one of the following methods:

a) Using Praxis disks (TDV, Santa Catarina, Brazil), the specimens were sequentially polished with coarse, medium, fine, and extra-fine aluminum oxide abrasive discs under running water at low speed for 60 s according to the manufacturer's instruction.

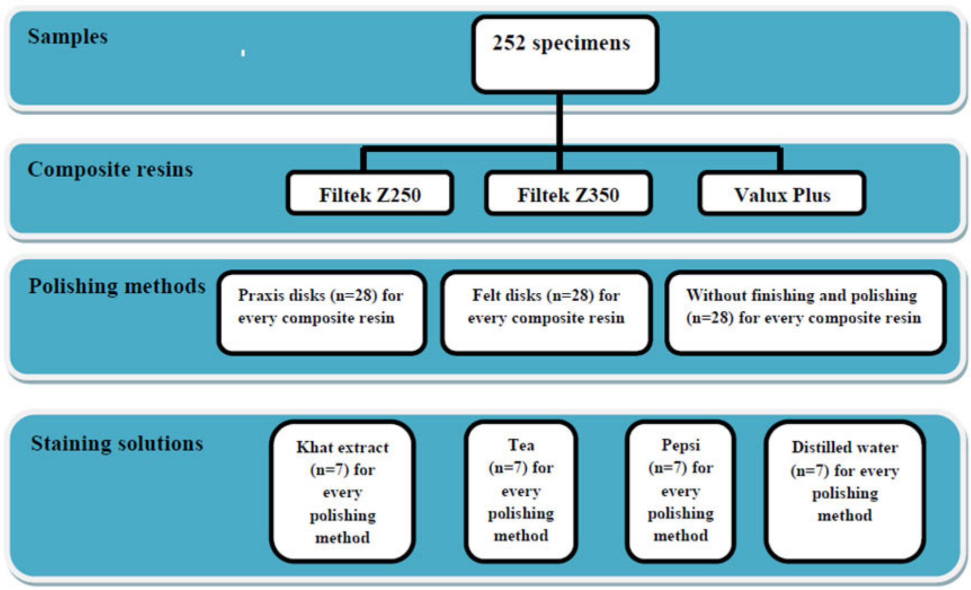

Figure 1. Distribution of the study sample.

Table 1. The characteristics of the composite resin materials and polishing systems used in this study.

A. Composite materials					
Composite material	**Shade**	**Filler type**	**Filler by volume**	**Composition**	**Manufacturer**
3M Filtek Z250 XT	A3	Nanohybrid	60%	Monomers: Bis-GMA, UDMA, and Bis-EMA Filler: Zircon and SiO2 0.6 mm (0.01-3.5 mm)	3M ESPE, St. Paul, MN, USA
3M Filtek Z350 XT	A3	Nanofiller	59.5%	Monomers: Bis-GMA, UDMA, TEGDMA, and Bis-EMA Filler: Aggregated zirconia (0.6-1.4 mm) and non-aggregated SiO2 (20 nm)	3M ESPE, St. Paul, MN, USA
3M Valux Plus	A3	Microhybrid	66%	Monomers: Bis-GMA and TEGDMA Filler: Single filler 100% zirconia/silica (0.01-3.5 mm)	3M ESPE, St. Paul, MN, USA

B. Polishing systems		
Polishing systems	Composition	Manufacturer
Praxis discs	4 grits codified by color of aluminum oxide, polyethylene terephthalate, synthetic rubber resin, aluminum, and water-based pigments	TDV, Santa Catarina, Brazil
Felt discs	Natural wool wheels	TDV, Santa Catarina, Brazil
Poligloss polishing paste	Aluminum oxide	TDV, Santa Catarina, Brazil

Bis-GMA=bisphenol-A-glycidyl methacrylate; Bis-EMA=bisphenol-A-ethoxylateglycidyl methacrylate; TEGDMA=triethylene glycol dimethacrylate; UDMA=urethane dimethacrylate.

b) The specimens were polished using felt disks (TDV, Santa Catarina, Brazil) in combination with Poligloss aluminum oxide polishing paste (TDV, Santa Catarina, Brazil) at low speed for 60 s according to the manufacturer's instruction.

All of the procedures were carried out by the same operator (specimen preparation, finishing, and polishing) [25].
The tea (Al-Kbous Tea, Sana'a, Yemen) was prepared by immersing 2 prefabricated tea bags (200 mg per bag) into 300 mL of boiling distilled water for 10 min according to the manufacturer's recommendation [26]. Khat (Ansy Khat, Sana'a, Yemen) was used for extract preparation. Initially, fresh leaves and twigs of Khat were cleaned, air-dried, and packed in plastic bags and sent to the laboratory. Khat extract was prepared by immersing 20 g aliquots of the dried material into 500 mL of distilled water. Afterward, it was shaken at 200 rpm for 5 h at 37°C. After 5 h of shaking, medium grade filter papers were

used to filter the water extracts. Then fresh stock solutions were prepared by dissolving 5 g of the dried extract in 25 mL of distilled water [27]. Aqueous Khat extract with mineral water was prepared by adding 100 g of Khat extract to 100 mL of mineral water at a ratio of 1:1. The same procedures were executed daily to obtain fresh solutions.

The tested materials were subdivided into 4 groups (*n*=7). All of the specimens in each group were immersed into distilled water, Pepsi, tea, and Khat extract. The solutions were replaced each day, and the specimens were washed with distilled water and dried with absorbent paper before the new immersion. The specimens of each subgroup were immersed in the tested solutions for 3 h daily for 7 days at 37°C [24]. Following completion of the immersion, the specimens were rinsed under running distilled water and air-dried.

After 24 h, when the polymerization was complete, *L**, *a**, and *b** (CIE system) values of the specimens were measured using a colorimeter (Portable Color Difference Meter TCD 100, PCE Instruments UK Ltd, Hampshire, UK) using the equation:

$$\Delta E = [(L^*1-L^*0)^2+ (a^*1-a^*0)^2+(b^*1-b^*0)^2]^{½}$$

Each specimen was measured twice and then the average values were calculated. Color measurement was recorded again, after 7 days of immersion in the solutions, for all of the groups using the same previously described method. For reproducible readings, the stained specimens were allowed to air-dry at room temperature for 30 min before the measurements. The second measurement served as the treatment for each specimen to enable the calculation of the color change (ΔE).

For reliability, 10% of the specimens were chosen randomly and tested. After 2 weeks, the tests were repeated again and then the results were compared using Cronbach's alpha test.

All of the data were collected and statistically analyzed using the Statistical Package for Social Science version 20.0 (SPSS Inc., Chicago, IL, USA). The assumptions of a normal distribution were met after results of the Shapiro-Wilk test ($p>0.05$), therefore parametric tests were used. Three-way ANOVA was used to assess the effects of different polishing methods, storage media, and composites on the color change values, including the possibility of interaction of the three factors. Because significant interaction effects were found, subgroup analysis using one-way ANOVA/Tukey's HSD was applied.

Results

The reliability test was performed after two weeks of re-examination of 10% of the specimens. Cronbach's Alpha value was 0.998, which indicates a high level of internal consistency for our sample data's.

The mean color change (ΔE values) of tested resin composites for all polishing methods in all staining media is exposed in Figure 2. Generally, Filtek Z250 XT showed more color change than Filtek Z350 XT, however least color change was found with Valux Plus in all polishing methods and staining media. According to three-way ANOVA, a significant interaction between three resin composites, staining media and polishing methods was noticed ($p<0.05$). Table 2 displays the mean color change (ΔE values) ± standard deviation of the materials for all polishing methods in all staining media after 7 days. Composite materials were compared according to the effects of different polishing methods in all staining media. There were no significant differences among the polishing methods ($p>0.05$).

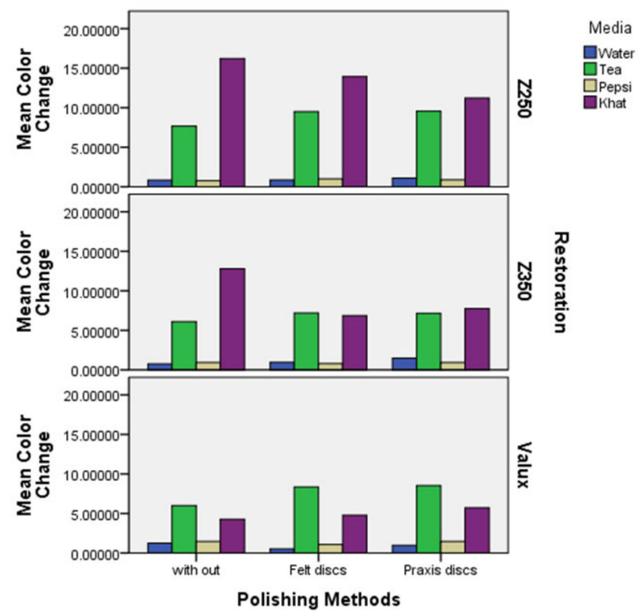

Figure 2. Mean color change (ΔE values) of the tested resin composites for all of the polishing methods in all of the staining media.

Table 3 compares the differences between the ΔE values of all staining media for all composites. Only Khat extract and tea produced clinically perceptible color change ($\Delta E>3.3$). Among the four treatment solutions, Khat extract induced the highest level of discoloration. Least discoloration was found with distilled water. Khat extract and tea revealed significantly more color change than pepsi and distilled water for all tested composites and all polishing methods ($p<0.05$). When polishing systems and staining media were fixed, there were significant differences between Filtek Z250 XT and Valux Plus ($p<0.05$). However, there were no significant differences between Filtek Z350 XT and Valux Plus and between Filtek Z250 XT and Filtek Z350 XT ($p>0.05$) as shown in Table 4.

Discussion

Composite resin restorations must match in shape and shade with the adjacent natural teeth. They must not only function properly but also fulfill aesthetic demands. However, discoloration of resin composites may be a major cause for replacement

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Table 2. Mean color change (ΔE values) ± standard deviation of the materials for all of the polishing methods in all of the staining media after 7 days.

Composite	Staining media	Without polishing	Praxis discs	Felt discs
3M Z250	Water	0.8±0.25	1.1±0.21	0.9±0.39
	Tea	7.7±0.91	9.5±0.67	9.5±1.08
	Pepsi	0.8±0.15	0.9±0.39	0.99±0.40
	Khat extract	16.2±7.47	11.2±3.57	13.9±5.14
3M Z350	Water	0.8±0.37	1.5±0.36	0.96±0.39
	Tea	6.1±0.45	7.2±0.60	7.2±0.66
	Pepsi	0.9±0.19	0.9±0.47	0.8±0.53
	Khat extract	12.8±1.88	7.8±0.94	6.9±2.92
3M Valux	Water	1.3±0.49	0.97±0.49	0.5±0.42
	Tea	6.0±1.49	8.5±1.18	8.4±1.12
	Pepsi	1.5±0.42	1.5±0.41	1.1±0.40
	Khat extract	4.3±0.82	5.7±0.88	4.8±1.58

Table 3. Comparison between three composites for each staining media in all of the polishing methods.

Polishing methods	Staining media		*P* value
Without polishing	Water	Tea	0.001
		Pepsi	1.001
		Khat extract	0.001
	Tea	Pepsi	0.001
		Khat extract	0.001
	Pepsi	Khat extract	0.001
Felt discs	Water	Tea	0.001
		Pepsi	0.997
		Khat extract	0.001
	Tea	Pepsi	0.001
		Khat extract	0.997
	Pepsi	Khat extract	0.001
Praxis discs	Water	Tea	0.001
		Pepsi	0.998
		Khat extract	0.001
	Tea	Pepsi	0.001
		Khat extract	0.987
	Pepsi	Khat extract	0.001

P<0.05 for one-way ANOVA. Tukey's HSD test for multiple comparisons was applied.

Table 4. Multiple comparisons of the mean color change (ΔE values) between three composites.

Composite		*P* value
3M Z250	3M Z350	0.055
	3M Valux	0.002
3M Z350	3M Valux	0.521

P<0.05 for one-way ANOVA. Tukey's HSD test for multiple comparisons was applied.

of restorations [28]. The color stability of composite resins is influenced by both extrinsic and intrinsic factors. Extrinsic factors include the duration and intensity of light emission during the curing process, exposure to environmental factors such as ambient and ultraviolet radiation, water, heat, and food colorants. Intrinsic factors include composition of the resin matrix, filler loading and particle size distribution, type of photoinitiator, and percentage of remaining C=C bonds [29]. Discoloration of dental materials can be evaluated by visual or instrumental techniques [30-32]. Visual methods involve comparison using the eyes [28], whereas instrumental methods involve the use of a colorimeter and spectrophotometer [33]. Color evaluation by visual comparison may not be a reliable method due to discrepancies inherent in the perception of color and the specification of observers. Instrumental techniques include colorimetry, spectrophotometry, and digital image analysis. The CIE Lab system for measuring chromaticity and recording color differences was used in this study because it is suitable for the identification of small color changes and has the advantages of repeatability, sensitivity, and objectivity [34]. A colorimeter (Portable Color Difference Meter TCD 100, PCE Instruments UK Ltd, Hampshire, UK) was used in this study. ΔE<3.3 was considered clinically acceptable, for color changes that cannot be detected by the human eyes [29,35,36].

Routine food habits can affect the esthetic quality of composite restorations. Consequently, studies have reported the susceptibility of resin materials to staining after immersion in solutions such as coffee [37-39], red wine [39,40], tea [39,41-43], soft drinks [39,42], and juices [8]. The present study addressed the problem of color stability of resin-based composites by investigating their susceptibility to stains caused by commonly consumed types of liquids (such as tea and Pepsi) and Khat, which is the most common social habit in daily life in Yemen (south of the Arabian Peninsula). To the best of our knowledge, there are no previous published studies concerning the color stability of composite resins with Khat. In Yemen, Khat chewing is one of the most common habits that may affect the color of esthetic restoration. Khat is the name generally used for Catha edulis forssk, a dicotyledonous evergreen shrub [44]. Khat has been chewed in Ethiopia since ancient times, and its use spread to east African countries [45] and South Africa [11]. It also has been used in Yemen since the sixth century [46]. Khat chewing has been considered a Muslim habit, and some authors relate its use to Muslim ceremonies However, its use has recently spread to various European, and Asian countries, and Australia [47].

The colorant's polarity can determine its degree of composite penetration. Less polar colorants may be easily absorbed inside the material, whereas more polar colorants tend to be adsorbed on the surface of the material [48]. In this study, immersion in staining media showed diverse effects on the color change of the three tested materials. The present findings indicated that Khat extract as well as tea induced the most color change in all of the materials, which were either polished or not polished. The color change values for all of the tea and Khat extract groups were greater than 3.3. These values were considered clinically unacceptable. The color change occurred due to a combination of intrinsic and extrinsic discoloration. Intrinsic discoloration may occur due to the penetration of yellow pigments through micro-cracks or interfacial gaps at the interface between the filler and the matrix. Extrinsic discoloration may be due to the adsorption of polar colorants and yellow pigments present in tea onto the surface of resin composite materials [49]. Many studies reported that the tannic acid naturally present in tea is responsible for significant color changes of composite resins [43,50-52]. However, crude Khat has tannins and some amount of fluoride, which may be the cause of staining [53,54], particularly in unpolished nanohybrids and nanofilled composite resins. In a case-control study by Yarom et al. investigating the effects of Khat chewing on oral and dental tissues among Yemeni Jews in Israel [55], teeth staining were found in 91.2% of the Khat chewers, while no staining was found in the controls. The authors attributed teeth discoloration to the stains caused directly by the chemicals (tannins) in Khat leaves.

Water absorption seems to affect the optical properties of composites in terms of their susceptibility to extrinsic stains and degradation. Water may decrease the durability of composite resins by expanding and plasticizing the organic matrix, which creates micro-cracks [38,56] and a high level of porosity that may facilitate fluid transport into and out of the polymer [56-58]. Thus, water may act as a vehicle for dye penetration [56,57]. All of the composite resins in this study that were immersed in distilled water showed clinically acceptable color changes. This observation confirms that water sorption itself did not alter the color of the composites to a significant extent because distilled water has no colorant components. However, some studies found that composite resins immersed in distilled water showed a gradual increase in staining of the composite resin when the samples were not subjected to finishing and polishing after 7 days. This could be the result of intrinsic discoloration [19].

Several studies have shown that the smoothest surface is obtained when the resin is polymerized against a polyester strip [3,40]. However, this smooth surface is rich in organic matrix [59] and presents a lower microhardness than polished surfaces, probably because this resin-rich layer has poor physical and mechanical properties [3,40]. The polyester strips used in the preparation of the specimens in all of the groups (with and without finishing/polishing) allowed a greater surface smoothness, thus decreasing the roughness of the resin and consequently the possibility of staining. According to Patel et al. [6], when resins are polymerized in air, the polymerization of the surface layer is inhibited by oxygen. Thus, the use of a polyester strip promotes greater surface smoothness in addition to preventing contact of the material with air, therefore eliminating the layer of unpolymerized resin. However, Dietschi et al. [60] found the area under the polyester strip appeared to have a lower degree of polymerization than the rest of the restoration. Theoretically, this could increase the restoration susceptibility to staining. In this situation, the finishing and polishing procedure could contribute to the color stability because it removes this surface resin matrix. Therefore, polishing tools used over the years range from multiple-step systems using fine and superfine diamond burs, aluminum oxide abrasive disks, and diamond and silicon impregnated rubber cups, to one-step polishing systems such as diamond-impregnated cups and silicon carbide brushes [61]. However, in this study, the removal of the surface layer of the resin by finishing/polishing did not affect the color stability. The smoothest surface was not necessarily the most stain resistant [62], which may support the hypothesis that discoloration is not exclusively dependent on the surface roughness but exposure to the staining media may change the surface quality and predispose materials to discoloration [63]. Similar results to the present study were also found by Bagheri et al. [7] and Nagem Filho et al. [64].

Another factor considered in the present study might be the types of disks used that have limitations due to their geometry [65,66]. Barbosa et al. [59] observed that the use of diamond burs produced scratches on the composite surface; discs caused some surface damage that may have been due to the loss of particles during polishing with abrasive materials [67]. Nonetheless, the polishing phase plays a critical role in the restoration process. However, polishing may result in an increase in surface roughness, with important consequences on plaque adhesion, surface pigmentation, and composite marginal integrity [65].

Among the three composite resins, Filtek Z250 XT was associated with the highest discoloration, whereas the least discoloration was associated with Valux Plus, irrespective of the immersion media, finishing, and polishing (**Figure 2**). Therefore the null hypothesis of this study were accepted which is the color stability of different composite resins would not be affected by Khat extract and other staining media after subjecting them to various polishing methods. Significant staining is generally associated with the hydrophilic nature of the composite matrix [48]. Hydrophobic materials are stained by hydrophobic solutions, and hydrophilic materials with high water absorption are stained by hydrophilic colorants in aqueous solutions [48].

The type of monomer in the resin was another factor responsible for increased staining. Awliya et al. [63] found that composites with Bis-GMA-based resins had greater water sorption than other composites. The water uptake in the Bis-GMA-based resins increased from 3% to 6% as a proportion of the triethylene glycol dimethacrylate (TEGDMA) increased from 0% to 1%. Furthermore, resins with higher concentrations of TEGDMA were more prone to color change. This monomer has a highly hydrophilic nature, which increases water and other fluid sorption [37]. In the present study, the Filtek Z250 XT had significantly greater color change than the Filtek Z350 XT. This may be because the Filtek Z350 XT has small amount of TEGDMA associated with Bis-EMA, which has a matrix with hydrophobic features [68].

However, the microhybrid resin (Valux Plus) showed the lowest ΔE* values compared to the other composite resins. The low staining susceptibility of Valux Plus was most probably linked to its low water sorption rate, which in turn was related to its hydrophobic matrix [69]. Filler loading also plays an important role in composite discoloration [70]. Kim et al. [71] found that increased filler loading resulted in lower water absorption. The filler loading by weight of Valux Plus was 66% whereas that of Z250 XT and Z350 XT were 60% and 59.5%, respectively. This might be the reason that Valux Plus had the lowest ΔE* values than the other composite resins used in this study. In addition, it is well known that vitamin C (ascorbic acid) has a low pH and can contribute to dental erosion [72]. A high concentration of ascorbic acid (vitamin C) was also found in Khat extract (25.72%). Every 1 g of Khat contained 257.20 mg of vitamin C [52]. Another factor that causes high water sorption is the greater erosive effect of acidic media on the material surface. Material that is exposed to acid provides less of a barrier for water molecules to enter the polymer network, thereby increasing the water sorption [73].

Methodological limitations are inherent to all *in vitro* studies. In the oral environment, however, saliva and other fluids may dilute the stains. In addition to the presence of water, temperature changes and pH levels in the oral cavity may affect the properties of dental composite restorations. In this study, flat specimen surfaces were used; clinically, composite resin restorations have irregular geometric structures of convex and concave surfaces. Furthermore, the colonization of bacteria on polished or unpolished surfaces should be investigated for possible differences in the kinetic cell growth and the hardness of bacterial plaque. In addition, the immersion of the specimens in different staining solutions and the absence of cleaning or brushing are significant factors affecting the susceptibility of the materials to staining.

Conclusions

Within the limitations of this *in vitro* study, it can be concluded that the composite resin type and staining solutions showed high color change effects. Stain susceptibility changed not only due to the material composition, but also by the staining solutions. Therefore, practitioners should attempt to minimize the discoloration of composite restorations by adopting suitable restoration types and educating patients on the staining effects and erosive potential of soft drinks and other staining solutions if they are heavily consumed.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

Authors' contributions	WAA	AAM	MAD	AAA
Research concept and design	✓	✓	--	--
Collection and/or assembly of data	✓	--	--	--
Data analysis and interpretation	✓	✓	--	--
Writing the article	✓	✓	✓	✓
Critical revision of the article	✓	✓	--	--
Final approval of article	✓	✓	--	--
Statistical analysis	✓	✓	✓	✓

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